Applications of DNA-barcoding in the identification and understanding of grass invasions in southern Africa

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Abstract

The spread of invasive species is one of the greatest threats to global biodiversity. Alien plant invasions also have serious economic impact in terms of the delivery of ecosystem goods and services. Studies of biological invasions in southern Africa have tended to overlook grasses (family Poaceae), although there are many naturalised species in the region. Only a few of these, all perennials, have been officially categorised as invasive in South Africa, but in the winter rainfall region of the Western Cape, grass invasion especially by Mediterranean European annuals have also been noted. These grasses can be difficult to identify. DNA barcoding has been suggested as an alternative method of identifying grasses in the hope of facilitating identification of existing invaders and preventing future invasions. In this study a list of all known naturalised grasses in South Africa was compiled, and a DNA barcoding reference database was assembled for these naturalised grass species as well as for native southern African grass species. The two official markers for plant DNA barcoding (*rbcLa* + *matK*) were used in barcoding and phylogenetic analyses, both individually and in combination. The barcoding data was assessed for identification efficacy using three distance-based metrics and one tree-based metric in the R package SPIDER, both including and excluding singleton data. This study lists 128 naturalised grass species and subspecies found in South Africa. In the DNA barcoding analyses, *matK* was found to perform better as a single barcode than *rbcLa*, with identification success rates of up to 84% for *matK* and 76% for *rbcLa*, using the most successful metric which was the Nearest Neighbour criterion for both of these markers in the data sets without singletons. The combined *rbcLa* + *matK* data set performed better than either of the two individual markers, with identification success rates of up to 91% in the data without singletons, with the most successful metric again being the Nearest Neighbour criterion. The combined *rbcLa + matK* data would therefore be the recommended DNA barcode for southern African grasses of the three data sets tested, based on the results of this study. Phylogenetic trees were constructed with the DNA barcoding data using Bayesian Inference (BI) and Maximum Parsimony (MP) to assess the usefulness of the data in phylogenetic studies and to confirm the efficacy of this grass DNA barcoding data when using tree-based methods of identification. Both the *matK* and combined datasets resolved all of the grass tribes represented in this study as monophyletic, but the *rbcLa* data did not. None of the datasets resolved the grass family tree to exactly match the most recently published trees, but both the *matK* and combined data sets provided useful phylogenetic information at tribal, generic and species level. Based on the results of this study, many naturalised and native grasses in South Africa can be identified if the two core DNA barcode markers are used in combination, which may allow for the early detection of future grass invasions in the country.

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List of abbreviations

ACDB – African Centre for DNA Barcoding

AGIS - Agricultural Geo-Referenced Information System

AIC - Akaike Information Criterion

BCM – best close match

BEP - Bambusoideae, Ehrhartoideae and Pooideae clade

BI – Bayesian Inference

BOLD – Barcode of Life Database

BP – bootstrap percentage

BSA – bovine serum albumin

˚C – degrees Celsius

CARA – Conservation of Agricultural Resources Act 43, 1983

CBOL - Consortium for the Barcode of Life

CCDB – Canadian Centre for DNA barcoding

CFR – Cape Floristic Region

CI – consistency index

CO1 - cytochrome oxidase sub-unit 1

DMSO - Dimethyl sulfoxide

DNA – deoxyribonucleic acid

EICA – Evolution of increased competitive ability

ERH – Enemy Release Hypothesis

GBSSI - Granule-Bound Starch Synthase

GPWG - Grass Phylogeny Working Group

iBOL – International Barcode of Life

Indels – insertions and deletions

IPNI – International Plant Names Index

ISP – invasive species programme

ITS - internal transcribed spacer

K2P – Kimura two-parameter

k-NN – nearest neighbour criterion

matK - maturase K

MCMC - Markov Chain Monte Carlo

MEGA - Molecular Evolutionary Genetics Analysis

mg - milligrams

$MgCl_2$ – magnesium chloride

ml – millilitres

ML – Maximum Likelihood

mM - millimolar

MP – Maximum Parsimony

MUSCLE - Multiple Sequence Comparison by Log-Expectation

MYA - millions of years ago

ndhF - Nicotinamide adenine dinucleotide dehydrogenase, subunit F

NEM:BA - National Environmental Management: Biodiversity Act, Act No.10 of 2004

NJ – neighbour-joining

nst – number of substitution types

ORF - open reading frame

PACMAD - Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae and Danthonioideae clade

PAUP – Phylogenetic Analysis using Parsimony

PCR – polymerase chain reaction

pers. comm. – personal communication

phyB – phytochrome B

POSA – Plants of Southern Africa online checklist

PP – posterior probability

PVP - polyvinylpyrrolidone

rbcLa - ribulose-1,5-bisphosphate carboxylase/oxygenase, large subunit "A"

RI – retention index

rpoC2 - RNA polymerase II, β" subunit

RNA – Ribonucleic acid

RUBISCO - Ribulose-1,5-bisphosphate carboxylase/oxygenase

SANBI – South African National Botanical Insitute

SAPIA - Southern African Plant Invaders Atlas

SLA – specific leaf area

SNP – single nucleotide polymorphism

SPIDER - SPecies IDentification and Evolution in R

TBR - tree-bisection reconnection

***trn*H – *psb*A** - spacer between the tRNA H and photosystem II protein D1 genes

trnK/matK - tRNA^{Lys} intron/ maturase K open reading frame

µl - microlitres

Chapter 1. Introduction and aims

1.1 Biological invasions

1.1.1 Definitions and models of invasion

The progress of globalisation, among other things, has led to the increased distribution of plant and animal species outside of their native ranges (De Lange & Van Wilgen 2010). The spread of invasive species is one of the greatest threats to the world's biodiversity and a major factor in the extinction of indigenous species (Mack *et al.* 2000, Bromilow 2010). Invasive species also affect the capacity of ecosystems to provide goods and services to humans, and thus can have a large impact on the economic productivity of a country (Van Wilgen *et al*. 2001, Richardson & van Wilgen 2004). Biological invasions can affect crops, forestry, grazing capacity, fishery productivity, cause decreases in available water, and can lead to the loss of native biodiversity that include medicinal plants used by local populations. Furthermore, it is estimated that plant invasions cost South Africa about R6.5 billion each year (De Lange & Van Wilgen 2010).

Richardson *et al.* (2000) define invasive plant species as naturalised species that produce an abundance of reproductive offspring at considerable distances from the parent plant, and that have the potential to spread over a vast area. The important factor is the potential to spread, as many alien (species whose presence in an area beyond their natural geographic range is due to introduction by human activity, whether accidental or purposeful) and naturalised (alien plants that reproduce without human intervention over many generations, with offspring usually recruited close to adults) plants do not end up invading the area in which they are transplanted or surrounding areas.

Transformer species, defined as invasive species that alter the character, condition, form or nature of ecosystems over a considerable area, are especially problematic in causing resource depletion or addition, and ecosystem instability (Richardson *et al.* 2000b), which can increase susceptibility of that ecosystem to fire, erosion, floods and other catastrophic events. Once an ecosystem is disturbed, either by a catastrophic event or by human activities such as overgrazing, ploughing, or addition of fertilisers to nutrient-poor soils, the system is open to further invasions by alien species, as disturbed conditions are known to be favourable for invasion (Hobbs & Huenneke 1992, Clements *et al.* 2004).

Various models for plant and animal invasions have been suggested (Williamson & Fitter 1996, Richardson *et al.* 2000b, Dietz & Edwards 2006). The most recently proposed invasion framework (Blackburn *et al*. 2011) combines the framework used by most plant ecologists when studying invasions proposed by Richardson *et al.* (2000) and the framework proposed by Williamson *et al.* in 1996, which is used by most animal ecologists.

The unified framework combines the concepts of stages of invasion from Williamson *et al.* and barriers to invasion (from Richardson *et al*. 2000b), and recognises that there are distinct stages of invasion in which certain barriers are faced. This unified framework does not distinguish between invasion of disturbed and undisturbed natural habitats, as do Dietz & Edwards (2006), because the level of disturbance is included in the "environmental barrier" and it varies from species to species as to how limiting the level of disturbance in the environment will be.

These barriers are (Blackburn *et al*. 2011):

Stage 1. Transport: Geographical barriers – the alien species must reach a new area outside of its natural range. Human activity often breaches this first barrier.

Stage 2. **Introduction:** Captivity or cultivation barriers – cultivated species are contained and must escape containment to reach the third stage.

Stage 3. Establishment: a) Survival barriers at the introduction site – these barriers will determine if propagules will survive. How the introduced plant copes with soil pH, moisture levels, nutrient availability and other such factors will determine survival at this stage of invasion.

b) Reproductive barriers – the introduced plant must establish a self-sustaining population. Any environmental components of the introduced environment that oppose the reproduction of the introduced species can be considered a reproductive barrier.

Stage 4. Spread: a) Dispersal barriers – in order to become invasive, the introduced species need to be able to spread outside of its area of introduction.

b) Environmental barriers - any condition in the new environment, which limits or prevents the growth or reproduction of the species.

If a species fails to cross any of the barriers at any of the stages of invasion, then that species will fail to become an invader.

1.1.2 Theories of biological invasion success

A driving question for invasion biologists is why some plants are better at invading than others, and why some areas are more susceptible to invasion than others (Richardson and Van Wilgen 2004). There are various theories as to how and why some alien plant species go on to become invasive and outcompete native species in their introduced range.

1.1.2.1 The enemy release hypothesis (ERH)

The ERH has frequently been used as an explanation for invasive success and states that once a plant species is removed from its native habitat it experiences a decrease in herbivory and a release from natural enemies, which may allow for an increase in abundance, size, and distribution (Elton 1958, Crawley & Keane 2002). This theory partly forms the basis for biological control, which has been successful in controlling invasive alien plants (McFadyen 1998). However, Crawley & Keane (2002) argue that the success of biological control is not proof of ERH, as herbicides are also effective at controlling invasive weeds, but the mechanism of invasion success in the weeds was not because of a lack of herbicide in the ecosystem.

Colautti *et al.* (2004) found contradictory evidence for the ERH in biogeographical studies (Fenner & Lee 2001, Callaway *et al.* 2004), and against the ERH in community studies (Gross *et al.* 2001, Agrawal & Kotanen 2003) and point out that there are other variables that could explain increased biomass of invasive species.

This hypothesis does not account for the fact that not all exotic species become naturalised or invasive, even when released from natural enemies. This suggests that the ERH may be too simplistic in explaining the mechanism of all plant invasions.

1.1.2.2 The evolution of increased competitive ability (EICA) hypothesis

The EICA theory builds on the ERH, and was first suggested in 1995 by Blossey & Nötzold. They hypothesised that the removal of a plant species from its native habitat to a new alien habitat reduces natural herbivore predation, and allows the plant to dedicate more of its resources to growth and development, so that alien invasive species tend to grow taller, accumulate greater biomass and produce more seeds than the same species in their native environment. The theory also states that invasive species are not immediately adapted to invade their new surroundings but adapt and evolve a competitive genotype in their new habitat. As a result exotic plant species should exhibit reduced herbivore resistance when reintroduced into their native ranges, as they have evolved a reduced herbivore response.

This theory has received support (Daehler & Strong 1997, Siemann & Rogers 2001), partial support (Hull-Sanders *et al*. 2007) and counter-evidence has been presented (Willis *et al*. 1999, Van Kleunen & Schmid 2003, Vila *et al.* 2003, Bossdorf *et al.* 2004, Handley *et al.* 2008, Williams *et al.* 2008), leading to the conclusion that, like the ERH, the EICA hypothesis does not apply in all cases of biological invasion. Bossdorf *et al*. (2004) hypothesise that an increase in competitive ability in some species' invasive ranges may have costs that include reduction in biomass allocation, and Reinhart *et al.* (2003), for example, found that increased biomass of *Prunus* L. seedlings in their non-native range was not linked to soil pathogen release.

1.1.2.3 Darwin's naturalisation hypothesis

There are also various theories which postulate that invading plants are able to occupy a vacant niche in their introduced range. It is thought that the invading species is able to occupy a niche that is not being utilised by the native species (Elton 1958, Stachowicz & Tilman 2005), which leads to the hypothesis that more diverse native communities have fewer niches vulnerable to invasion (Levine 2000). Disturbance is also thought to create novel niches that are susceptible to invasion (MacDougall & Turkington 2005).

Darwin's theory of naturalisation states that introduced species that are distantly related to the native species in the introduced range are more likely to become invasive (Darwin 1859) as there are more vacant niches available to them and they are less likely to be competing for the same resources. Darwin also suggested that the converse theory may be true, that introduced species with congeneric relatives in the native flora were more likely to naturalise, and that this may be due to the fact that introduced species that are closely related to the native flora are more likely to be pre-adapted to survive in the introduced environment. These two opposing theories are known as Darwin's naturalisation conundrum (Diez *et al.* 2008).

Darwin's theory of naturalisation has received support (Ricciardi & Atkinson 2004, Strauss *et al.* 2006, Jiang *et al.* 2010, Schaefer *et al*. 2011), partial support (Bezeng *et al*. 2012, Diez *et al*. 2008) and contradiction (Duncan & Williams 2002) in the literature. Thuiller *et al*. (2010) argued that it is crucial to consider the scale of the study when testing Darwin's hypothesis, and that any analysis conducted at genus or family level are not suitable to accurately test the hypothesis, as the dissimilarities being compared will be too broad.

1.1.2.4 Invasive traits

An important question in invasion biology is whether some species are inherently better suited for invasion than others (Pyšek & Richardson 2007). Crawley *et al*. (1996), point out that all plants must have the ability to increase their populations, which is an important invasion criterion, otherwise they run the risk of extinction. Identifying traits which allow an introduced species to outcompete a native one is therefore not always straightforward.

Numerous traits have been shown or are proposed to be linked to the invasive ability of an introduced plant species. These include wide range environmental tolerance, tallness, phenotypic plasticity, rapid evolutionary adaptation, high growth rates and biomass production, high reproductive potential and large specific leaf area (SLA) (Baker 1965, Richards *et al.* 2006, Gallagher *et al*. 2011, Te Beest *et al.* 2011, Skálová *et al.* 2012).

Zedler & Kercher (2004) argue that plants can potentially be invasive when they have multiple attributes that allow them to capitalise on opportunities presented by disturbed habitats. In general, invasive species have been demonstrated to germinate earlier, flower earlier, later, or for longer periods, produce larger or more abundant flowers (Brown *et al*. 2002), grow more quickly, produce greater biomass, exhibit vegetative growth, have an extended growing season (Zedler & Kercher 2004), recover more quickly from fire or herbivory, produce more seeds, draw water more quickly (D'Antonio & Vitousek 1992), use up soil nutrients more quickly or alter soil nutrients in their favour, use a different photosynthetic pathway (Milton 2004), or use allelopathy (Zedler & Kercher 2004) to outcompete native species.

Selfing or autogamy is a trait that has often been linked to invasive potential (Harmon-Threatt *et al*. 2009). Selfing as an invasive reproductive strategy allows for fairly rapid reproduction even in the presence of only one propagule, avoids pollinator limitations, reduces the biomass expenditure required for floral displays to attract pollinators, and ensures that progeny are similar to the successful parent coloniser (Clements *et al*. 2004). While selfing is commonly present in many invasive species, both selfing and outcrossing is present in others, and some successful invaders are obligate outcrossers, thus selfing is not a necessary requirement for invasion.

In terms of the ability of invasive species to self-reproduce, (Baker's Rule, Baker 1965), it is not always clear whether the autogamy that is prevalent in invasive species (Harmon-Threatt *et al.* 2009, Hao *et al.* 2011) is present at the time of introduction, or is an evolved adaptation due to pollinator limitation, or an adaptation evolved to enhance competition for resources (Rambuda & Johnson 2004). Fenster & Barrett (1994) reported that the recessive modifier alleles that promote selfing were present in low quantities of native Brazilian populations of *Eichhornia paniculata* (Spreng.) Solms (Brazilian water hyacinth) but that these alleles were selected for and increased rapidly in the introduced population on the Caribbean islands, possibly due to the lack of pollinators for this species on the islands.

1.1.2.5 Phenotypic plasticity

Instead of the existence of a general, static "invasive phenotype" (Baker 1965), some invasion biologists have reported that alien plant species can adapt rapidly to new environments (Clements *et al.* 2004). The proposed mechanisms of this rapid evolution are often linked to the trait of phenotypic plasticity, which may allow for greater ecological niche breadth, and may be influenced by standing variation found in the introduced individuals, inter- and intra-specific hybridisation, polyploidy and adaptation to stressful novel environments via epigenetics or transposon activation (Prentis *et al.* 2008, Te Beest *et al.* 2011). Multiple introductions have also been reported to increase the genetic variation in invasive plant populations (Lavergne & Molofsky 2007). Invasive species may either exhibit inherently greater phenotypic plasticity than native species due to standing genetic variation, or they may evolve greater phenotypic plasticity than native species (Richards *et al.* 2006).

Polyploidy has been observed in significantly higher numbers in invasive compared with non-invasive angiosperms (Prentis *et al.* 2008, Pandit *et al.* 2011). Polyploids have larger cells and organs and they produce more biomass and larger seeds, which may allow for increased competitive ability, as well as allowing for increased phenotypic plasticity due to the potential for multiple alleles per gene (Te Beest *et al*. 2011).

Some studies have found that invasive species show more phenotypic plasticity when compared to the same species from the native range (Kaufman & Smouse 2001), and when compared to native species in the introduced range (Milberg *et al*. 1999, Skálová *et al.* 2012). Others have found no evidence of phenotypic plasticity as a factor in the invasive success of a species (DeWalt *et al.* 2004), which suggests that phenotypic plasticity cannot always be used to explain the invasive potential of a plant species.

1.2 Impacts of plant biological invasions

In general, invasive plants, and in particular transformer species, may have a significant impact on ecosystems. They may be able to outcompete native species by differential or excessive use of nutrients and/or water, in many cases forming dense, monotypic stands, which completely exclude native species, hampering their germination and growth. These invasive species can also alter soil stability, as well as water usage and quality (Zedler & Kercher 2004), and can affect ecosystem processes such as primary productivity, fire regime (D'Antonio & Vitousek 1992, Brooks *et al.* 2004), decomposition, and nutrient cycling (Vitousek *et al.* 1996, Richardson & Van Wilgen 2004). Introduced species can also be vectors of plant, animal, and human diseases (Vitousek *et al.* 1996). Symbiotic relationships between indigenous plants, animals, fungi and microorganisms are threatened when invasive alien plant species replace native populations (Richardson & Van Wilgen 2004).

Aquatic alien weeds can form dense mats on water bodies, and alter the light penetrance, water quality and nutrient balance of these bodies. This can affect animals and other plants living in these aquatic ecosystems (Van Wilgen *et al.* 2001).

Invasive species affect the capacity of ecosystems to provide goods and services. For example, invasive weeds can provide challenges for farmers if weedy species outcompete crops or displace grasses that are suitable for grazing with unpalatable species (Richardson & Van Wilgen 2004). Loss of recreational revenue is also an issue – in South Africa, altered sedimentation leading to sand depletion on beaches invaded by rooikrans (*Acacia cyclops* G.Don), loss of native wildflower species important for ecotourism and flower harvesting due to tree and shrub invasions, and the clogging of dams and rivers used for watersports with aquatic weeds are some of the challenges to the tourism industry (Richardson & Van Wilgen 2004).

As mentioned, biological invasions pose a major threat to the world's biodiversity. This is of great concern in South Africa, which is home to three of the world's biodiversity hotspots, namely the Succulent Karoo, the Maputaland-Pondoland-Albany, and the Cape Floristic

Region (CFR), the latter of which is home to more than 9 000 species and more than 6 000 endemic species (Manning & Goldblatt 2012). The CFR is also the country's most invaded biome (Richardson & Van Wilgen 2004).

South Africa has a ~350 year history of colonialism, which brought with it many alien species for use in agriculture, forestry and ornamental gardens (Rouget *et al.* 2003). Tree species from the genera *Acacia* Mill., *Hakea* Schrad. & J.C.Wendl., and *Pinus* L. have invaded the fynbos and renosterveld biomes of the CFR (Van Wilgen *et al*. 2001, Richardson & Van Wilgen 2004). Dense stands of these species can reduce the seedbanks of native species, leading to a decrease in biodiversity of the area (Musil 1993, Holmes & Cowling 1997) and can increase fuel loads in these ecosystems. While many fynbos species are serotinous, an increased fire regime can prevent recovery of these species.

Also of great concern in South Africa is the differential use of water by invasive species in comparison to indigenous ones, as water is a scarce resource in many parts of the country. Riparian, estuarine and wetland ecosystems are especially vulnerable to plant biological invasion, probably because they are nutrient rich, water-abundant and naturally disturbed. A range of alien species have colonized these ecosystems in South Africa, from the giant reed *Arundo donax* L, and tree species such as *Pinus* species, *Acacia mearnsii* De Wild and *Eucalyptus camaldulensis* Dehnh., to the evergreen shrub *Lantana camara* L. (Richardson & Van Wilgen 2004). Often these ecosystems are the starting points for invasions that spread away from the water with time. It is estimated that invasion by alien species at water catchment areas in South Africa may have reduced river flow by up to 6.7% (Versveld *et al.* 1998, Le Maitre *et al*. 2002).

Free floating and submerged aquatic invaders, such as *Azolla filiculoides* Lam., and *Eichhornia crassipes* (Mart) Solms (water hyacinth) are also problematic in South Africa as they are responsible for decreasing water flow, blocking light penetration and oxygen flow into the water, and reducing water quality and biodiversity (Richardson & Van Wilgen 2004, Hoveka 2014). Water hyacinth is widespread throughout South Africa and it is particularly problematic in six provinces, stretching from Mpumalanga to the Western Cape (Richardson & Van Wilgen 2004). Current methods of control for water hyacinth are usually mechanical and/or chemical control, but both of these strategies are costly, short-term strategies (Van Wyk & Van Wilgen 2002). Biological control is considered to be an affordable longer term solution, but currently biological control of water hyacinth in South Africa has not been as successful as elsewhere, in part due to the low temperatures and frosts in the highveld region, which is not ideal for the insects currently used to control the weed in subtropical or tropical parts of the world (Coetzee *et al.* 2011). Research is currently being carried out investigating potential new biological control agents (Coetzee *et al.* 2011) or combinations of existing agents (Marlin *et al.* 2013) for water hyacinth in South Africa.

1.3 Family Poaceae

The grass family (Poaceae) includes some of the most important crop species upon which humans, wild and domesticated herbivores are dependent for a major portion of their diet, including maize, rice, sugarcane, wheat, sorghum, barley, oats, rye, and millet. Other grasses such as *Panicum virgatum* L. (switchgrass) and *Miscanthus x giganteus* have been identified as potential sources of biofuel (Byrt *et al*. 2011). Grasslands and other grass-dominated ecosystems cover approximately one third of the earth's surface (Shantz 1954). The family is composed of about 10 000-11 000 species accommodated in 600-700 genera (Clayton & Renvoize 1986, Watson & Dallwitz 1992, Kellogg 2001, GPWG 2012). This family also contains large numbers of species that have become invasive around the world, and is therefore one of the most important families for invasion biologists to study.

Poaceae is a monophyletic, monocotyledenous family. The origin of grasses has been fixed at between 55 and 70 million years ago (MYA), based on the appearance of grass pollen in the fossil record in South America and Africa (Linder 1985, Jacobs *et al*. 1999) and the non-parametric rate-smoothing analysis of *rbcL* molecular data (Bremer 2002). Grass pollen is distinct and can be identified by microscopic channels that perforate the outer pollen wall (Linder & Ferguson 1985). Almost all grasses are wind-pollinated. Grass flower structure is therefore reduced, as is common in wind-pollinated groups (Gibbs Russell *et al*. 1991).

Grasses (particularly those in sub-Saharan Africa) have co-evolved with herbivores over millions of years (Wang *et al*. 1994, Jacobs *et al*. 1999) and this has resulted in widespread tolerance of grass species to grazing via adaptations such as leaf fibres and increased leaf silica content. Intercalary meristems are an important adaptation of grasses to repeated grazing and fire, and allow for rapid regeneration after disturbances where apical meristems are removed or damaged (Gibbs Russell *et al*. 1991). Perennial grasses survive as dormant rootstocks or with vegetative rhizomes or stolons, and annuals survive via their seedbanks.

1.3.1 Grass phylogeny

Much work has been done to complete a phylogeny of the grasses. The most comprehensive studies have been carried about by the Grass Phylogeny Working Groups (GPWG) I and II. In 2001, GPWG I published a phylogeny based on 59 representative species with a combination of data from previous studies, namely: chloroplast genes *ndhF* (NADH dehydrogenase, subunit F) (Clark *et al*. 1995), *rpoC2* (RNA polymerase II, β" subunit) (Barker *et al.* 1999) *rbcL* (ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) large subunit "a") (Barker *et al.* 1995), the nuclear regions, *phyB* (phytochrome B) (Mathews *et al*. 2000), GBSSI (Granule-Bound Starch Synthase) (Mason-Gamer *et al*. 1998), ITS (internal transcribed spacer) (Hsiao *et al*. 1999), restriction site maps of the chloroplast genome (Soreng & Davis 1998), and 53 morphological characteristics (Kellogg *et al*. 2001). Another tree was published in 2012 by GPWG II, this time using *rbcL*, *ndhF* and *trnK/matK* (tRNA^{Lys} intron/ maturase K ORF) data for 531 species, representing 311 genera.

These and other studies on Poaceae phylogeny agree that there are 12 monophyletic sub-families (Sánchez-Ken *et al*. 2007, Bouchenak-Khelladi *et al*. 2008, GPWG II, 2012). Three of these, the Anomochlooideae, Pharoideae, and Puelioideae were early-diverging, with Anomochlooideae being the earliest to diverge (Figure 1.1). The other nine subfamilies form a clade that is further divided into two clades; the BEP clade (Bambusoideae, Ehrhartoideae, Pooideae), and the PACMAD clade (Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae and Danthonioideae, Figure 1.1), which encompasses all of the C_4 grasses. C_4 photosynthesis is estimated to have evolved 22-24 times within the PACMAD clade (GPWG II 2012). Originally, a thirteenth sub-family was characterised, the

Centothecoideae, but more recent studies (Sánchez-Ken & Clark 2007, Zuloaga *et al.* 2007, Sánchez-Ken & Clark 2010) proposed that as monophyly of this sub-family is weakly supported and it appears to be polyphyletic within the Panicoideae, circumscription of the Panicoideae should be expanded to include the Centothecoideae.

The sister-clade to Poaceae has frequently been resolved as Joinvilleaceae. Poaceae has three chloroplast inversions, two of which are shared with other closely related families and one which is shared with Joinvilleaceae only. The third is unique to the grasses (Doyle *et al.* 1992). This was confirmed by Bouchenak-Khelladi *et al*. (2008) with their large multi-gene tree (using *rbcL*, *matK* and *trnL-F* markers), although other molecular studies have placed Ecdeiocoleaceae as sister to Poaceae (Bremer 2002, Michelangeli *et al*. 2003), and *rbcL* data from Briggs *et al.* (2000) indicated that the sister clade to Poaceae should be Joinvilleaceae + Ecdeiocoleaceae, a relationship supported by Bouchenak-Khelladi *et al*. (2014).

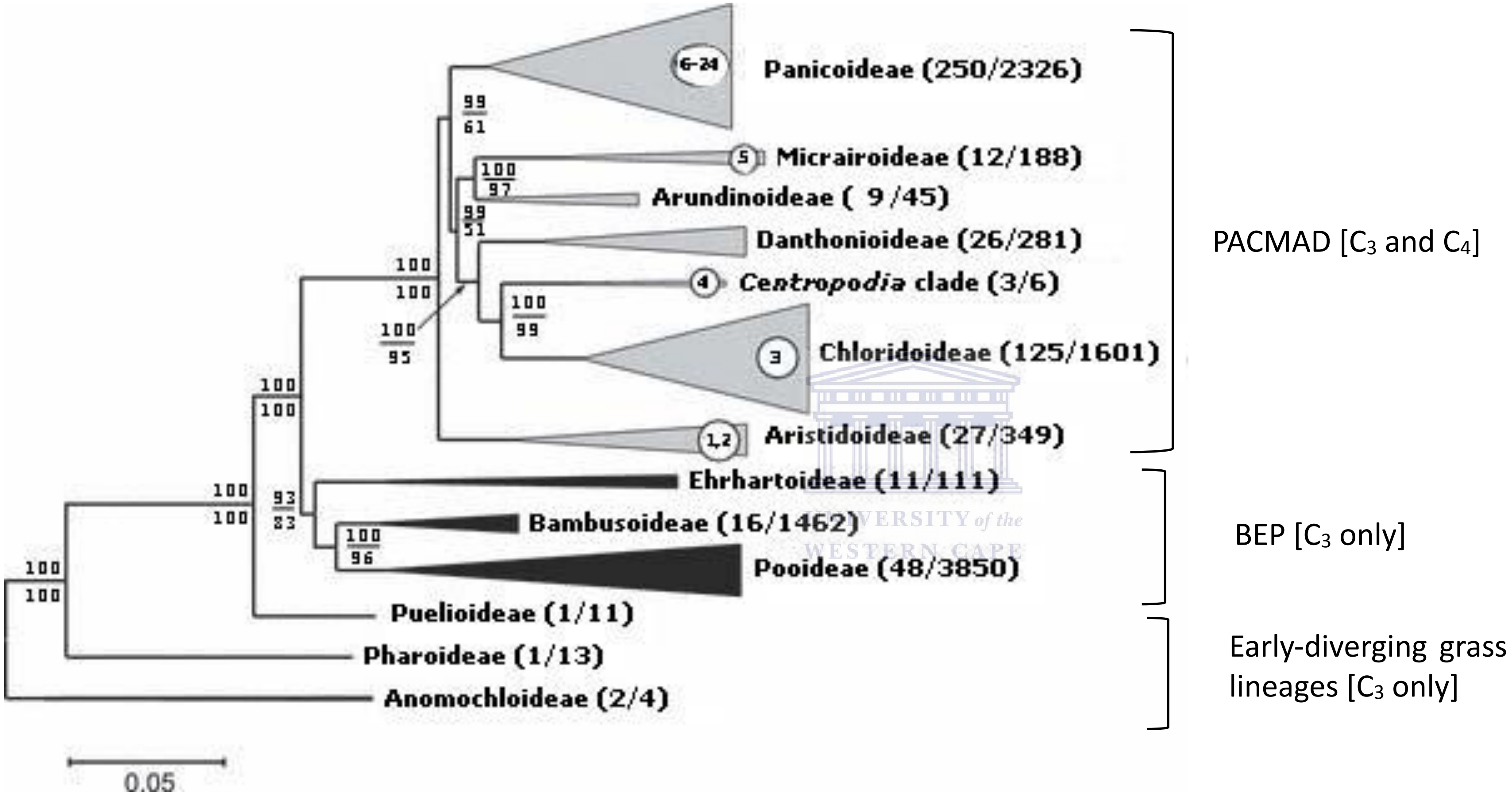


Figure 1.1 Phylogenetic tree of Poaceae, showing the early diverging lineages Anomochloideae, Pharoideae and Puelioideae, as well as the BEP and PACMAD clades, based on Bayesian inference (edited from GPWG II 2012). The numbers within the sub-family clades in the PACMAD clade represent the instances of the evolution of C_4 photosynthesis.

1.4 Grass invasions

1.4.1 Impacts

Grass invasions are widespread throughout the world, although examples in Africa are relatively rare (D'Antonio & Vitousek 1992). African grasses have co-evolved with large grazing herbivores for millions of years and are well-adapted to fire, grazing and other disturbances**.** This has led to invasions by African grasses in many parts of the world (D'Antonio & Vitousek 1992).

Invasive grasses alter ecosystems by increasing groundcover and intercepting incident light, thereby outcompeting slower-growing native species by preventing germination and growth, competing for water and nutrient resources, affecting herbivore grazing habits, increasing litter which alters humidity and soil properties, interfering with crop species, transforming riparian, wetland and estuarine ecosystems, and altering fuel loads and fire regimes (D'Antonio & Vitousek 1992, Musil *et al*. 2005).

Large rhizomatous grasses such as *Arundo donax* L., *Phragmites australis* (Cav.) Trin. ex Steud. and *Spartina alterniflora* Loisel., invade riparian and estuarine ecosystems and form dense monotypic stands, which can decrease water levels, alter water flow and cause silting (Guthrie 2007, Baumel *et al.* 2002, Gray *et al*. 1990, Adams *et al.* 2012).

Both annual and perennial grass species have the potential to become invasive. Annual grasses are opportunistic pioneer species that generally germinate quickly and grow rapidly, which enables them to deplete water and nutrient resources before native species can access them. Annuals also produce abundant seed banks. Perennial grasses on the other hand may be able to persist and regenerate after fires and grazing, and numerous invasive perennial grasses are large in size, with large flowers. Many of the most invasive perennial grasses are polyploids, which is linked to phenotypic plasticity, as well as larger cells and organ size, increased biomass and larger seeds (Te Beest *et al*. 2011).

Many grasses have evolved to be adapted to fire, and some grass species have reproductive cycles that are stimulated by fire. Grass tissue dries rapidly and burns readily. In some cases alien grasses burn more readily than native species, and are stimulated to germinate by fire. The presence of alien grasses in an ecosystem may reduce the number of years between natural fires, which could hamper native plants and recovery of animal species, in a positive feedback loop that leads to alien-dominated grasslands. A good example is the invasion of the winter European annual *Bromus tectorum* L. (cheatgrass) into the western intermountain region of North America (Knapp 1996). This grass increased the frequency of fires in the area from every 60-110 years to every 3-5 years (Whisenant 1990), which has caused erosion, the displacement of native species in the area, and cost the US government millions of dollars in terms of fire management and rehabilitation.

1.4.2 Grass invasions in South Africa

Grass invasions in South Africa are considered to be poorly characterised and understood, and until recently were considered of little concern in comparison to other invasive plant species in the country (Milton 2004). However, there are reported grass invasions throughout South Africa, mainly on disturbed lands such as old mine dumps, along roads and paths, and on abandoned agricultural fields. They also occur in wetland and riparian ecosystems and are currently a problem in the winter rainfall areas of the Western Cape, particularly in the renosterveld, which is a highly disturbed and fragmented ecosystem (Milton 2004).

Only ~12% of grass species in southern Africa are naturalised alien species (Milton 2004). Of these, only a few have come under legislation in South Africa, requiring control. The first list of invasive alien species requiring control in South Africa was published in the Conservation of Agricultural Resources Act 43, 1983 (CARA, updated in 2001). According to this legislation, Category 1 invaders must be removed and destroyed immediately, Category 2 invaders must be grown under controlled conditions only, and Category 3 invaders may no longer be planted, but existing plants may remain (Bromilow 2010) (Table 1.1).

The National Environmental Management: Biodiversity Act, Act No.10 of 2004 (NEM:BA) is intended to replace the CARA legislation. On 22 February 2014, the government published draft regulations relating to the NEM:BA Act, and on 1st August 2014 the final regulations were published. These regulations list invasive species and stipulate the control measures to be taken for various categories of invasive species. Fifteen grass species (all perennials) that are found on the mainland of South Africa are listed (Table 1.1). Category 1a and 1b invasive species in the NEM:BA act may not be owned, imported into South Africa, moved, sold, may not be given as a gift, or dumped in waterways. Category 1a species require compulsory intervention by the Department of Environmental Affairs if they are found on private property, and Category 1b must be contained, with government assistance if required. Category 2 species can remain in gardens with a permit, and Category 3 species are allowed to remain in gardens but are not allowed to be sold or propagated (NEM:BA 2014).

Table 1.1 Invasive grasses in South Africa as classified by CARA (from Bromilow 2010, with addition from NEM:BA 2004 (published 1 August 2014). X under the CARA legislation is the proposed category.

Alien grass species	**longevity**	**CARA**	**Current NEM:BA (1 August 2014)**
Ammophila arenaria (L.) Link	P	1b	3
Arundo donax L.	P	1	1b
Cortaderia jubata (Lem.) Stapf	P	1	1b
Cortaderia selloana (Schult. & Schult.f.) Asch. & Graebn.	P	1	1b
Cynodon dactylon (L.) Pers.	P	X2	-
Glyceria maxima (Hartm.) Holmb.	P	-	1b in protected areas and wetlands, not listed elsewhere
Lolium multiflorum Lam.	P	X2	-
Lolium perenne L.	P	X2	-
Nassella tenuissima (Trin.) Barkworth	P	1	1b
Nassella trichotoma (Nees) Hack. ex Arechav.	P	1	1b

Alien grass species	**longevity**	**CARA**	**Current NEM:BA (1 August 2014)**
Paspalum quadrifarium Lam.	P	-	1a
Pennisetum clandestinum Hochst. ex Chiov.	P	X2	1b in protected areas and wetlands, not listed elsewhere.
Pennisetum purpureum Schumach.	P	X2	1b
Pennisetum setaceum (Forssk.) Chiov.	P	1	1b
Pennisetum villosum R.Br. ex Fresen.	P	-	1b
Sasa ramosa (Makino) Makino & Shibata	P	-	3
Sorghum halepense (L.) Pers	P	2	2
Spartina alterniflora Loisel.	P	-	1a

Although only perennial grasses have been legislated as invasive in South Africa, both annual and perennial alien grasses have succeeded in invading parts of the country. Most of the annual naturalised grasses in South Africa are C_3 grasses from the sub-family Pooideae. The most commonly found alien annuals are from the genera Avena L., *Briza* L., *Bromus* L., *Hordeum* L., *Lolium L., Phalaris* L., *Poa* L. *and Vulpia* C.C.Gmel. (Midoko-Iponga 2004, Miton 2004, Shiponeni & Milton 2006, Muhl 2008, Sharma *et al.* 2010). C_3 grasses prefer cool, wet growing seasons, whereas C_4 grasses (most southern African grasses are C_4) are best adapted to warm, drier growing seasons. These alien annuals originate from the fire-prone Mediterranean European winter rainfall region, and these grasses are successfully invading the Cape Floristic Region (particularly the remaining patches of renosterveld vegetation) which shares a similar climate to that of the Mediterranean (Milton 2004, Musil *et al.* 2005). In Hawaii, C_3 grasses also grow in higher temperatures, but there is a marked precipitation distribution gradient, with C_3 grasses found in wetter and C_4 in more arid areas. C_4 photosynthesis is more water efficient (Edwards & Still 2008).

These annual alien grasses presently mostly invade abandoned agricultural land and nearby vegetation patches that have high nitrogen concentrations in the soil due to past fertilizer application, but there is concern that they may spread further into the renosterveld patches in the future. C_3 grasses require more nitrogen than C_4 species, so these C_3 aliens generally only invade in nitrogen-rich disturbed soils, whereas most of the indigenous grass species are C_4 species and are able to grow in low-nitrogen soils (Richardson *et al.* 2000a). There is concern that global climate change will cause increased CO_2 levels, which could maximise the efficiency of C_3 photosynthesis and also cause these C_3 grasses to have increased nitrogen-use efficiency, which would allow them to invade a wider range of soils (Milton 2004).

A study that modelled potential future ranges of alien annual grasses in South Africa based on predicted climate variables in the year 2050 found that in general, future climate warming will cause range contractions for almost all of these grasses (Parker-Allie *et al.* 2009) with range shifts into areas of higher elevations and cooler temperatures for the C_3 grasses. However, this study does not include the possible effects of higher CO_2 levels, which may counteract the negative effects of increased temperature on these C_3 grasses, as discussed above.

Perennial alien species have mostly invaded riparian and wetland areas throughout South Africa, which may lead to water-depletion in an already water-scarce country. *Arundo donax* is a particular problem in South Africa along rivers (Guthrie 2007) due to its rapid growth, high transpiration rates, and flammability, and has been mentioned as one of the six most important plant invaders in terms of its impact in the country (Henderson 1998).

Spartina alterniflora Loisel., another large perennial hydrophyte, has been known to form hybrids with other *Spartina* species, such as *Spartina maritima* (Curtis) Fernald and *Spartina foliosa* Trin., which after polyploidisation events have gone on to become highly invasive (Gray *et al*. 1990, Baumel *et al.* 2002). *Spartina alterniflora* has been found in the Great Brak estuary in South Africa (Adams *et al.* 2012), and *S. maritima* is also present in South Africa, so there is concern that unless *S. alterniflora* is eradicated, similar invasive hybrids could be formed in South Africa in future.

Pennisetum setaceum (Forssk.) Chiov. is a polyploid perennial grass (native to the Middle East and North Africa) that is unpalatable to grazers due to barbs on its leaves. This allows *P. setaceum* to dominate, which also increases fire risk to the invaded area (Milton 2004). This species is highly invasive in Hawaii (Wagner *et al.* 1990). In South Africa it is currently mainly confined to mine dumps and roadsides but has also invaded some riparian sites (Milton 2004). *Pennisetum setaceum* populations that have invaded the Gamka River have developed pseudo-vivipary, which allows the plant to reproduce even when the inflorescence is submerged (Milton *et al.* 2008).

1.4.3 Control of invasive grasses in South Africa

Considering the costs of managing and clearing invasive species, considerable focus has been applied to the prediction of potentially invasive species in order to prevent their entry into the country, prevent further use in the country, or to allow for their eradication before they become problematic (Wilson *et al.* 2013). The South African National Biodiversity Institute Invasive Species Programme (ISP) focuses on these preventative stages of biological invasions.

The Working for Water programme was started in 1995 to carry out the management of already invasive alien species in South Africa (Richardson & Van Wilgen 2004). Methods of control of the mostly annual alien grasses in Western Cape renosterveld that have been tested include mowing, hand collection, burning and herbicide application (Musil *et al.* 2005). Intensive burning, although the cheapest method of control, was found to be ineffective because it promoted the recruitment of alien invasive annual and perennial grass species and inhibited the recruitment of native geophytes. Mowing of the infested area prior to seed maturation and the collection of the mown grass for use as fodder was found to be the most effective method of alien annual grass control in this study. Perennial species can be more difficult to control, as mechanical removal of underground vegetative organs may be required to fully eradicate such species. There are currently no biological control protocols for invasive grasses in South Africa, although a bio control system for *Arundo donax* is being investigated (Canavan K., personal communication).

1.5 Issues of identification and DNA barcoding

Grasses are known to be difficult to identify. Identification is usually based on flower morphology, and if a grass is not flowering, it can be particularly challenging to distinguish. Phenotypic variation within a species can also lead to incorrect identification. There is a lack of taxonomists who are trained in grass identification in South Africa. Also, correct identification is very important in invasion biology, as an incorrect identification can have an impact on management (Pyšek *et al.* 2013). For example, crabgrass *Digitaria violascens* Link is morphologically very similar to the European native *Digitaria ischaemum* (Schreb.) Muhl. *Digitaria violascens* has now become a common invader in parts of Italy and Spain, including protected heathlands, where it was incorrectly identified as *Digitaria ischaemum,* and so not managed as an invasive species (Verloove 2010). *Digitaria violascens* is also naturalised in South Africa, where many native *Digitaria* species are found, so it is possible that similar identification issues could occur in this region. As a result of this and other identification issues, alternative methods such as DNA barcoding have been considered for grass species identification in certain situations.

In an effort to categorise and monitor the earth's biodiversity, the concept of a DNA barcode was initially proposed by Hebert *et al.* (2003) and extensively researched by the Consortium for the Barcode of Life (CBOL), an international collaboration of researchers across 25 institutions, which was launched in 2004. This technique would ideally allow for easy, rapid and affordable identification of morphologically indistinct or fragmented samples using a short, standardised DNA sequence, and would have applications in biosecurity, monitoring illegal trade of commercial species, crime scene analysis, identifying alien invasive species, and monitoring biodiversity (Hebert *et al*. 2003). Grasses in particular are often associated with crime scenes, and grass seeds or fragments found on the clothes of victims or suspects can provide useful evidence (Ward *et al.* 2009). The decision to use molecular data was based on the current scarcity of taxonomic specialists, and in terms of plants, the hope was to alleviate the problems of identification based on morphology at various life-stages, phenotypic variation, and the problems associated with identifying plant fragments.

CBOL aimed to standardise the DNA barcoding methods and criteria for international usage. An accessible database, the Barcode of Life Datasystems (BOLD) was established as a repository of DNA barcoding data from around the world, to allow for widespread sharing of data and protocols (Ratnasingham & Hebert 2007).

The criteria for a successful DNA barcode have been established as (CBOL Plant Working Group 2009):

1. Universality - the marker needs to be present and sequenceable in all taxa being examined.
2. Quality and ease of use - it should be easily sequenced with good quality traces.
3. Discrimination - the barcode should be able to distinguish between a large number of closely related taxa.

The mitochondrial gene *CO1* (cytochrome oxidase sub-unit 1) was proposed as a suitable marker for animals by Hebert *et al.* in 2003, as it fulfils all three criteria for a successful DNA barcode and had 100% identification success amongst 200 closely related lepidopteran species. This locus was not suitable for distinguishing plant species due to low substitution rates in the mitochondrial genome in plants in comparison to animals (Wolfe *et al.* 1987, Muse 2000).

The Consortium for the Barcode of Life (CBOL) Plant Working Group proposed that two plastid genes, *rbcLa* and *matK* (both genes already used in plant phylogenetics studies, including those mentioned in 1.3.1 above) as a core barcode for land plants (CBOL Plant Working Group 2009). The *rbcLa* marker is often not variable enough to distinguish species or even closely related genera when used on its own (Gielly & Taberlet, 1994, Rousseau 2012). The barcoding gap for *rbcLa* was found to be the least discriminatory out of 13 candidate barcodes for distinguishing species when using distance-based calculation methods and tree-based assignation methods in a study by Pettengill & Neel (2010). It does however fulfil criteria 1 and 2 for a successful DNA barcode marker as it is universally present, relatively strongly conserved and is easy to sequence across most plant taxa (CBOL Plant Working Group 2009, De Vere *et al*. 2012, Rousseau 2012, Gere *et al.* 2014, Hoveka 2014, Kabongo 2014).

The *matK* marker is more variable and better able to distinguish between species (Fazekas *et al.* 2008, Lahaye *et al*. 2008) but although it is universally present in plant taxa it is more difficult to amplify (as it is not as conserved as *rbcLa*), especially if universal primers are to be used. Universal primers have not always been successful in amplifying across plants groups, which sometimes leads to the need for designing group-specific *matK* primers (Kress & Erickson 2007, De Vere *et al.* 2012, GPWG II 2012).

The combination of these two genes resolved 72% of species in the large study of 550 species representing the major land plant lineages carried out by the CBOL Plant Working Group (2009). Other studies examining a broad taxonomic range have found the combined barcode to distinguish roughly 70-80% of species (Kress & Erickson 2007, Fazekas *et al.* 2008, De Vere *et al.* 2012). This success rate is lower than that found in animals, and studies carried out on closely related taxonomic groups suggest that even this success rate is overestimated for very closely related species, depending on the identification metric used (Hollingsworth *et al.* 2011, Clement & Donahue 2012, Gere *et al*. 2013). For this reason, nuclear regions have also been considered for supplemental use in plant DNA barcoding if required, as synonymous substitutions in nuclear genes are greater than those in plastid genes (Wolfe *et al*. 1987).

The intergenic spacer *trnH-psbA* was suggested by CBOL Plant Working Group (2009) as an additional third land plant DNA barcoding region, as this region is highly variable, is easy to amplify, and has high discriminatory power (Kress & Erickson 2007, CBOL Plant Working Group 2009, Pang *et al*. 2012, Hoveka 2014). This region does pose challenges for species-specific identification across distantly related taxa, because of the variable length of the region, which is often due to the presence of insertions and deletions (indels) (Kress *et al.* 2005). This might mean the use of taxon-specific primers will be required for this region.

The highly variable nuclear internal transcribed spacer (ITS) region was also suggested as an additional plant identification marker as it has shown better identification success than the currently accepted *matk* + *rbcLa* DNA barcode in many studies (Yao *et al.* 2010, China Plant BOL Group 2011, Yan *et al*. 2011). ITS does also provide PCR amplification challenges as there are multiple (sometimes paralogous) copies of this region in many species, although the use of ITS2 only reduces this issue (China Plant BOL Group 2011, Hollingsworth 2011, Hollingsworth *et al.* 2011).

Much of the initial criticism of DNA barcoding related to the idea of using the technique to replace taxonomic studies of species delimitation, instead of using DNA barcoding solely for the identification of already defined species (Will & Rubinoff 2004, DeSalle *et al.* 2005, Wheeler 2005, Ebach *et al*. 2008). Tautz *et al.* in 2003 called for DNA-based data to become central to taxonomic studies, rather than auxiliary to it. They argued that DNA data is not subjective and interpretive data and is reproducible and would allow for world-wide digital communication of information. Defenders of morphology-based studies argued that molecular data are not always informative in isolation as each gene has its own evolutionary history, and that in the study of fossils in comparison to current species, for example, morphological studies are vital as ancient DNA is too degraded for PCR analysis beyond ~100 000 years (Stuessy 2009).

However, over time, the aims of DNA barcoding have been refined to focus on using DNA barcoding as an identification tool, not as a way of replacing taxonomic delineation of species (DeSalle 2006). DNA barcoding should also be separated from phylogenetic studies that are usually directed at refining and confirming taxonomic species delimitations, in that DNA barcoding focuses on identification over species delimitation or evolutionary relationships, although DNA barcoding data does contain phylogenetic information and can be used as such to a certain extent (Pettengill & Neel 2010, Pyšek *et al.* 2013). DNA barcode data may provide an initial indication of the discovery of a new species, but in-depth multi-locus molecular and taxonomic studies are required to confirm and define a new species.

Currently, debates around DNA barcoding tend to focus on the particular methods used to measure species discrimination success and the DNA barcode gap (Srivathsan & Meier 2012, Collins & Cruikshank 2013) rather than on the merits of DNA barcoding itself, which has already demonstrated its usefulness in fields such as illegal trade of protected species (Holmes *et al.* 2009, Alacs *et al.* 2010, Kabongo 2014), incorrect labelling of ingredients in traditional and herbal medicine (Mangka *et al.* 2013, Newmaster *et al.* 2013), food contamination (D'Amato *et al*. 2013) and invasion biology (Chown *et al.* 2008, Briski *et al*. 2011).

1.6 Objectives of this study

Grass invasions are an emerging issue in South Africa. It is therefore preferable to monitor naturalised grass species so that decisions about control can be made before they become difficult to eradicate. Furthermore it is important to monitor grasses that are known to be invasive in parts of the world with similar climates to the parts of South Africa where they are naturalised. As these grasses can be challenging to identify, this study aims to:

Compile a comprehensive list of the naturalised grasses in South Africa from literature. This will be completed in collaboration with the National Working Group on Alien Grasses.

Assemble a DNA barcoding database for both naturalised and native grasses in South Africa, consisting of the two CBOL approved markers for plant DNA barcoding (*rbcLa* and *matK*). The DNA barcoding data can be used as an alternative method of identifying alien grasses if taxonomic expertise is inadequate.

Test the barcode data for identification efficacy using the R package, SPIDER.

Construct a phylogeny of grasses in South Africa from the DNA barcoding data as a further evaluation of the efficacy of this data to distinguish between grasses in South Africa. The phylogeny can also be used as a backbone to which other phylogenetic and taxonomic data can be added to construct a more in-depth phylogeny of South African grasses.

Chapter 2. Materials and Methods

2.1 List compilation

Milton (2004) compiled a list of all of the known naturalised grass genera in South Africa, and this paper was used as a guide for compiling a list of naturalised grass species in the country, along with the books *Grasses of southern Africa* (Gibbs Russell *et al*. 1991) (upon which Milton's paper was based), *Guide to Grasses of southern Africa* (van Oudtshoorn 2006), *Problem plants and alien weeds of South Africa* (Bromilow 2010*), Plants of the Greater Cape Floristic Region Vol. 1: the Core Cape Flora* (Manning & Goldblatt 2012), *Plants of the Greater Cape Floristic Region, Vol.2: the Extra Cape Flora* (Snijman 2013); as well as the South African Plant Invaders Atlas (SAPIA) database, version 2.0, 2006 (AGIS 2007), and SAPIA newsletters, specifically newsletter 7 (2008). Grass expert Ms Lyn Fish was also consulted for up to date advice. Scientific names were checked on the International Plant Names Index (IPNI) (2012) and the Plant List (2013).

Most of the information on naturalised grasses in southern Africa was found in Gibbs Russell *et al.* (1991), and therefore the definition that the authors used for "naturalised" grasses was applied when deciding which grasses to include in the list. The definition used is: introduced grasses that "form self-sustaining populations under local conditions".

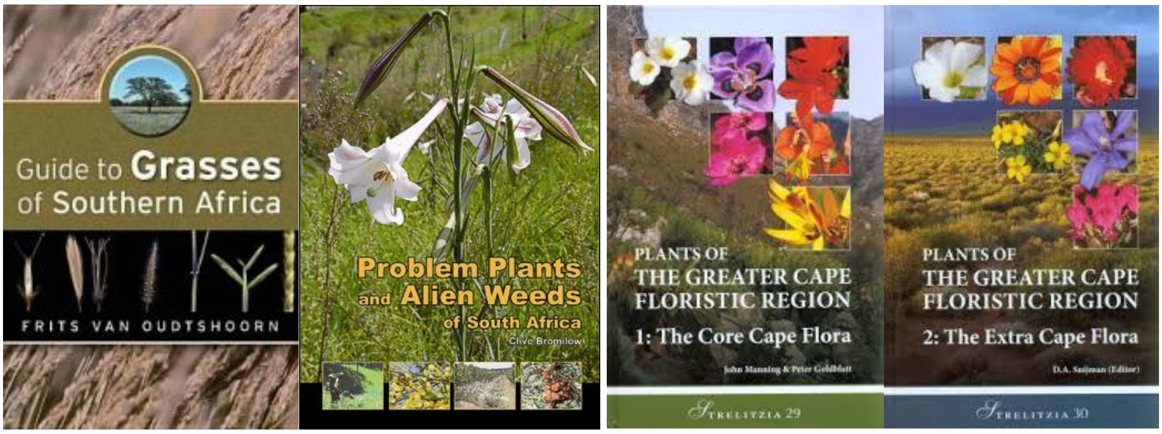
Gibbs Russell *et al.* (1991) is based on herbarium records found in the national herbaria in South Africa up until the publication date, and these records can be found on the PRECIS database, Plants of Southern Africa – an online checklist (POSA 2009). Some species of grass that are found in South Africa are considered of uncertain origin, possibly indigenous or possibly naturalised (Lyn Fish, pers. comm.). These grasses were not included in the list of naturalised species, however many of them were included in the phylogenetic and barcoding analyses, which cover grasses in South Africa generally. There is no exhaustive list of species of unknown origin, but rather a working list of species based on expert knowledge (Lyn Fish, pers. comm.).

Table 2.1 List of grasses considered to be of uncertain origin in South Africa, based on Bromilow (2010), Gibbs Russell *et al.* (1991), and Lyn Fish (pers. comm.). Not all of these species are included in this study. Abbreviations: EC = Eastern Cape; FS = Free State; G = Gauteng; KZN = KwaZulu-Natal; L = Limpopo; M = Mpumalanga; NC = Northern Cape; NW = North West province; WC = Western Cape.

Scientific name	**Sub-family**	**Distribution in South Africa**	**Included in this study**
Alloteropsis cimicina (L.) Stapf	Panicoideae	G	No
Arthraxon lanceolatus Hochst.	Panicoideae	L, M	Yes
Arundinella nepalensis Trin.	Panicoideae	EC, FS, G, KZN, L, M, NC, NW, WC	No
Brachiaria eruciformis (Sm.) Griseb.	Panicoideae	Throughout SA	Yes
Bromus pectinatus Thunb.	Pooideae	EC, FS, G, M, NC, NW, WC	Yes
Calamagrostis epigejos (L.) Roth var *capensis* Stapf	Pooideae	EC, G, M, NC, WC	Yes (species only)
Chloris gayana Kunth	Chloridoideae	EC, FS, G, KZN, L, M, NC, NW, WC	Yes
Chloris pycnothrix Trin.	Chloridoideae	EC, FS, G, KZN, L, M, NW	Yes
Chloris virgata Sw.	Chloridoideae	EC, FS, G, KZN, L, M, NC, NW, WC	Yes
Cynodon transvaalensis Burtt Davy	Chloridoideae	EC, FS, G, KZN, L, M, NC, NW, WC	Yes
Digitaria ciliaris (Retz.) Koeler	Panicoideae	ThroughoutSA	Yes
Digitaria debilis (Desf.) Willd.	Panicoideae	FS, G, KZN, L, M, WC	No
Digitaria didactyla Willd	Panicoideae	G, KZN, L, M	Yes
Digitaria nuda Schumach.	Panicoideae	EC, KZN, LIM, M	No
Digitaria sanguinalis (L.) Scop.	Panicoideae	EC, FS, G, KZN, L, M, NC, NW, WC	Yes
Echinochloa colona (L.) Link	Panicoideae	EC, FS, G, KZN, L, M, NC, NW, WC	Yes

Scientific name		Distribution in South Africa	Included in this study
Echinochloa crus-galli (L.) P.Beauv.	Panicoideae	EC, FS, G, KZN, L, M, NC, NW, WC	Yes
Echinochloa crus-pavonis (Kunth) Schult.	Panicoideae	EC, KZN, WC	No
Echinochloa stagnina (Retz.) P.Beauv.	Panicoideae	FS, G, L, M, NC	No
Eleusine coracana Gaertn. subsp. *africana* (Kenn.-O'Byrne) K.W.Hilu & de Wet	Chloridoideae	EC, FS, G, KZN, L, M, NC, NW, WC	No
Eragrostis cilianensis (All.) Vignolo ex Janch.	Chloridoideae	EC, FS, G, KZN, L, M, NC, NW, WC	Yes
Eragrostis pilosa (L.) P.Beauv.	Chloridoideae	EC, FS, G, KZN, L, M, NC, WC	Yes
Eragrostis racemosa (Thunb.) Steud.	Chloridoideae	EC, FS, G, KZN, L, M, NC, NW, WC	No
Hyparrhenia anamesa Clayton	Panicoideae	EC, FS, G, KZN, L, M, NC, NW, WC	No
Imperata cylindrica (L.) P.Beauv.	Panicoideae	EC, FS, G, KZN, L, M, NC, NW, WC	Yes
Leptochloa panicea (Retz.) Ohwi	Chloridoideae	KZN, L, M	No
Melinis repens (Willd.) Zizka subsp. repens	Panicoideae	EC, FS, G, KZN, L, M, NC, NW, WC	Yes
Microstegium nudum (Trin.) A.Camus	Panicoideae	EC, KZN, LIM, M, WC	No
Odontelytrum abyssinicum Hack.	Panicoideae	FS, M	No
Olyra latifolia L.	Bambusoideae	EC, KZN	No
Oryza longistaminata A.Chev.& Roehr	Ehrhartoideae	Lim(Nylsvlei)	No
Oryza punctata Kotschy ex Steud	Ehrhartoideae	KZN	No
Paspalidium obtusifolium (Delile) Simpson	Panicoideae	KZN, LIM	Yes
Paspalum vaginatum Sw.	Panicoideae	EC, FS, KZN, WC	Yes
Pennisetum macrourum Trin.	Panicoideae	EC, G, KZN, L, M, NC, WC	Yes
Pennisetum unisetum (Nees) Benth	Panicoideae	KZN	No

Scientific name	Sub-family	Distribution in South Africa	Included in this study
Phragmites australis (Cav.) Steud.	Arundinoideae	EC, FS, G, KZN, L, M, NC, NW, WC	Yes
Poa bulbosa L.	Pooideae	NC, WC	Yes
Rottboellia cochinchinensis (Lour.) Clayton	Panicoideae	KZN, LIM, M	Yes
Setaria pumila (Poir.) Roem. & Schult	Panicoideae	EC, FS, G, KZN, L, M, NC, NW, WC	Yes
Setaria verticillata (L.) P.Beauv.	Panicoideae	EC, FS, G, KZN, L, M, NC, NW, WC	Yes
Spartina maritima (Curtis) Fernald	Chloridoideae	EC, WC	No
Sporobolus virginicus (L.) Kunth	Chloridoideae	EC, KZN, L, NC, WC	Yes
Stenotaphrum dimidiatum (L.) Brongn.	Panicoideae	KZN, WC	No
Stenotaphrum secundatum (Walter) Kuntze	Panicoideae	EC, KZN, WC	Yes
Tragus berteronianus Schult.	Chloridoideae	EC, FS, G, KZN, L, M, NC, NW, WC	Yes



(a) (b) (c) (d)

Figure 2.1 Covers of some of the scientific literature used for the compilation of the list of naturalised grasses in southern Africa. **(a)** Guide to Grasses of southern Africa (van Oudtshoorn 2006), **(b)** Problem plants and alien weeds of South Africa (Bromilow 2010), **(c)** Plants of the Greater Cape Floristic Region *Vol. 1*: the Core Cape Flora (Manning & Goldblatt 2012), **(d)** Plants of the Greater Cape Floristic Region *Vol.2*: the Extra Cape flora (Snijman 2013).

2.2 Taxon sampling

Once the list of naturalised grass species in South Africa was completed, three main sources were searched for sequences for the two DNA barcoding marker regions; a portion of the *matK* plastid gene and a portion of the plastid *rbcL* gene. The online databases Barcode of Life Data Systems (BOLD) (Ratnasingham & Hebert 2007) and GenBank (Benson *et al.* 2005) were searched for representatives of these two markers. The African Centre for DNA Barcoding (ACDB) has a large database of the two DNA barcoding marker for grasses of South Africa, where many native and alien grasses collected in the field or from herbarium collections were subjected to DNA barcoding (Van der Bank *et al.* unpublished, see Appendix 1B), and sequences from this database were also used. As identification of an unknown grass species would not necessarily be successful if only alien grasses were included in the DNA barcoding data, it was decided that native species also be included in the analyses so that a more definitive identification can be made in the future. As it would not be possible to collect sequences for every native grass in South Africa in the timeframe available for this study, sampling of native taxa was based on availability in the ACDB Grasses of southern Africa database, with replicates collected from BOLD and GenBank. For each native and naturalised species, three replicates of each marker were collected when available, as the DNA barcoding metrics best test the data when replicates are present. However, in many cases three replicates were not available.

For species for which three replicates were not available, herbarium specimens were used to collect leaf tissue for DNA extraction, with the aim of completing the database. Specimens were taken from the National Herbarium in Pretoria (PRE) and the Compton Herbarium at Kirstenbosch (NBG). Photographs of each herbarium specimen were taken, to be uploaded onto the BOLD database. Table 2.2 lists all of the herbarium specimens, native and naturalised, from which DNA was extracted for this study, as well as PCR success for the two barcoding regions. Although the aim was to collect at least three replicates for each gene region for each species represented in the study, in many cases this was not possible and only one or two replicates were retrieved (see Appendix 1A for the number of replicates per species).

As many of the current annual grass invasions that have been reported in South Africa occur in the Western Cape, particularly in the renosterveld, a list of common native renosterveld grasses was compiled from recent papers and books that mentioned grasses and grass invasions in the renosterveld (Midoko-Iponga 2004, Musil *et al*. 2005, Shiponeni & Milton 2006, Muhl 2008, Todd 2008, Manning & Goldblatt 2012). An effort was made to ensure that specimens from these renosterveld grasses were represented in the native taxa sampled (see Appendix 1A for a comprehensive list of all species included in this study). This was with the aim of improving identification of unknown grasses in general in this region, to rule out or discover possible invasions.

Table 2.2 List of southern African naturalised and native grass taxa (family Poaceae) from which DNA was extracted for this study.

*Specimen details include the herbarium specimen ID, date sampled, collector, and specimen number (if available).** Herbarium abbreviations: PRE = National Herbarium, Pretoria; NBG = Compton Herbarium, Kirstenbosch.

Extraction no.	**Scientific name**	**Specimen details***	**Herbarium ** **	**Origin**	**PCR success *rbcLa***	**PCR success matK**
POA1	*Agrostis avenacea* J.F.Gmel.	144091(1982, Crook A.O. 2430)	NBG	naturalised	X	X
POA199	*Agrostis gigantea* Roth	842733(2004, De Wet S.F. 912)	PRE	naturalised	✓	X
POA102	*Agrostis montevidensis* Spreng. ex Nees	7680085(2006, Smook L. 11945)	PRE	naturalised	⍰	partial
POA103	*Agrostis montevidensis* Spreng. ex Nees	768077(2006, Smook L. 11736)	PRE	naturalised	⍰	partial
POA2	*Agrostis montevidensis* Spreng. ex Nees	49307(1961, Dlamini B. s.n.)	NBG	naturalised	X	X
POA47	Agrostis lachnantha Nees	157066(1996, Boucher C. 6038)	NBG	native	X	X
POA3	*Aira cupaniana* Guss.	269463(2011, Nicolson G. & Roets D. 493)	NBG	naturalised	X	X
POA4	*Aira cupaniana* Guss.	272711(2009, Husted L. LBH897)	NBG	naturalised	X	partial
POA104	*Alopecurus arundinaceus* Poir.	979505(1991, Lampinen R. & Lampinen T. 13475)	PRE	naturalised	⍰	X
POA197	*Arthraxon lanceolatus* (Roxb.) Hochst.	0661701(1983, Spies J.J. 1423)	PRE	native	⍰	partial

Extraction no.	Scientific name	Specimen details*	Herbarium **	Origin	PCR success *rbcLa*	PCR success *matK*
POA198	*Arthraxon lanceolatus* (Roxb.) Hochst.	709152(1985, Krynauw S. 852)	PRE	native	✓	✓
POA5	*Avena barbata Avena barbata* Pott ex Link	205067(2001, Joubert C.E. 464A)	NBG	naturalised	X	X
POA6	*Avena barbata Avena barbata* Pott ex Link	162650(1994, Kemper J. IPC 687)	NBG	naturalised	X	X
POA111	*Avena sativa* L.	568560(2000, Brand R. 213)	PRE	naturalised	X	partial
POA110	*Avena sativa* L.	573006(1997, Immelman K.L. 97/coll/9)	PRE	naturalised	✓	partial
POA108	Avena byzantina K.Koch	658121(1969, Von Breitenbach 59)	PRE	naturalised	X	X
POA109	Avena byzantina K.Koch	975978(1984, De Lotito D.T. 73.697)	PRE	naturalised	X	X
POA105	*Axonopus fissifolius* (Raddi) Kuhlm.	573759(1994, Smook L. 8768)	PRE	naturalised	X	✓
POA107	*Axonopus fissifolius* (Raddi) Kuhlm.	708244(1985, Smook L. 5436)	PRE	naturalised	X	✓
POA106	*Axonopus fissifolius* (Raddi) Kuhlm.	832416(2005, De Wet S.F. 920)	PRE	naturalised	X	partial
POA112	*Bambusa balcooa* Roxb. Ex Roxb.	559757(1980, Smook L. & Soderstrom T.R.)	PRE	naturalised	✓	partial
POA113	*Bambusa balcooa* Roxb. Ex Roxb.	709389(1986, Henderson L. 715)	PRE	naturalised	✓unusable sequence	✓
POA114	*Brachiaria advena* Vickery	0603647(1979, Harding G. 2629)	PRE	naturalised	✓	✓

Extraction no.	**Scientific name**	**Specimen details***	**Herbarium ****	**Origin**	**PCR success *rbcLa***	**PCR success *matK***
POA116	*Brachiaria advena* Vickery	580033(1990, Moss H. 1253)	PRE	naturalised	X	X
POA115	*Brachiaria advena* Vickery	740999(1982, Granger J.E. 347b)	PRE	naturalised	X	X
POA117	*Brachiaria umbellata* (Trin.) Clayton	768090(2005, Smook L. 11950)	PRE	naturalised	✓	✓
POA48	Brachiaria serrata (Thunb.) Stapf	206052(2004, Forest F. *et al.* 778)	NBG	native	X	✓
POA7	*Brachypodium distachyon* (L.) P.Beauv.	166488(1971, Crook A.O. 1043)	NBG	naturalised	X	partial
POA8	*Brachypodium distachyon* (L.) P.Beauv.	166495(1976, duToit P.V.C. 1716)	NBG	naturalised	X	X
POA9	*Briza maxima* L.	269033(2011, Paijmans D. DMP 247)	NBG	naturalised	✓	partial
POA10	*Briza maxima* L.	269467(2011, Nicolson G. & Roets D. 512)	NBG	naturalised	✓	✓
POA12	*Briza minor* L.	172752(2000, Pretorius W.A.J. 603)	NBG	naturalised	X	partial
POA118	*Briza subaristata* Lam.	9904040-00300(Liebenbeg L.C.C. 4221)	PRE	naturalised	X	✓
POA13	*Bromus alopecuros* Poir.	248868(2008, Boucher C. 7568)	NBG	naturalised	X	partial
POA14	*Bromus hordeaceus* subsp. molliformis	157032(1994, Kemper J. IPC721)	NBG	naturalised	X	X
POA119	*Bromus rigidus* Roth	973725(1992, Jessop J.P. s.n.)	PRE	naturalised	✓	partial
POA15	*Bromus rigidus* Roth	166557(1994, Kemper J. IPC691)	NBG	naturalised	✓	partial

Extraction no.	Scientific name	Specimen details*	Herbarium **	Origin	PCR success *rbcLa*	PCR success *matK*
POA121	*Bromus rubens* L.	973747(1991, Badman F.J. 5056)	PRE	naturalised	X	partial
POA120	*Bromus rubens* L.	979484(2000, Symon D.E & Symon J. s.n.)	PRE	naturalised	X	partial
POA124	*Cenchrus biflorus* Roxb.	568135(1996, Du Toit G.J. 5055)	PRE	naturalised	X	✓
POA123	*Cenchrus biflorus* Roxb.	585733(2001, Hoare D.B. 3049)	PRE	naturalised	X	✓
POA122	*Cenchrus biflorus* Roxb.	74866(2001, Klaasen E. Uiras M.M. EK 382)	PRE	naturalised	✓	partial
POA125	*Cenchrus brownii* Roem. & Schult.	590745(2001, Siebert S.J. 2129)	PRE	naturalised	✓	✓
POA126	*Cenchrus brownii* Roem. & Schult.	671774(1985, Spies J.J. 2368)	PRE	naturalised	X	partial
POA127	*Cenchrus brownii* Roem. & Schult.	783834(1989, Ellis R.P. 6022)	PRE	naturalised	X	partial
POA16	*Cenchrus incertus* M.A.Curtis	171480(1999, February N. s.n.)	NBG	naturalised	X	X
POA17	*Cenchrus incertus* M.A.Curtis	179301(1994, Theron P.J. & Fellingham A.C. 1633)	NBG	naturalised	X	partial
POA49	*Chaetobromus involucratus subsp. dregeanus* (Nees) Verboom	198519(2004, Forest F. et al. 629)	NBG	native	X	partial
POA128	*Chloris truncata* R.Br.	965036(1997, Saunders A.C. 20138)	PRE	naturalised	X	partial
POA129	*Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn.	976977(1984, Lippman M.C. 101)	PRE	naturalised	unusable sequence	✓
POA18	*Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn.	270665(2011, Koordom M. MEK023)	NBG	naturalised	✓	✓

Extraction no.	Scientific name	Specimen details*	Herbarium **	Origin	PCR success *rbcLa*	PCR success *matK*
POA130	*Corynephorus fasciculatus* Boiss. & Reut.	727046,(1987, Spies.J.J. 3690)	PRE	naturalised	X	partial
POA50	*Cymbopogon marginatus* (Steud.) Stapf ex Burtt-Davy	247878(2009, Jardine I. & Jardine C. 1064)	NBG	native	X	partial
POA51	Cymbopogon nardus (L.) Rendle	160432(1981, Smook L. 3667)	NBG	native	X	X
POA131	*Cymbopogon pospischilii* (K.Schum.) C.E.Hubb	591028(2000, Du Toit G.J. 5451)	PRE	native	✓	✓
POA133	*Cynodon aethiopicus* Clayton & Harlan	835722(1991, Ward C.J. s.n.)	PRE	naturalised	X	✓
POA132	*Cynodon aethiopicus* Clayton & Harlan	962903(1989, Kayombo J. and Kayombo M.J. 49)	PRE	naturalised	X	✓
POA134	*Cynodon nlemfuensis* Vanderyst	625920(1980, Ellis R.P. 3422)	PRE	naturalised	X	✓
POA135	Cynosurus coloratus Lehm. ex Steud	685312(1986, Spies J.J. s.n.)	PRE	naturalised	X	✓unusable sequence
POA19	Cynosurus coloratus Lehm ex Nees	209260(2006, Rösch H. 638)	NBG	naturalised	X	✓
POA136	*Dichanthium aristatum* (Poir.) C.E.Hubb.	463884(2000, Siebert S.J & du Plessis F. 1517)	PRE	naturalised	X	✓
POA137	*Digitaria abyssinica* (A.Rich.) Stapf.	85\30421(2005, De Wet S.F. s.n.)	PRE	naturalised	X	✓
POA138	*Digitaria abyssinica* (A.Rich.) Stapf.	851028(2008, De Wet S.F. 1059)	PRE	naturalised	✓	✓
POA52	*Digitaria argyrograpta* (Nees) Stapf	210554(2005, Mudau A.C. 9)	NBG	native	X	partial

Extraction no.	**Scientific name**	**Specimen details***	**Herbarium ****	**Origin**	**PCR success *rbcLa***	**PCR success *matK***
POA139	*Digitaria violascens* Link	646901(1984, Ellis R.P. 4416)	PRE	naturalised	X	partial
POA140	*Digitaria violascens* Link	960526(1978, Lloyd N. s.n.)	PRE	naturalised	X	X
POA56	*Ehrharta calycina* Sm.	269565,(2011, Nicolson G. & Roets D. 492)	NBG	native	✓	✓
POA55	*Ehrharta calycina* Sm.	272851(2011, Jardine I. 1662)	NBG	native	✓	✓
POA57	*Ehrharta capensis* Thunb.	269675(2011, Boucher C., 7734)	NBG	native	✓	✓
POA59	*Ehrharta erecta* Lam.	267863(2008, Clark V.R., Daniels R.J., Fabricius M. & Le Roux J.A. 109)	NBG	native	X	X
POA146	*Ehrharta erecta* Lam.	979486(2000, Symon D.B. 16246)	PRE	native	X	X
POA147	*Ehrharta stipoides* Labill.	973382(1982, Gordon-Gray K.D. s.n.)	PRE	naturalised	X	X
POA148	*Ehrharta stipoides* Labill.	973388(1988, Bates R 16460)	PRE	naturalised	✓	✓
POA62	*Ehrharta thunbergii* Gibbs Russ.	272853(2011, Jardine I. 1665)	NBG	native	✓	partial
POA64	*Ehrharta villosa* Schult.f.	161782(1978, Boucher C. 3983)	NBG	native	X	X
POA63	*Ehrharta villosa* Schult.f.	161812(1986, Taylor H.C. 11577)	NBG	native	X	partial
POA54	*Ehrharta bulbosa* Sm.	160933(1976, du Toit P.V.C. 1499)	NBG	native	X	X
POA58	*Ehrharta delicatula* Stapf	211530(2004, Steyn H.M., 594)	NBG	native	X	✓unusable sequence

Extraction no.	**Scientific name**	**Specimen details***	**Herbarium ****	**Origin**	**PCR success *rbcLa***	**PCR success *matK***
POA60	*Ehrharta longiflora* Sm.	221464(2006, Rösch H. HR551)	NBG	native	✓	✓
POA61	*Ehrharta melicoides* Thunb.	1181488(2001, Pretorius W.A.J. 733)	NBG	native	X	X
POA142	*Eleusine multiflora* Hochst. Ex A.Rich.	579999(1990, Moss H. 1206)	PRE	naturalised	partial	✓
POA141	*Eleusine multiflora* Hochst. ex A.Rich.	746207(1990, Smook L. 7285)	PRE	naturalised	✓	✓
POA143	*Eleusine multiflora* Hochst. ex A.Rich.	PRE742392(1989, Turner B.J. 1508)	PRE	naturalised	X	✓
POA144	*Eleusine tristachya* (Lam.) Lam.	857320(1995, Lourens , Fellingham 1665)	PRE	naturalised	X	✓
POA20	*Eleusine tristachya* (Lam.) Lam.	169033(1995, Hansford G. s.n.)	NBG	naturalised	X	partial
POA145	*Entolasia olivacea* Stapf	746896(1985, Johannsmeier M.F. 372)	PRE	naturalised	partial	✓
POA149	*Eragrostis barrelieri* Daveau	0834833(1998, Smook, L. 10141)	PRE	naturalised	✓	✓
POA195	*Eragrostis barrelieri* Daveau	834752(1998, Smook L. 10041)	PRE	naturalised	✓	partial
POA66	*Eragrostis capensis* (Thunb.) Trin.	248347(2006, Turner R.C. 1467)	NBG	native	X	X

Extraction no.	Scientific name	Specimen details*	Herbarium **	Origin	PCR success *rbcLa*	PCR success *matK*
POA65	*Eragrostis capensis* (Thunb.) Trin.	273661(2011, Nicholson G. & Roets D. 59)	NBG	native	✓	✓
POA67	*Eragrostis curvula* (Schrad.) Nees	249567(2010, Jardine I. & Jardine C. 1302)	NBG	native	✓	✓
POA151	*Eragrostis curvula* (Schrad.) Nees	579278(1998, De Castro A., Brits M. 31)	PRE	native	✓	✓
POA150	*Eragrostis curvula* (Schrad.) Nees	856637(2009, Jacobsen N.H.G 6479)	PRE	native	✓	✓
POA152	*Eragrostis tef* (Zucc.) Trotter	0809528(1994,Kroon N.V. 11511)	PRE	naturalised	X	X
POA154	*Eragrostis tef* (Zucc.) Trotter	733847(1989, Saaiman T. 357)	PRE	naturalised	X	X
POA153	*Eragrostis tef* (Zucc.) Trotter	738963(1989,Barker N.P. 580)	PRE	naturalised	X	X
POA156	*Eragrostis virescens* J.Presl	572537(1998,Immelman K.L. 99/coll/5)	PRE	naturalised	✓	✓
POA155	*Eragrostis virescens* J.Presl	603640/603641(1979, Harding G s.n.)	PRE	naturalised	X	X
POA68	*Festuca scabra* Vahl	205127(2005, Jardine I. & Jardine C. 206)	NBG	native	✓	partial
POA157	*Hainardia cylindrica* (Willd.) Greuter	67553(1971, Ellis R.P. 664)	PRE	naturalised	X	partial
POA69	*Harpochloa falx* (L.f.) Kuntze	207471(2004, Forest F., Trinder-Smith T.H. et al. 798)	NBG	native	X	X
POA70	*Helictotrichon capense* Schweick.	183969(2000, Helme N.A. 2342)	NBG	native	✓	X
POA158	*Helictotrichon imberbe* (Nees) Veldkamp	851469(2006, Siebert S.G. 3255)	PRE	native	✓	partial
POA71	*Helictotrichon hirtulum* (Steud.) Schweick.	162647(1994, Kemper J. IPC681)	NBG	native	X	X

Extraction no.	**Scientific name**	**Specimen details***	**Herbarium ****	**Origin**	**PCR success *rbcLa***	**PCR success *matK***
POA196	*Hordeum murinum* L. subsp. leporinum (Link) Arcang.	747579(1986, Cloete & Haselau 242)	PRE	naturalised	✓	partial
POA21	*Hordeum murinum* L. subsp. leporinum (Link) Arcang.	268254(2011, Tywalana M. 18)	NBG	naturalised	✓	✓
POA72	*Hyparrhenia hirta* (L.) Stapf	(1976, du Toit P.V.C. 1527)	NBG	native	X	✓
POA73	*Koeleria capensis* Nees	249565(2010, Jardine I. & Jardine C. 1308)	NBG	native	✓	✓
POA22	*Lagurus ovatus* L.	99026100(2011, Paijmans D. DMP250)	NBG	naturalised	✓	✓
POA161	*Lamarckia aurea* (L.) Moench	316145(1998, Koekemoer M. 1417)	PRE	naturalised	✓	partial
POA23	*Lamarckia aurea* (L.) Moench	120481(1979, Fairall P. 1711)	NBG	naturalised	X	X
POA53	*Leptochloa fusca* (L.) Kunth.	209575(2006, Pretoriu, W.A.J. 818)	NBG	native	X	X
POA162	*Lepturus repens* (G.Forst.) R.Br.	619760(1982, Ellis R.P. 4062)	PRE	naturalised	✓	partial
POA24	*Lolium multiflorum* x perenne	198371(2004, C.R.E.W. 264)	NBG	naturalised	✓	partial
POA74	*Melica racemosa* Thunb.	205061(2000, Joubert C.E. 420)	NBG	native	✓	partial
POA75	*Melinis nerviglumis* (Franch.) Zizka	273025(2012, Komape D.M., Mabe L.I. & Siebert S.J. KMS353)	NBG	native	✓	✓
POA40	*Nassella neesiana* (Trin. & Rupr.) Barkworth	269195(2011, Paijmans D. DMP 179)	NBG	naturalised	X	✓
POA192	*Nassella neesiana* (Trin. & Rupr.) Barkworth	7351621988,(Zeitsman P.C. 342)	PRE	naturalised	✓	✓

Extraction no.	Scientific name	Specimen details*	Herbarium **	Origin	PCR success *rbcLa*	PCR success *matK*
POA192	*Nassella neesiana* (Trin. & Rupr.) Barkworth	7351621988,(Zeitsman P.C. 342)	PRE	naturalised	✓	✓
POA164	*Nassella tenuissima* (Trin.) Barkworth	933386(1975,Wells, Erb 43)	PRE	naturalised	X	X
POA165	*Nassella trichotoma* (Nees) Hack. ex Arechav.	827936(1995, Victor J.E. 792)	PRE	naturalised	✓	partial
POA78	*Panicum schinzii* Hack.	208399(2007, Helme N.A. 4638)	NBG	native	✓	✓
POA167	*Parapholis incurva* (L.) C.E.Hubb.	726909(1987, Spies J. 3648)	PRE	naturalised	✓	X
POA168	*Parapholis incurva* (L.) C.E.Hubb.	979475(Syman D.E. 15855)	PRE	naturalised	✓	partial
POA26	*Paspalum distichum* L.	268518(2011, Boatwright J.S. 515)	NBG	naturalised	✓	✓
POA173	*Paspalum notatum* Flüggé	576966(2000, De Castro A, Brits M. s.n.)	PRE	naturalised	X	✓unusable sequence
POA171	*Paspalum notatum* Flüggé	659429(2002, Van Wyk E. evw452)	PRE	naturalised	X	✓unusable sequence
POA172	*Paspalum notatum* Flüggé	833639(2005, De Wet S.F. 941)	PRE	naturalised	X	✓
POA169	*Paspalum quadrifarium* Lam.	963856(1968, Sargent F.H. 9648)	PRE	naturalised	X	X
POA170	*Paspalum quadrifarium* Lam.	963859(1950, Montes J.E. 727)	PRE	naturalised	X	X
POA27	*Pennisetum clandestinum* Hochst. ex Chiov.	271633(2012' Koordom M. & Kritzinger-Klopper M.E.K. 228)	NBG	naturalised	✓	X

Extraction no.	Scientific name	Specimen details*	Herbarium **	Origin	PCR success *rbcLa*	PCR success *matK*
POA28	*Pennisetum clandestinum* Hochst. ex Chiov.	272126(2012, Koordom M. & Kritzinger-Klopper M.E.K. 228)	NBG	naturalised	✓	✓
POA174	*Pennisetum glaucum* (L.) R.Br.	905810(1973, Simon B.K. 2347)	PRE	naturalised	✓	partial
POA29	*Pennisetum glaucum* (L.).	160829(1979, Thompson M.F. 3960)	NBG	naturalised	✓	✓
POA79	*Pennisetum macrourum* Trin.	269131(2011, Nicolso, G. & Roets D. 630)	NBG	naturalised	✓	partial
POA80	*Pennisetum macrourum* Trin.	273167(2012, Komape D.M., Mabe L.I. & Siebert S.J. KMS 345)		naturalised	✓	✓
POA81	*Pennisetum thunbergii* Kunth	196673(2000, Pond U. UP 248)	NBG	native	X	□unusable sequence
POA175	*Pennisetum villosum* R.Br. ex Fresen.	?(1966, Copley B. 101)	PRE	naturalised	✓	✓
POA83	*Pentaschistis airoides* (Nees) Stapf	198790(2004, Forest F. et al. 573)	NBG	native	X	X
POA82	*Pentaschistis airoides* (Nees) Stapf	208792(2006, Jardine I. & Jardine C. 500)	NBG	native	✓	✓unusable sequence
POA84	*Pentaschistis eriostoma* (Nees) Stapf	277151(2007, Pienaar E M640)	NBG	native	partial	partial
POA85	*Pentaschistis glandulosa* (Schrad.) H.P.Linder	199671(2000, Helme N.A.1928)	NBG	native	✓	✓
POA176	*Pentaschistis pallida* (Thunb.) H.P.Linder	?(2006, Clark V.R., Ramdhani 121)	PRE	native	X	X
POA86	*Pentaschistis pallida* (Thunb.) H.P.Linder	162860(1975, Ellis R.P. 2489)	NBG	native	X	partial

Extraction no.	**Scientific name**	**Specimen details***	**Herbarium ****	**Origin**	**PCR success *rbcLa***	**PCR success *matK***
POA87	*Pentaschistis rigidissima* H.P.Linder	168441(1993, Taylor H.C. 12230)	NBG	native	X	✓unusable sequence
POA30	*Periballia minuta* (L.) Asch. & Graebn.	81158(1943, Salter T.M. 8766)	NBG	naturalised	X	X
POA33	*Phalaris canariensis* L.	161849(1989, Pretorius W.A.J. 155)	NBG	naturalised	X	X
POA179	*Phalaris canariensis* L.	973562(1983, Sanchez E. 1360)	PRE	naturalised	X	X
POA36	*Phalaris paradoxa* L.	268250(2011, Tywalana M. 15)	NBG	naturalised	✓	✓
POA181	*Phalaris paradoxa* L.	774937(1989, Alldermann s.n.)	PRE	naturalised	X	X
POA180	*Phalaris paradoxa* L.	979546(1994, Lambinon J.E.J and Van den Sande G. s.n.)	PRE	naturalised	✓	✓
POA31	*Phalaris angusta* Nees ex Trin.	19499(1953,Middlemost A.J.M. 1865)	NBG	naturalised	X	X
POA177	*Phalaris angusta* Nees ex Trin.	67515(1969, Ellis R.P. 70)	PRE	naturalised	X	X
POA32	*Phalaris aquatica* L.	155913(1992, Kruger I.J. 429)	NBG	naturalised	X	X
POA178	*Phalaris aquatica* L.	726900(1987, Spies J. 3676)	PRE	naturalised	X	X
POA34	*Phalaris minor* Retz.	198693(2004, Forest F. et al. 577)	NBG	naturalised	X	X
	Pentaschistis rigidissima H.P.Linder					

Extraction no.	**Scientific name**	**Specimen details***	**Herbarium ****	**Origin**	**PCR success *rbcLa***	**PCR success *matK***
POA39	*Sphenopus divaricatus* (Gouan) Rchb.	248557(2001, Mucina L. & Santos Guerra A. FSA 7239/6)	NBG	naturalised	X	X
POA38	*Sphenopus divaricatus* (Gouan) Rchb.	248809(2001, Mucina L. & Santos Guerra A. FSA 7269/6)	NBG	naturalised	X	X
POA90	*Sporobolus africanus* (Poir.) Robyns & Tournay	205088,(2004, Joubert C.E. 654)	NBG	native	✓	✓
POA166	*Steinchisma hians* (Elliott) Nash & Small	969520(1981, Mallo M., Medan D., Toitsa R. s.n.)	PRE	naturalised	X	X
POA91	*Stipa capensis* Thunb.	189548(2001, Boucher C. 6786)	NBG	native	X	✓unusable sequence
POA189	*Stipa capensis* Thunb.	618829(1981, Smook L. 3617)	PRE	native	X	✓
POA190	*Stipa clandestina* Hack.	559037(1976, Hiscock L.E. 58285)	PRE	naturalised	X	X
POA191	*Stipa clandestina* Hack.	726495(1988, Clark B. 1799)	PRE	naturalised	✓	✓
POA42	*Stipa papposa* Nees	120659(1980, Crook A.O 2221)	NBG	naturalised	X	X
POA41	*Stipa papposa* Nees	128372(1980, Crook A.O. 2221)	NBG	naturalised	X	X
POA43	*Stipa papposa* Nees	139478(1980, Crook A.O 221)	NBG	naturalised	X	partial
POA44	*Stipa variabilis* Hughes	168444(1981, Smook L. 3617)	NBG	naturalised	X	X

Extraction no.	Scientific name	Specimen details*	Herbarium **	Origin	PCR success *rbcLa*	PCR success *matK*
POA193	*Stipa variabilis* Hughes	976280(1970, Blaylock B.J. 1618)	PRE	naturalised	✓	✓
POA77	*Tenaxia disticha* (Nees) N. P. Barker & H. P. Linder	172131(1999, Palmer P.A. 359)	NBG	native	✓	✓
POA76	*Tenaxia disticha* (Nees) N. P. Barker & H. P. Linder	210429(2005, Mudau A.C. 82)	NBG	native	✓	✓
POA93	*Trachypogon spicatus* (L.f.) Kuntze	206054(2004, Forest F. et al. 830)	NBG	native	X	X
POA92	*Trachypogon spicatus* (L.f.) Kuntze	273166(2012, Komape D.M., Mabe L.I. &	NBG	native	X	partial
POA96	*Tribolium echinatum* (Thunb.) Renvoize	168706(1995, Le Roux 4575)	NBG	native	X	partial
POA101	*Tribolium hispidum* (Thunb.) Desv	0248927(2008, Jardine I. and Jardine C. 1018)	NBG	native	✓	✓
POA97	*Tribolium hispidum* (Thunb.) Desv	272844(2011, Jardine I. 1622)	NBG	native	✓	partial
POA98	*Tribolium obtusifolium* (Nees) Renvoize	201666(1999, Pienaar A. PB180)	NBG	native	✓	unusable sequence
POA159	*Tribolium purpureum* (L.f.) Verboom & H.P.Linder	463830(1975, Ellis R.P. 2470)	PRE	native	✓	✓
POA160	*Tribolium purpureum* (L.f.) Verboom & H.P.Linder	853508(2003, Kraaij T. 29599)	PRE	native	X	X
POA99	*Tribolium uniolae* (L.f.) Renvoize	249014(2004, Forest F., & Trinder Smith T.H. 779)	NBG	native	X	X

Extraction no.	**Scientific name**	**Specimen details***	**Herbarium ****	**Origin**	**PCR success *rbcLa***	**PCR success *matK***
POA100	*Tribolium uniolae* (L.f.) Renvoize	269464(2011, Nicolson G. & Roets D. 494)	NBG	native	✓	X
POA95	*Tribolium acutiflorum* (Nees) Renvoize	164807(1981, Smook L. 3606)	NBG	native	X	X
POA94	*Tribolium acutiflorum* (Nees) Renvoize	196677(2000, Pond U. UP 220)	NBG	native	X	✓
POA194	*Vulpia fasciculata* (Forssk.) Samp.	742008(Spies J.J. 3900)	PRE	naturalised	X	X
POA45	*Vulpia muralis* (Kunth) Nees	205072(2001, Joubert 478)	NBG	naturalised	X	partial
POA46	*Vulpia muralis* (Kunth) Nees	205215(2005, Boucher C 7362)	NBG	naturalised	X	X

2.3 DNA extraction

Laboratory work was carried out at the African Centre for DNA Barcoding (ACDB) at the University of Johannesburg. Total DNA was extracted from herbarium tissue using the Macherey-Nagel NucleoSpin® Plant II Mini kit. 20-40mg of dry leaf tissue was used for each extraction. Plant tissue was ground using liquid nitrogen. DNA was visualised on a 1% agarose gel (2g/200ml with 1X TBE, containing 8µl Ethidium Bromide, run for 30 minutes at 110-125V) under ultraviolet light to check for DNA quantity and quality. DNA samples were stored at -20˚C.

2.4 Polymerase Chain Reactions (PCR)

Thermoprime 1.1X PCR Master Mix with 1.5mM $MgCl_2$ (Thermo Scientific Inc. Waltham, USA) and TopTaq Master Mix (Oiagen Inc. Hilden, Germany) were used for PCR reactions for both the *matK* and *rbcLa* markers. It was noted that TopTaq Master Mix gave superior PCR results and was used for the bulk of my PCR reactions.

Bovine serum albumin (BSA, 0.1mg/ml final concentration) was added to the reaction mix to improve primer annealing and to stabilise the *Taq* polymerase enzyme. For the *matK* reactions, DMSO was also added (final concentration 4% of the total reaction volume). Primers were added at a final concentration of 1µM per reaction.

For the *rbcLa* marker, the PCR cycling program was: 94˚C for 3 minutes, followed by 35 cycles of: 94˚C for 1 minute, 50˚C for 40 seconds, 72˚C for 50 seconds, and a final extension of 72˚C for 5-10 minutes.

For *matK* the cycling program was: 94˚C for 3 minutes, followed by 40 cycles of: 94˚C for 1 minute, 48˚C for 40 seconds, 72˚C for 50 seconds, and a final extension of 72˚C for 5-10 minutes.

PCR products were run on a 1% agarose gel (as detailed above) to check PCR success. Successful PCR products were cleaned using QIAGEN QIAquick PCR purification kits, and rerun on a 1% agarose gel to check for clean-up success.

For *rbcLa*, the universal primers *rbcLa*_F and *rbcLa*_R were used (Cuénoud *et al.* 2002). (Table 2.2). For *matK*, all of the commonly used universal primers listed in Table 2.3 were trialled for amplification of the *matK* barcoding region.

Table 2.3 Primers tested for amplification of core barcoding regions *matK* and *rbcLa*.

Locus	Primer name	Sequence (5’-3’)	Reference
matK	*matK*_390F*	CGATCTATTCATTCAATATTC	Cuénoud *et al.* 2002
	*matK*_1326R*	TCTAGCACACGAAAGTCGAAGT	Cuénoud *et al*. 2002
	3F_KIM*	CGTACAGTACTTTTGTGTTTACGAG	CBOL Plant Working Group 2009
	1R_KIM*	ACCCAGTCCATCTGGAAATCTTGGTTC	CBOL Plant Working Group 2009
	matK _1248R*	GCTRTRATAATGAGAAAGATTTCTGC	Yu *et al.* 2011
	matK _472F*	CCCRTYCATCTGGAAATCTTGGTTC	Yu *et al.* 2011
	matK _MALPR 1*	ACAAGAAAGTCGAAGTAT	Dunning & Savolainen 2010
	matK _X F*	TAATTTACGATCAATTCATTC	Ford *et al*. 2009
	*matK*_F318poales**	TTCTGAGTTTTATTCTCAGATT	De Vere *et al.* 2012
	*matK*_R1460poales**	AGGGTTGTTTTGGTGAACATCAAAG	De Vere *et al.* 2012
	*matK*_F875poales**:	TTATGGATCCTCTTATGCATTATGT	De Vere *et al.* 2012
	*matK*_900R**	ATTSACAANGTAAGATTTCCAT	GPWG II 2012
rbcLa	*rbcLa*_F*	ATGTCACCACAAACAGAGACTAAAGC	Levin *et al.* 2003
	*rbcLa*_R*	GTAAAATCAAGTCCACCYCG	Kress & Erickson 2007

*These primers are all universal primers commonly used in DNA barcoding studies.

** These primers were designed specifically for order Poales or family Poaceae.

The only primer combinations which produced PCR products for *matK* of herbarium samples were (Table 2.3): *matK*_F390 + *matK*_900R (~531 bp) and *matK*_F875poales + *matK*_1326R (~509 bp). These primers split the original *matK* barcoding fragment into two smaller, overlapping fragments. The final *matK* barcoding fragment was edited to a single fragment ~811bp long and the *rbcLa* fragment was ~555bp long.

For the trial reactions carried out with the addition of polyvinylpyrrolidone (PVP) (see Chapter 4.1), a final concentration of 0.8% PVP was used, with the addition of 1ul undiluted DNA template.

2.5 DNA sequencing

Sequencing for this project was carried out at ACDB. Sequencing for the grasses of South Africa project was carried out at the Canadian Centre for DNA Barcoding (CCDB). The sequencing reactions were carried out on purified PCR products using ABI PRISM® BigDye® Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems Inc. California, USA) and the same primers used for PCR. The PCR cycling program used for the Big Dye reaction was: 94˚C for 10 seconds; 50˚C for 5 seconds; 60˚C for 4 minutes (26-40 cycles).

The resulting products were then purified with ethanol and sodium acetate and sequenced on an ABI 3130 *xl* Genetic Analyser (ABI).

2.6 Sequence alignment and assembly

Sequences were assembled using Sequencher 3.1 (Gene Codes, Ann Arbor, Michigan, USA). Assembled sequences for both markers were aligned in MEGA version 6 (Tamura *et al.* 2013) using the Multiple Sequence Comparison by Log-Expectation (MUSCLE) v. 3.8.31 (Edgar 2004) algorithm.

As *rbcLa* is known from previous studies (Gielly & Tabernet 1994, Pettengill & Neel 2010) to not always resolve to species level, *rbcLa* sequences were chosen based on having a *matK* partner, as the combined data frame was likely to be the most informative. Therefore, if a specific sample had a sequence for *rbcLa* only and not for *ma*tK, that *rbcLa* sequence was not included in the *rbcLa* analysis. The number of *rbcLa* sequences with no *matK* partner available was very large and would have created a huge dataset that is not very informative. However, as *matK* is known to be a more informative marker on its own at species level (Lahaye *et al.* 2008, CBOL Plant Working Group 2009, De Vere *et al.* 2012, Hoveka 2013, Kabongo 2013), any samples with only *matK* sequences available were included in the *matK* analysis.

Partial sequences generated from incomplete sequencing, or only one of the two *matK* PCRs being successful, were initially included in both the phylogenetic and barcoding analyses.

2.7 Phylogenetic analyses

Phylogenetic analyses were carried out on *rbcLa*, *matK*, and combined matrices using PAUP* v.4.0b.10 (Swofford 2002) and MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003).

In PAUP, trees were constructed using Maximum Parsimony (MP) in a heuristic search. 10 trees were retained per step, using tree-bisection reconnection (TBR) branch swapping, with 1000 replicates of random taxon addition. Strict consensus trees were then constructed, and bootstrap resampling was performed to assess node support using the same search strategy as the heuristic search (Felsenstein 1985). Bootstrap values are considered as providing weak node support from 50%-74%, moderate support from 74%-84% and strong support from 85%-100% (Hillis & Bull 1993). Bootstrap values were manually plotted onto the strict consensus MP trees in Photoshop 7.0.

Bayesian inference (BI) was used as a maximum likelihood approach to infer phylogenetic relationships using MrBayes v.3.1.2. Firstly, the most appropriate model of sequence evolution for each single locus data partition was assessed using jModelTest v.2.1.4 (Guindon & Gasquel 2003, Darriba *et al*. 2012). Based on both the Akaike Information Criterion (AIC) and AIC with corrections for small sample sizes (AICc), the best substitution model for both the *matK* and the *rbcLa* data partitions was found to be GTR+I+G.

In MrBayes, the rate was set to gamma and the number of substitution types (nst) was set to 6. The Markov Chain Monte Carlo (MCMC) analysis was run initially for 5 000 000 generations for estimation purposes, with sampling at every 1000 generations. In order for the standard deviation of split frequencies to reach below 0.01, the *rbcLa* data partition had to run for 19 000 000 generations, the *matK* for 9 000 000, and the combined data frame for 11 000 000 generations. A 50% majority rule consensus tree with posterior probabilities (PP) was produced for each data set, with PP values 0.50 - 0.94 considered as low support, and 0.95 – 1.00 considered as strong support.

For both MP and BI analyses, species from the basal grass genera were also included as outgroups, namely *Anomochloa* Brongn., *Streptochaeta* Schrad. ex Nees*, Puelia* Franch. and *Pharus* P. Browne*,* although these genera are not found in South Africa. Other outgroups included were species from the order Poales: sister clades Ecdeiocoleaceae and Joinvilleaceae, as well as the closely related clade Restionaceae. Trees were rooted with *two* species from family Restionaceae*, Elegia squamosa* Mast. and *Baloskion tetraphyllum* (Labill.) B.G.Briggs & L.A.S.Johnson.

Once the MP and BI trees were constructed, the MP bootstrap values were manually mapped onto the Bayesian majority rule consensus trees. Tree nodes therefore include both bootstrap values where significant, and posterior probability values. Tree branches were rotated and rooted in FigTree v.1.4.1 and then exported and edited in the R (v.3.1.0) package Ape v.3.1-2 (Paradis *et al.* 2004). Final editing was completed in Photoshop 7.0.

2.8 Assessment of the chosen markers as a DNA barcode for identification purposes

The suitability of the *rbc*L*a*, *matK*, and combined data sets as species identification tools for the taxa of family Poaceae found in southern Africa was assessed using the R package SPIDER

(SPecies IDentification and Evolution in R) version 1.2-0 (Brown *et al.* 2012). Each of the three data partitions was further sorted into a set that included singleton sequences and a set that excluded singletons.

For the datasets that excluded singletons, varieties and subspecies were listed only at species level in order to reduce the number of singletons that needed to be removed from the data, unless these subspecies or varieties had replicate sequences.

SPIDER measures the presence or absence of a barcode gap by subtracting the largest intraspecific distance within a species from the smallest interspecific distance between species (Meyer & Paulay 2005). If the distance within a species is smaller than the distance between a species and its closest con-specific, then a barcoding gap is present. A Kimura 2-Parameter distance matrix (Kimura 1980) was generated with the option pairwise.deletions = TRUE in SPIDER (Brown *et al.* 2012). The significance of the differences between these distances was assessed using the Wilcoxon signed-rank test.

Four metrics were performed on each data frame to assess identification accuracy (three distance-based measures and one tree-based measure). In each test, every individual is treated as an unknown and is tested against the rest of the sequences in the data set. When species represented by a single sequence are included in the data set, the nearest match will always be a different species to the query. The following four metrics were tested at both species and genus level for each data set:

Nearest neighbour - this metric finds the closest individual or nearest species index to the target individual.

Threshold identification – this metric mimics the BOLD species identification method for animals. It searches for all specimens which match a query within a given threshold. If more than one species is matched to a query within the distance threshold, then a species-level identification cannot be made. The default threshold is 1% genetic distance, but this can be optimised for each data set.

Meier's best close match (Meier *et al*. 2006). – This metric functions similarly to the nearest neighbour criterion, but it utilises a threshold (default 1%) which again can be optimised for each data set. This metric finds the individual closest to the target within the threshold. If more than one species is equally close to the target within the threshold, then a correct species identification cannot be made.

Species monophyly criterion – determines if each species is monophyletic over a neighbour joining (NJ) tree. Species represented by an individual sequence are either considered to be always monophyletic [TRUE] by default or never monophyletic [FALSE], depending on the settings chosen. (Brown *et al.* 2012). Bootstrap support can also be incorporated into the analysis. In SPIDER, bootstrap support values greater than 70% are considered correct identifications. In this study, the bootstrap calculation could not be completed for the *matK* data sets and R would crash, so the bootstrap calculation is not included in the results. Rosenberg's probability of reciprocal monophyly (Rosenberg 2007) tests whether the monophyly observed is in fact a chance outcome of random branching or not.

For the *matK* only data (with and without singletons), SPIDER inferred that there was potentially missing data in the distance matrix due to certain samples that generated very short *matK* sequences for which only one half of the *matK* fragment or less was sequenced (Table 2.4). These individuals were therefore removed from the *matK* SPIDER analyses but were still included in the *matk+rbcLa* combined data frame if they had an available *rbcLa* pair (the combined data was informative enough for a distance matrix to be generated for all samples) and in the phylogenetic analyses mentioned above. 36 individuals were removed from the *matK* data without singletons, and 55 from the *matK* data with singletons.

Table 2.4. List of specimens left out for *matK* only (SPIDER analyses) with and without singletons, due to short sequence fragment size.

Scientific name	Voucher number*	Project or database sequence is from
Agrostis montevidensis Spreng. ex Nees	PRE7680085	This study
Agrostis montevidensis Spreng. ex Nees	PRE768077	This study
Arthraxon lanceolatus (Roxb.) Hochst.	PRE0661701	This study
Avena sativa Avena sativa L.	PRE573006	This study
Avena sativa L.	PRE568560	This study
Axonopus fissifolius (Raddi) Kuhlm.	PRE832416	This study
Bambusa balcooa Roxb. Ex Roxb.	PRE559757	This study
Brachiaria advena Vickery	PRE0603647	This study
Brachypodium distachyon (L.) P.Beauv.	NBG166488	This study
Briza maxima L.	NBG269033	This study
Briza minor L.	NBG172752	This study
Bromus alopecuros Poir.	NBG248868	This study
Bromus rigidus Roth	PRE973725	This study
Bromus rubens L.	PRE979484	This study
Bromus rubens L.	PRE973747	This study
Cenchrus biflorus Roxb.	PRE74866	This study
Cenchrus biflorus Roxb.	PRE671774	This study
Cenchrus biflorus Roxb.	PRE783834	This study
Cenchrus incertus M.A.Curtis	NBG179301	This study

Scientific name	**Voucher number***	**Project or database sequence is from**
Chloris truncata R.Br.	PRE965036	This study
Coix lacryma-jobi L.	BM2008/346	Genbank
Corynephorus fasciculatus Boiss. & Reut.	PRE727046	This study
Dactylis glomerata L.	AP080	Genbank
Digitaria violascens Link	PRE646901	This study
Eleusine tristachya (Lam.) Lam.	NBG169033	This study
Eragrostis barrelieri Daveau	PRE834752	This study
Hainardia cylindrica (Willd.) Greuter	PRE67553	This study
Hordeum murinum L. subsp. *leporinum* (Link) Arcang.	PRE747579	This study
Lamarckia aurea (L.) Moench	PRE316145	This study
Lepturus repens (G.Forst.) R.Br.	PRE619760	This study
Lolium multiflorum Lam. x *Lolium perenne* L.	NBG198371	This study
Rostraria pumila (Desf.) Tzvelev	PRE758821	This study
Parapholis incurva (L.) C.E.Hubb.	PRE979475	This study
Pennisetum glaucum (L.) R.Br.	PRE905810	This study
Pennisetum villosum R.Br. ex Fresen.	PRE463	ACDB grasses of southern Africa
Puccinellia fasciculata (Torr.) E.P.Bicknell	PRE527237	This study
Puccinellia fasciculata (Torr.) E.P.Bicknell	PRE527237	This study
Stipa papposa Nees	NBG139478	This study

Scientific name	**Voucher number***	**Project or database sequence is from**
Vulpia fasciculata (Forssk.) Samp.	NMW908	BOLD
Chaetobromus involucratus subsp. *dregeanus* (Nees) Verboom	NBG198519	This study
Cymbopogon marginatus (Steud.) Stapf ex Burtt-Davy	NBG247878	This study
Ehrharta calycina Sm.	NBG272851	This study
Ehrharta villosa Schult.f.	NBG161812	This study
Festuca scabra Vahl	NBG205127	This study
Helictotrichon imberbe (Nees) Veldkamp	PRE851469	This study
Melica racemosa Thunb.	NBG205061	This study
Pennisetum macrourum Trin.	NBG269131	This study
Pentaschistis eriostoma (Nees) Stapf	NBG277151	This study
Pentaschistis glandulosa (Schrad.) H.P.Linder	NBG199671	This study
Trachypogon spicatus (L.f.) Kuntze	NBG273166	This study
Tribolium echinatum (Thunb.) Renvoize	NBG168706	This study
Tribolium hispidum (Thunb.) Desv	NBG272844	This study
Pennisetum mezianum Leeke	PRE467	ACDB grasses of southern Africa
Pentaschistis reflexa H.P.Linder	PRE408	ACDB grasses of southern Africa
Tetrapogon tenellus (Roxb.) Chiov.	KNP160	ACDB grasses of southern Africa

*Voucher numbers for this study were constructed from the herbarium abbreviation and the herbarium specimen identification number.

Chapter 3. List of naturalised grass species in South Africa

3.1 Introduction

The National Environmental Management: Biodiversity Act, Act No.10 of 2004 (NEM:BA) is a set of regulations regarding the management and control of invasive species in South Africa. A list of species considered to be invasive and in need of control was published on 1st August 2014. Fifteen perennial grass species were listed under various categories in this Act, all requiring some level of management and control in the country (Table 1.1).

While these fifteen naturalised perennial grasses are considered to be the most invasive in the country, there have been reports of annual grass invasions in parts of South Africa (Milton 2004, Musil 2005) as well. There is some concern that these annual grass invasions may be an emerging problem that will require greater intervention in the future, if they are not monitored. Some of these annual grasses have become very invasive in other parts of the world with similar climates, for example *Bromus tectorum* L. (cheatgrass) invasions in the western intermountain region and semi-desert shrublands of North America. This highly flammable grass has considerably shortened the fire interval in the area and has altered the vegetation in its introduced habitats in North America from shrubland and perennial grasses to annual grassland (Knapp 1996). *Bromus tectorum* invasions have cost the US government millions of dollars in terms of fire management, native species loss and erosion rehabilitation, and the grass is not a reliable forage crop, as it does not germinate in dry years (Young & Allen 1997). This species is currently found in South Africa, particularly in the winter rainfall regions of the CFR, although invasions of this severity have not yet been reported.

While naturalised grasses do not necessarily become invasive, a list of all naturalised grass species currently found in South Africa would be useful for monitoring what species are already established in the country, and help to allow for the detection of future invasions. Once the list is available, identification tools such as a DNA Barcoding database can be implemented and impact assessments and monitoring plans can be carried out.

The National Working Group on Alien Grasses was formed in 2013 with the aim of listing all alien grasses in South Africa. The construction of this list is still in progress. This group aims to document every alien grass species in the country, including those used in horticulture, agriculture and industry, and those that are not yet naturalised. Once this list has been compiled, decisions will be made as to which species to prioritise for monitoring and future management. The list of naturalised grasses compiled in the present study will be included in this list of alien grasses in South Africa.

3.2 Results

3.2.1 List of naturalised grasses in South Africa

There are about 912 grass species in southern Africa according to Gibbs Russell *et al*. (1991), although this count is based on a survey of all national herbarium specimens in 1990 and is not the most up to date figure for the country. According to van Oudtshoorn (2006) there are about 967 grass species in southern Africa. 406 species, six subspecies and one variety (413 taxa in total, both naturalised and native) are represented in this study, about 43-45% of all species in South Africa (see Appendix 1A for all species included in this study).

The list of naturalised grasses in South Africa compiled in this study consists of 128 species and subspecies, which represents about 13-14% of the grasses found in the country (see Table 3.1). This total does not include *Bromus hordeaceus* and *Hordeum murinum* species as their subspecies are all listed as naturalised in South Africa and are included in the list. This is not an exhaustive list and does not include species of unknown origin, or species used in horticulture and agriculture, or for other commercial uses. Only grasses that are known to be naturalised are listed. Other species that may be naturalised or may be native are listed in Table 2.1 (Chapter 2). It is possible that there are more grasses that have been introduced into South Africa than we are currently aware of.

A more exhaustive list of all alien grass species (not just naturalised species) in South Africa is currently being generated in collaboration with the National Working Group on Alien Grasses, but it is not yet published. An updated edition of the book Grasses of Southern Africa (Gibbs Russell *et al*. 1991) is also in the process of publication and may have further information on naturalised grasses in South Africa, although consultation with one of the authors (Lyn Fish, pers. comm.) aided in keeping the list in this study as up to date as possible.

Table 3.1 List of all known naturalised grass species in South Africa, based on literature cited in Chapter 2. Abbreviations: A = annual; E = East; EC = Eastern Cape; FS = Free State; KZN = KwaZulu-Natal; L = Limpopo; M = Mpumalanga; N = North; NC = Northern Cape; NW = North West province; P = perennial; S = South; W = West; WC = Western Cape.

Scientific name	**Common synonyms used in South Africa**	**Distribution in South Africa**	**Native to:**	**Sub-family**	**Longevity**	**C_3/C_4**
Arundo donax L.		Throughout SA	Asia, tropical Africa	Arundinoideae	P	C_3
Bambusa balcooa Roxb. Ex Roxb.		KZN, M, WC, LIM	India	Bambusoideae	P	C_3
Sasa ramosa (Makino) Makino & Shibata			E. Asia, New Zealand	Bambusoideae	P	C_3
Chloris truncata R.Br.		NC, WC	Australia	Chloridoideae	P	C_4
Cynodon aethiopicus Clayton & Harlan		EC, G, KZN, LIM	tropical Africa	Chloridoideae	P	C_4
Cynodon nlemfuensis Vanderyst		G, KZN, M, LIM	Kenya	Chloridoideae	P	C_4
Eleusine indica (L.) Gaertn.	*Eleusine indica* subsp. *indica*	EC, G, KZN, M, NW	India	Chloridoideae	A	C_4
Eleusine multiflora Hochst. ex A.Rich.		FS, G, M	tropical E. Africa to Ethiopia	Chloridoideae	A	C_4
Eleusine tristachya (Lam.) Lam.		FS, G, M, NC, WC	Tropical Africa, S. America, N. America	Chloridoideae	P	C_4
Eragrostis barrelieri Daveau		EC, FS, G, LIM, M, NC, NW	Europe	Chloridoideae	A	C_4
Eragrostis minor Host		G	Europe	Chloridoideae	A	C_4
Eragrostis tef (Zucc.) Trotter		Throughout SA	North Africa	Chloridoideae	A	C_4

Scientific name	**Common synonyms used in South Africa**	**Provinces found in South Africa**	**Native to:**	**Sub-family**	**Longevity**	**C_3/C_4**
Eragrostis virescens J.Presl		EC, FS, G, KZN, LIM, M, NC, NW, WC	S. America	Chloridoideae	A	C_4
Lepturus repens (G.Forst.) R.Br.		KZN	shores of Indian Ocean to Polynesia & N. Australia	Chloridoideae	P	C_4
Spartina alterniflora Loisel.		Great Brak, WC	N. America to Argentina	Chloridoideae	P	C_4
Cortaderia jubata (Lem.) Stapf		FS, G, LIM, M, WC	S. America	Danthonioideae	P	C_3
Cortaderia selloana (Schult. & Schult.f.) Asch. & Graebn.		EC, G, KZN, LIM, M, WC	S. America	Danthonioideae		C_3
Ehrharta stipoides Labill.	*Microlaena stipoides*	LIM	Australasia	Ehrhartoideae	P	C_3
Axonopus fissifolius (Raddi) Kuhlm.	*Axonopus affinis*	EC, KZN, LIM, M	Tropical America	Panicoideae	P	C_4
Brachiaria advena Vickery		EC, FS, G, M	Australia	Panicoideae	A/P	C_4
Brachiaria umbellata (Trin.) Clayton		In SANBI Nelspruit	Mauritius, Madagascar, Indian Ocean Islands	Panicoideae	P	C_4
Cenchrus biflorus Roxb.		LIM	tropical America	Panicoideae	A	C_4
Cenchrus brownii Roem. & Schult.		KZN	tropical America	Panicoideae	A	C_4
Cenchrus incertus M.A.Curtis		FS, LIM, NC, WC	tropical America	Panicoideae	A	C_4

Scientific name	**Common synonyms used in South Africa**	**Provinces found in South Africa**	**Native to:**	**Sub-family**	**Longevity**	**C_3/C_4**
Coix lacryma-jobi L.		G, KZN, M	East Indies	Panicoideae	A	C_4
Dichanthium aristatum (Poir.) C.E.Hubb.		FS, G, KZN, LIM	Asia	Panicoideae	P	C_4
Digitaria abyssinica (A.Rich.) Stapf	*Digitaria scalarum*	WC	tropical Africa	Panicoideae	P	C_4
Digitaria violascens Link		KZN	Asia and Americas	Panicoideae	A	C_4
Entolasia olivacea Stapf		LIM	Tanzania, Uganda, Cameroon	Panicoideae	P	C_3
Panicum miliaceum L.		G, L, NC	Asia	Panicoideae	A	C_4
Paspalum dilatatum Poir.		Throughout SA	S. America	Panicoideae	P	C_4
Paspalum distichum L.		Throughout SA	S. America	Panicoideae	P	C_4
Paspalum notatum Flüggé		FS, G, KZN, LIM, M, WC	S. America	Panicoideae	P	C_4
Paspalum quadrifarium Lam.		KZN	S. America, USA, Italy	Panicoideae	P	C_4
Paspalum urvillei Steud.		EC, G, KZN, LIM, M, WC	S. America	Panicoideae	P	C_4
Pennisetum clandestinum Hochst. ex Chiov.	*Cenchrus clandestinus*	EC, G, KZN, NC, WC	tropical E. and N. E. Africa	Panicoideae	P	C_4
Pennisetum glaucum (L.) R.Br.	*Cenchrus spicatus*	G, KZN, LIM, M	India	Panicoideae	A	C_4

Scientific name	Common synonyms used in South Africa	Provinces found in South Africa	Native to:	Sub-family	Longevity	C_3/C_4
Pennisetum macrourum Trin.	*Cenchrus caudatus*	EC, G, KZN, L, M, NC, WC	Tropical Africa, Arabia, Australasia	Panicoideae	P	C_4
Pennisetum setaceum (Forssk.) Chiov.	*Cenchrus setaceus*	Throughout SA	E.,N. Africa, tropical Africa, Middle East and S.W. Asia	Panicoideae	P	C_4
Pennisetum villosum R.Br. ex Fresen.	*Cenchrus longisetus*	FS, G, KZN, M, NC	Ethiopia	Panicoideae	P	C_4
Setaria italica (L.) P.Beauv.		Throughout SA	Asia	Panicoideae	A	C_4
Setaria parviflora (Poir.) M.Kerguelen		WC	Tropical America	Panicoideae	P	C_4
Sorghum bicolor L. Moensch subsp. *drummondii* (Steud.) de Wet			Tropical N. Africa	Panicoideae	A	C_4
Sorghum halepense (L.) Pers.		Throughout SA	Mediterranean	Panicoideae	P	C_4
Steinchisma hians (Elliott) Nash & Small	*Panicum hians*	KZN	N. America	Panicoideae	P	C_3 /C_4 intermediate
Agrostis avenacea J.F.Gmel.		EC, FS, G, KZN, M, WC	Australia	Pooideae	A	C_3
Agrostis gigantea Roth		EC, KZN, M, WC	Europe	Pooideae	P	C_3
Agrostis montevidensis Spreng. ex Nees		KZN, M, WC	S. America	Pooideae	A	C_3
Aira cupaniana Guss.		EC, KZN, NC, WC	Europe	Pooideae	A	C_3
Aira praecox L.		WC	Europe	Pooideae	A	C_3
Alopecurus arundinaceus Poir.		M	Eurasia	Pooideae	P	C_3

Scientific name	Common synonyms used in South Africa	Provinces found in South Africa	Native to:	Sub-family	Longevity	C_3/C_4
Ammophila arenaria (L.) Link		EC, WC	Europe	Pooideae	P	C_3
Anthoxanthum odoratum L.		EC, KZN	Europe	Pooideae	P	C_3
Arrhenatherum elatius (L.) P.Beauv. ex J.Presl & C.Presl		KZN	Mediterranean	Pooideae	P	C_3
Avena barbata Pott ex Link		EC, NC, WC	Europe	Pooideae	A	C_3
Avena byzantina K.Koch		G, WC	Europe	Pooideae	A	C_3
Avena fatua L.		EC, FS, G, M, NC, WC	Europe	Pooideae	A	C_3
Avena sativa L.			Europe	Pooideae	A	C_3
Avena sterilis L.		EC, G, KZN, LIM, M, NC, WC	Europe	Pooideae	A	C_3
Brachypodium distachyon (L.) P.Beauv.		NC, WC	Mediterranean	Pooideae	A	C_3
Briza maxima L.		NC, WC	Mediterranean	Pooideae	A	C_3
Briza minor L.		EC, FS, G, KZN, LIM, NC, WC	Mediterranean	Pooideae	A	C_3

Scientific name	**Common synonyms used in South Africa**	**Provinces found in South Africa**	**Native to:**	**Sub-family**	**Longevity**	**C_3/C_4**
Briza subaristata Lam.		EC, WC	S. America	Pooideae	P	C_3
Bromus alopecuros Poir.		WC	Mediterranean	Pooideae	A	C_3
Bromus catharticus Vahl		Throughout SA	Europe, Australia, USA, S. America	Pooideae	A/P	C_3
Bromus commutatus Schrad		EC, FS, KZN, WC	Europe, Asia, USA, S. America, Africa	Pooideae	A	C_3
Bromus diandrus Roth		EC, FS, KZN, LIM, M, NC, WC	Mediterranean	Pooideae	A	C_3
Bromus hordeaceus L.		EC, M, WC	Europe	Pooideae	A	C_3
Bromus hordeaceus subsp ferronii (Mabille) P.M. Sm.		EC	France, Britain	Pooideae	A	C_3
Bromus hordeaceus subsp. *molliformis* (J.Lloyd) Maire & Weiller		EC, M, WC	Europe	Pooideae	A	C_3
Bromus hordeaceus subsp. *divaricatus* (Bonnier & Layens) Kerguélen			Europe	Pooideae	A	C_3
Bromus rigidus Roth		EC, WC	Mediterranean	Pooideae	A	C_3
Bromus rubens L.		NC	Europe	Pooideae	A	C_3

Scientific name	Common synonyms used in South Africa	Provinces found in South Africa	Native to:	Sub-family	Longevity	C_3/C_4
Bromus tectorum L.		G, NC	Mediterannean	Pooideae	A	C_3
Catapodium rigidum (L.) C.E.Hubb.		EC, WC	Mediterannean	Pooideae	A	C_3
Corynephorus fasciculatus Boiss. & Reut.		WC	Europe	Pooideae	A	C_3
Cynosurus coloratus Lehm ex Nees		NC, WC	Europe	Pooideae	A	C_3
Cynosurus echinatus L.		EC, FS, WC	Europe	Pooideae	A	C_3
Dactylis glomerata L.		EC, FS, KZN, M, WC	Europe	Pooideae	P	C_3
Deschampsia cespitosa (L.) P.Beauv.		EC	Europe	Pooideae	P	C_3
Deschampsia flexuosa (L.) Trin.		WC	Europe	Pooideae	P	C_3
Elymus repens (L.) Gould	*Elytrigia repens*	EC, LIM, WC	Mediterranean	Pooideae	P	C_3
Festuca arundinacea Schreb.		EC, FS, G, KZN, LIM, M, WC	Europe, temperate Asia	Pooideae	P	C_3

Scientific name	**Common synonyms used in South Africa**	**Provinces found in South Africa**	**Native to:**	**Sub-family**	**Longevity**	**C_3/C_4**
Gastridium phleoides (Nees & Meyen) C.E.Hubb.	*Gastridium ventricosum*	WC	Mediterranean	Pooideae	A	C_3
Glyceria maxima (Hartm.) Holmb.	*Poa aquatica*	KZN	Eurasia	Pooideae	P	C_3
Hainardia cylindrica (Willd.) Greuter		EC, WC	Mediterranean	Pooideae	A	C_3
Holcus lanatus L.		EC, G, KZN, LIM, M, WC	Europe, Asia	Pooideae	P	C_3
Hordeum marinum subsp. *gussoneanum* (Parl.) Thell.	*Hordeum geniculatum*	WC	Europe	Pooideae	A	C_3
Hordeum murinum L.	*Hordeum murinum* subsp. *murinum*	EC, NC, WC	Europe	Pooideae	A	C_3
Hordeum murinum subsp. *glaucum* (Steud.) Tzvelev		EC, KZN, NC, WC	Europe	Pooideae	A	C_3
Hordeum murinum subsp. *leporinum* (Link) Arcang.		EC, NC, WC	Europe	Pooideae	A	C_3
Hordeum stenostachys Godr.		EC, FS, NC	probably naturalized from S. America	Pooideae	A	C_3
Lagurus ovatus L.		EC, WC	Mediterranean	Pooideae	P	C_3
Lamarckia aurea (L.) Moench		WC	Mediterranean basin	Pooideae	A	C_3
Lolium multiflorum Lam.		Throughout SA	Mediterranean	Pooideae	short-lived P	C_3

Scientific name	Common synonyms used in South Africa	Provinces found in South Africa	Native to:	Sub-family	Longevity	C_3/C_4
Lolium multiflorum Lam. x *Lolium perenne* L		EC, FS, KZN, LIM, M, WC	Europe	Pooideae	A/P	C_3
Lolium perenne L.		EC, FS, G, KZN, M, NC, WC	Europe	Pooideae	P	C_3
Lolium rigidum Gaudin		EC, KZN, NC, NW, WC	Mediterranean	Pooideae	A	C_3
Lolium temulentum L.		EC, FS, G, KZN, M, NC, NW, WC	Mediterranean	Pooideae	A	C_3
Nassella neesiana (Trin. & Rupr.) Barkworth	*Stipa neesiana*	EC, FS	S. America	Pooideae	P	C_3
Nassella tenuissima (Trin.) Barkworth	*Stipa tenuissima*	EC	S. America	Pooideae	P	C_3
Nassella trichotoma (Nees) Hack. ex Arechav.	*Stipa trichotoma*	EC, WC	S. America	Pooideae	P	C_3
Parapholis incurva (L.) C.E.Hubb.		NC, WC	Europe	Pooideae	A	C_3
Periballia minuta (L.) Asch. & Graebn.		WC	Med. Basin	Pooideae	A	C_3
Phalaris angusta Nees ex Trin.		KZN, WC	S. America	Pooideae	A	C_3
Phalaris aquatica L.		EC, FS, G, KZN, M, WC	Mediterranean	Pooideae	P	C_3
Phalaris arundinacea L.		EC, FS, G, KZN, M, NW, WC	Northern USA	Pooideae	P	C_3

Scientific name	**Common synonyms used in South Africa**	**Provinces found in South Africa**	**Native to:**	**Sub-family**	**Longevity**	**C_3/C_4**
Phalaris canariensis L.		EC, FS, G, KZN, M, NC, NW, WC	Canary Islands, N. Africa	Pooideae	A	C_3
Phalaris minor Retz.		EC, FS, G, KZN, M, NC, WC	Mediterranean	Pooideae	A	C_3
Phalaris paradoxa L.		G, NW, WC	Mediterranean	Pooideae	A	C_3
Poa annua L.		Throughout SA	Europe	Pooideae	A	C_3
Poa pratensis L.		EC, G, KZN, M, WC	Europe	Pooideae	P	C_3
Poa trivialis L.		G	Europe	Pooideae	P	C_3
Polypogon monspeliensis (L.) Desf.		Throughout SA	Eurasia	Pooideae	A	C_3
Polypogon viridis (Gouan) Breistr.		Throughout SA	S. Europe	Pooideae	P	C_3
Puccinellia distans L. Parl.		EC, NC, WC	Europe	Pooideae	P	C_3
Puccinellia fasciculata (Torr.) E.P.Bicknell		EC, NC, WC	Europe	Pooideae	P	C_3
Rostraria cristata (L.) Tzvelev	*Lophochloa cristata*	EC, WC	Europe, Mediterranean	Pooideae	A	C_3
Rostraria pumila (Desf.) Tzvelev	*Lophochloa pumila*	NC, WC	Europe	Pooideae	A	C_3
Sphenopus divaricatus (Gouan) Rchb.		NC, WC	Europe	Pooideae	A	C_3

Scientific name	**Common synonyms used in South Africa**	**Provinces found in South Africa**	**Native to:**	**Sub-family**	**Longevity**	**C_3/C_4**
Stipa clandestina Hack.	*Achnatherum clandestinum*	EC, FS, G, WC	Mexico	Pooideae	P	C_3
Stipa papposa Nees	*Jarava plumosa*	WC	S. America	Pooideae	P	C_3
Stipa variabilis Hughes	*Austrostipa variabilis*	WC	Australia	Pooideae	P	C_3
Vulpia bromoides (L.) Gray		EC, FS, KZN, M, NC, WC	Europe	Pooideae	A	C_3
Vulpia fasciculata (Forssk.) Samp.		WC	Europe	Pooideae	A	C_3
Vulpia muralis (Kunth) Nees		EC, FS, WC	Europe	Pooideae	A	C_3
Vulpia myuros (L.) C.C.Gmel.		EC, FS, KZN, NC, WC	Europe	Pooideae	A	C_3

Of the 128 naturalised grass species and subspecies, 82 (~64%) belong to the sub-family Pooideae, a group of cool growing season C_3 grasses from predominantly the temperate Northern Hemisphere (Table 3.1). Sub-family Panicoideae contains 28 grasses (~22%). This sub-family contains both C_3 and C_4 grasses, and these grasses generally tolerate hotter and drier conditions than the Pooideae. 12 (~9%) belong to the Chloridoideae (C_4 only), 2 (~3%) belong to the Danthonioideae, (a C_3 sub-family that has many native representatives in South Africa, many of which are found in the Western Cape), and 2 (~3%) belong to the Bambusoideae (C_3 only. A single grass (~1%) belongs to Arundinoideae (C_3 only) and 1 (~1%) belongs to Ehrhartoideae (another C_3 grass sub-family with native representation in South Africa). There are no representatives of the sub-family Aristidoideae (C_4 photosynthesis only) in this list of naturalised grasses, but this sub-family is well represented in the native taxa found in southern Africa and in this study (Appendix 1A).

There are also no representatives of sub-family Micrairoideae in either the native or naturalised taxa in this study. *Isachne rigens* (Sw.) Trin. is a native grass represented in this study (Appendix 1A) that is thought to be synonymous with *Panicum arbusculum* Sieber ex Griseb, (The Plant List 2013). *Isachne* R.Br. belongs to Micrairoideae (Sánchez-Ken *et al.* 2007), but *Panicum* L. falls within sub-family Panicoideae. Although other *Isachne* species were found to group in a monophyletic clade within the well-supported subfamily Micrairoideae by Teerawatananon *et al.* (2011), in this study (Chapter 5), this particular species is nested with other *Panicum* species in the sub-family Panicoideae. For the purposes of this study, both names are listed on the phylogenetic trees in Chapter 5, but this species is considered to nest within sub-family Panicoideae and not Micrairoideae. *Isachne rigens* is currently the preferred name (The Plant List 2013).

Of the naturalised grasses in South Africa 90 exhibit C_3 photosynthesis (~70%) and 38 exhibit C_4 photosynthesis (~30%). *Steinchisma hians* (Elliott) Nash & Small, is a C_3/C_4 intermediate (GPWG II 2012) (Table 3.1).

There are 67 annual species on the naturalised grasses list (~52%) and 58 species are perennial (~45%). There are also three species that exhibit both annual and perennial variants (Table 3.1).

I was not able to collect any molecular data for 12 out the 128 naturalised grasses in this study, either because there were no specimens available in herbaria, or because PCR was not successful for herbarium specimens (see Table 3.2 below). *Sasa ramosa* (Makino) Makino & Shibata was listed in Bromilow (2010) as being under CARA legislation, and it is on the current preliminary NEM:BA list (Table 1.1, Chapter 1) but no specimens of this species were found in either of the herbaria I searched (POSA 2009), nor were any found in BOLD or GenBank databases. *Sasa ramosa* is a bamboo species native to Northern Europe, Asia and New Zealand (Clayton *et al.* 2006 - ongoing). It is uncertain as to why this species is listed as invasive in South Africa, but sampling and further molecular work is required to obtain representatives of this species for the barcoding database of naturalised grasses in South Africa.

The only herbarium sample for *Spartina alterniflora* Loisel.that was available for sampling in the two herbaria used in this study was from 1964 (Table 2.2 in Chapter 2) and had very degraded DNA, and no PCR products were generated from this sample. However, invasions of this grass have recently been reported in South Africa, thus it should be possible to obtain fresher tissue for DNA extraction from this species in the future (Adams *et al.* 2012).

There are no *Periballia* Trin. species represented in the molecular data for this study, as there were very few herbarium specimens of *Periballia minuta* (L.) Asch. & Graebn. available and PCR was unsuccessful on the one specimen from 1943 for which DNA was extracted (Table 2.2). Further efforts are required to find tissue for sampling to enable DNA barcoding of this species.

Bromus hordeaceus L. is well represented in the DNA barcoding database at species level, so the lack of data for the three subspecies (Table 3.2) is not necessarily an issue, as correct identification at species level would be enough to confirm that a sample of this grass is a naturalised grass.

The genera *Avena* L., *Cortaderia* Stapf, *Cynosurus* L. and *Phalaris* L. are not indigenous to southern Africa (Gibbs Russell *et al.* 1991). These genera are represented by other species in the DNA barcode database in this study (Appendix 1A), and a successful identification of an unknown grass at genus level would be sufficient to indicate an alien species for the rest of the species mentioned in Table 3.2 below.

Table 3.2 List of naturalised grass species in South Africa not represented by any molecular data in this study.

	Scientific name
1	*Avena byzantina* K.Koch
2	*Bromus hordeaceus L. subsp* ferronii (Mabille) P.M. Sm.
3	*Bromus hordeaceus* subsp. *molliformis* (J.Lloyd) Maire & Weiller
4	*Bromus hordeaceus* subsp. *divaricatus* (Bonnier & Layens) Kerguélen
5	*Cortaderia jubata* (Lem.) Stapf
6	*Cynosurus coloratus* Lehm ex Nees
7	*Periballia minuta* (L.) Asch. & Graebn.
8	*Phalaris angusta* Nees ex Trin.
9	*Phalaris aquatica* L.
10	*Phalaris minor* Retz.
11	*Spartina alterniflora* Loisel.
12	*Sasa ramosa* (Makino) Makino & Shibata

3.2.2 Usage of some names in this list of naturalised grass species

Although specimens identified as *Bambusa balcooa* Roxb. Ex Roxb. have been collected in South Africa and are listed in herbaria collections (Gibbs Russell *et al.* 1991, POSA 2009), it should be noted that it is not actually certain which specific bamboo species is currently found in South Africa, and this identification is currently an approximation (Lyn Fish, pers. comm.). Two specimens that were identified as *Bambusa balcooa* in South Africa were included in this study (see Table 2.2, Chapter 2) and one specimen was taken from the GenBank database (sampled in India, Appendix 1C). *matK* sequences were available for all three of these specimens (although one specimen sampled in South Africa (voucher number PRE559757) only had partial *matK* sequence information, Table 2.2).

When aligned in MUSCLE v. 3.8.31 (Edgar 2004), two nucleotide differences are observed between *Bambusa balcooa* PRE709389 (Table 2.2) and the specimen from India (voucher number AUS_TB2, Appendix 1C) within the 597bp in which the two sequences overlap. Both BOLD and BLAST searches of *B. balcooa* voucher PRE709389 found 99% identity *to B. balcooa* voucher AUS_TB2 and 100% identity over 720bp of query sequence to various *Bambusa* Schreb. and *Dendrocalamus* Nees species (*Dendrocalamus* is also from sub-family Bambusoideae). *Bambusa* is considered polyphyletic with some *Dendrocalamus* species nested within *Bambusa* (Sun *et al*. 2005, Pattanaik & Hall 2011). *Bambusa balcooa* PRE559757 had 100% identity with many *Bambusa* and *Dendrocalamus* species over 330bp of query sequence in both BLAST and BOLD ID searches, and 99% identity (one nucleotide difference) with *B.balcooa* voucher AUS_TB2.

According to Verloove (2012), as *Pennisetum* Rich. and *Cenchrus* L. are very closely related, with *Cenchrus* species nested within *Pennisetum*, it is recommended to merge the two genera (Chemisquy *et al.* 2010), and as the generic name *Cenchrus* has taxonomic priority over *Pennisetum*, all *Pennisetum* species should be transferred to *Cenchrus*. However, the accepted names for many of these species are still listed under *Pennisetum* on the Plant List (2013) and some of these grasses are still currently commonly identified and listed under *Pennisetum* in South Africa (Klopper *et al.* 2006). For this reason, those members of the *Pennisetum/Cenchrus* clade that are still known as *Pennisetum* in this country are listed as so in Table 3.1 above, and throughout this study, in order to avoid confusion when comparing this list to herbarium specimens in the country.

3.3 Discussion

This list aims to document as thoroughly as possible all of the naturalised grasses in South Africa. However, the bulk of the information on naturalised grasses comes from a book published more than 20 years ago (Gibbs Russell *et al.* 1991) and it is known that there are many grass species of unknown origin in the country. In-depth molecular and taxonomic studies could unravel the origins of many of the grass species considered to be of unknown origin in the future. There is also concern that collectors favour species that they know are native when collecting field specimens for herbaria, so sampling bias may mean that naturalised grass species are underrepresented in herbaria collections. Efforts should also be made in the future to complete the DNA barcoding database for these naturalised grasses so that they are fully represented in the database, with a focus on the species for which no data could be collected, as mentioned in Table 3.2.

This list is therefore not exhaustive and should be added to as more information is accumulated about alien grasses in the country. A new version of the book *Grasses of southern Africa is* expected to be published in the near future, and should contain more up to date information about naturalised grasses in the country, although Lyn Fish (South African grass expert) provided preliminary information from this book towards the present study.

Due to the increasing interest in biofuels as an alternative source of energy, there is concern about the possible introduction of bamboo species and other biofuel plants into South Africa. This may be the reason why *Sasa ramosa* appears on the list of species to be controlled in the country (NEM:BA 2014). There is already at least one naturalised bamboo species in the country (Gibbs Russell *et al*. 1991) and from my sequencing results it appears that this bamboo belongs to either *Bambusa* or *Dendrocalamus*. However, the amount of investigation into this species carried out in this study is not sufficient to make an accurate species identification, and it is worth sequencing further herbarium samples of this species and carrying out more in-depth taxonomic, phylogenetic and DNA barcoding studies on the bamboo species already in the country, as well as those that may be introduced into the country in the future, to aid in the monitoring of future invasions.

As can be seen from Table 3.1, the naturalised grasses in South Africa can be split roughly into two groups; those that are predominantly C_3 and annual and mostly from the family Pooideae (most of which were brought by colonialists from Europe as forage grasses, Henderson 2001, Milton 2004), and those that are predominantly C_4 and perennial grasses (most of which were brought from Asia and South America as ornamentals and for various uses including preventing soil erosion) (Milton 2004). The mostly annual European C_3 grasses are found predominantly in the winter rainfall regions of the country, whereas the mostly C_4 perennials are found in both winter and rainfall regions, often in riparian and wetland habitats.

As of yet, there have been few studies investigating why and how currently invasive grasses are able to gain advantage over native species in South Africa. Of particular interest is the theory that certain traits of invasive grasses may be conferring an advantage over native grasses.

A common trait that is considered in invasion biology is longevity (Musil *et al.* 2005, James 2008, Stewart 2009, Schmidt & Drake 2011). In the case of the naturalised grasses in South Africa, both annual and perennial grasses have been introduced, and both types have become invasive in the country. It is possible that either annularity or perenniality may confer advantages that lead to invasion, depending on the habitat in which they are found. Annual grasses in the winter rainfall area may be able to germinate and flower more quickly than native species, thus crowding out native species and preventing their germination or regeneration. Large perennial grasses found in riparian and wetland habitats may outcompete native species in terms of size, and they can regenerate after fires or grazing, allowing them to persist. However, it appears that as both annual and perennial grasses are successful invaders in the country, life-cycle may not be useful as a general trait to predict invasiveness in these grasses as a whole.

Another trait that was considered worth investigating in invasive grasses was photosynthetic pathway (Milton 2004) but again in South Africa both C_3 and C_4 grasses have successfully invaded, thus this is not necessarily useful as a general trait that predicts invasiveness in grasses across South Africa as a whole.

C_4 grasses outperform C_3 grasses at higher temperatures, yet many C_3 grasses have naturalised in South Africa, where temperatures are generally considered to be high and where C_4 grasses thrive. The invasive C_3 grasses mainly do well in the winter rainfall areas of the Western Cape (where native C_3 grasses are also found), which have similar rainfall patterns to those of Mediterranean Europe where these grasses are from. They do most of their growing in the cooler, wet season. These C_3 grasses generally invade disturbed soils on abandoned fields that have been exposed to nitrogen fertilisers and run-off, as C_3 grasses require more nitrogen than C_4 grasses (as mentioned in Chapter 1). Due to this nitrogen requirement it is possible that invasions will be contained in old agricultural lands and will not spread, as undisturbed soil in the region is usually nitrogen poor. However, increased disturbance and global change leading to increased atmospheric nitrogen may increase soil nitrogen levels and allow these C_3 grass invasions to spread further in the future (Milton 2004).

Another trait that is commonly linked to plant invasion is polyploidy (Te Beest *et al.* 2011). In the case of grasses, some of the most invasive species in South Africa and the world are polyploids, for example, many species from the genera *Pennisetum* Rich., *Paspalum* L., and *Cortaderia* Stapf, as well as *Arundo donax* L. and *Spartina alterniflora* Loisel. (Goldblatt & Johnson (eds) 1979 - ongoing). Polyploidy is likely to influence the large size of these particular invasive grasses, amongst other things. However many native South African grass species are also polyploids and the grass family as a whole contains many polyploids. Further investigation may reveal whether polyploidy is a factor influencing the success of grass invasions in South Africa.

Finally, phenology, particularly flowering time, has been suggested as a trait that may influence invasive potential in plants (Brown *et al*. 2002, Pyšek & Richardson 2007, Marushia *et al.* 2010). Wolkovich & Cleland (2010) list four hypotheses for how phenology may influence the success of invasive species:

1. **Vacant Niche** - invaders may bloom or leaf earlier or later than (essentially out of sync with) natives to make use of temporally available resources.
2. **Priority Effects** - invaders tend to establish earlier than native species and utilise available resources first to help them establish. These invaders often have rapid growth rates.
3. **Niche Breadth** - Invaders with longer phenological phases than native species will have greater niche breadth and extended access to resources.
4. **Plasticity and Climate** - Invaders exhibit phenotypic plasticity and adapt rapidly to weather change or new environments, whereas native species may not adapt to a change in climate.

Almost all grasses are wind pollinated, so competition between native and invasive grasses for pollinators is somewhat reduced, but pollen volumes and the timing of pollen release could be factors that give an invasive out-crossing grass an advantage over a native out-crossing grass. Also a longer flowering period may increase the amount of time that a flower is exposed to pollen and thus increase fertilisation potential.

An example that could potentially be investigated further in South Africa is a comparison of the flowering time and duration in the invasive grasses found in the renosterveld versus the commonly found native species in the area. This a region in which grass invasion is fairly well-documented and it may be interesting to see if flowering time is conferring an advantage on these alien grasses over the native plant species in the area. Flowering time information for renosterveld plant species, both native and naturalised, can be found in Manning & Goldblatt (2012).

Chapter 4. DNA barcoding the grasses of southern Africa

4.1 Introduction

Plant invasions are a major cause of biodiversity loss and species extinction (Mack *et al.* 2000, Bromilow 2010), and are known to dramatically affect an ecosystem's potential to provide goods and services to a country. These economic losses, along with the detection, control and eradication of plant invasions are estimated to cost South Africa billions of rand each year (De Lange & Van Wilgen 2010).

Grass invasions are generally considered of lesser concern in South Africa at present in comparison to invasions by other plant groups, but grass invasions do exist in South Africa, as mentioned in Chapter 1, particularly in wetland and riparian ecosystems, roadsides and other disturbed areas, as well as in ecosystems in the winter rainfall regions of South Africa, where C_3 invasive grass species are successful (Milton 2004). There are concerns that with global change, grass invasions may increase in South Africa (Milton 2004). Grass invasions can alter ecosystem fire regimes, alter water flow and quantity in rivers and wetlands, affect livestock and wild herbivore grazing, replace native species, decrease incident light, and alter soil humidity, all of which can lead to biodiversity and resource loss and economic disruption (D'Antonio & Vitousek 1992, Musil *et al.* 2005).

Grasses are often difficult to identify, due to their relatively homogenous morphology, and are usually classified by their flower structures. If a grass is not flowering then it can be challenging to make a conclusive identification. There are currently few grass identification experts in South Africa, and the time taken to accurately identify a potentially naturalised or invasive grass may hinder the early detection process. As a result, alternative methods such as DNA barcoding have been considered for invasive grass species identification.

The Consortium for the Barcode of Life was formed in 2004 with the aim of DNA barcoding as much of the world's biodiversity as possible. This would allow easy and rapid identification of morphologically indistinct or fragmented samples using a short, standardised DNA sequence, and would have applications in biosecurity, monitoring illegal trade of commercial species, crime scene analysis, identifying alien invasive species, and monitoring biodiversity (Hebert *et al.* 2003).The main use of DNA barcoding would be identification, and it is not meant to replace taxonomic and phylogenetic studies, although DNA barcoding data does contain phylogenetic information and can contribute to these studies (Pyšek *et al.* 2013).

After much investigation into suitable DNA markers for barcoding plant species, two plastid gene markers which are also commonly used in phylogenetic studies, namely *matK* and *rbcLa*, were selected as the official plant DNA barcode markers (CBOL 2009). There is still much debate as to whether these two markers are the best performing markers for species identification in plants, with research still directed towards testing other markers, particularly the nuclear regions ITS and *trnH-psbA,* both of which are considered to be highly variable, with better PCR success rates than *matK* (CBOL 2009, China Plant BOL Group 2011, Hollingsworth 2011, Pang *et al*. 2012, Hoveka 2014).

The aim of this chapter is to assess the suitability of DNA barcoding as a suitable method of identification of both native and naturalised grasses in southern Africa, specifically with the two markers currently recommended by the CBOL Plant Working Group (2009). The identification efficacy of this data was tested with the R package SPIDER using distance-based and tree-based criteria of identification.

4.2 Results

4.2.1 PCR success rates

Out of 199 naturalised and native specimens collected for DNA extraction, an overall low PCR success rate was achieved. Only 83 out of 199 specimens produced a successful PCR product for *rbcLa* (44%), using the commonly used universal barcoding primers listed in Table 2.2 (Chapter 2), although issues with sequencing reduced the number of full *rbcLa* sequences further. Partial *rbcLa* sequences were included in both the barcoding and phylogenetic analyses. It was hypothesised that the low PCR success rate may in some cases be due to the degraded nature of some of the DNA extracted from the herbarium samples, especially from older specimens. However the majority of specimens included in this study were collected from 1990 -2012, so the age of the DNA is not the cause of PCR failure in the majority of cases. Another potential issue was high concentrations of tannins and other polyphenolic compounds that are commonly found in grass specimens. These are known to inhibit PCR.

In a few cases another issue encountered was that sometimes DNA from very old and degraded samples generated a PCR product, but after sequencing it was found that the product was from a DNA contaminant that was usually not from the sub-family Poaceae. This particular issue was noted early on, and efforts to increase experimental sterility minimised the issue.

Initially, no PCR products were generated for *matK*, using the common universal primer combinations listed in Table 2.3. Although these primers are universal, none of them had perfect or very close matches to many of the Poaceae sequences, and it was concluded that more specific primers should be trialled. The universal primers F390 and R1326 matched fairly well to most Poaceae sequences (Cuénoud *et al.* 2002), but for my samples they did not produce any PCR products when used as a pair, probably due to the issues of degradation and contaminants mentioned above.

Previous barcoding and phylogenetic studies including sub-family Poaceae have also encountered problems of amplification for *matK* from herbarium samples, and have designed more specific primers to counteract the issue (Kress & Erickson 2007, GPWG II 2012, De Vere *et al.* 2012). It should be noted that in other previous studies, some of these universal *matK* primers have successfully amplified Poaceae samples (from mostly fresh tissue) (Ragupathy *et al.* 2009, Drumwright *et al.* 2011, Saarela *et al.* 2013). After examining various Poaceae sequences and comparing these to primer sequences, the specific primers most likely to amplify Poaceae were selected from the literature (De Vere *et al.* 2012, GPWG II 2012, Table 2.3). Initially, full *matK* PCRs were attempted with these specific primers, but they were not successful. As mentioned in Chapter 2.4., it was decided to divide the original *matK* barcoding fragment into two smaller overlapping fragments to increase the possibility that degraded DNA would be amplified. The specific primers *matK* _900R and *matK* _F875poales were used in combination with the universal primers F390 and R1326 to produce two smaller overlapping *matK* fragments, as mentioned in Chapter 2.

Once these more specific primer pairs were selected, a total of 127 out of 199 specimens (64%) yielded PCR products, either for both fragments or for one fragment (Table 2.2). Again, partial sequences were included in the analysis (except for the specimens mentioned in Chapter 2, Table 2.4, which were excluded from the *matK* SPIDER analyses only). Of the two *matK* fragments, 69 out of 199 PCRs (35%) were successful for the first fragment (*matK*_F390 + *matK*_900R), and 111 out of 199 (56%) were successful for the second fragment (*matK*_F875poales + *ma*t*K*_1326R). The second set of primers were more successful at amplification than the first set. However not all of these PCR products produced useable sequences. Another minor issue was that a few specimens when sequenced were found to be the incorrect species according to their herbarium record, although they still belonged to sub-family Poaceae. In some cases this could be due to incorrect identification, or again PCR contamination could be an issue. These taxa were not included in the analysis.

Due to the lack of PCR success in many relatively recently collected herbarium samples (see Table 2.2), it was decided to attempt PCR with the addition of polyvinylpyrrolidone (PVP) for these samples, as PVP has been shown to reverse the inhibitory effects of polyphenolic compounds on PCR (Koonjul *et al.* 1999). Due to time constraints, only a trial PCR was carried out (Figure 4.1). 0.8% PVP (final concentration) was trialled with various DNA template dilutions (undiluted, 1 in 10, 1 in 50, 1 in 100) and these dilutions were also trialled without the addition of PVP. PCRs of the undiluted template with the addition of 0.8% PVP successfully produced PCR products with the universal *rbcLa* primers on some samples that I was unable to PCR previously. The template dilutions, with and without the addition of PVP, did not work. The double fragment seen in lane 1 (Figure 4.1) for *Trachypogon spicatus* is likely due to mispriming, and may be eliminated with stronger PCR stringency, for instance by raising the annealing temperature.

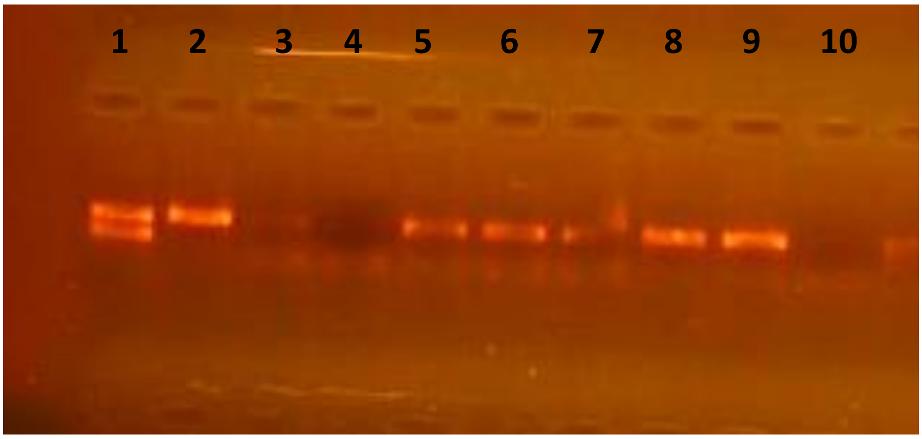


Figure 4.1. 1% gel of PCR products from samples that would not PCR with *rbcLa* primers under normal PCR conditions. 0.8% PVP was added to the PCR reactions. Undiluted template was used in these reaction. Lanes (include sample name, species name and date sampled): **1**: POA92, *Trachypogon spicatus* (2012); **2**: POA 40, *Nassella neesiana* (2011); **3**: POA 34, *Phalaris minor* (2004); **4**: POA 35, *Phalaris minor* (2005) – did not work; **5**: POA 45, *Vulpia muralis* (2001); **6**:POA 46, *Vulpia muralis* (2005); **7**: POA 49, *Chaetobromus involucratus* subsp. *dregeanus* (2004); **8**: POA 50, *Cymbopogon marginatus* (2009); **9**: POA 52, *Digitaria argyrograpta* (2005); **10**: negative control.

4.2.2 DNA barcoding analysis

4.2.2.1 Summary statistics

A total of 199 native and alien grass leaf samples were collected from herbarium specimens from the National Herbarium in Pretoria (PRE) and the Compton Herbarium at Kirstenbosch (NBG) for extraction and sequencing (Table 2.2). As mentioned above, DNA sequences were not retrieved for all of the sampled specimens. In addition, 395 *matK* and 279 *rbcLa* sequences were retrieved from the BOLD and GenBank databases and 340 *matK* and 339 *rbcLa* sequences were downloaded from the ACDB Grasses of southern Africa project database.

In total, for the DNA barcode analysis, the *matK* matrix consisted of 779 sequences with singletons included, and 576 sequences with these excluded (Table 4.1). Originally, 838 sequences were available in the *matK* matrix, but as mentioned in Chapter 2 (Table 2.4), some *matK* sequences were considered too short by SPIDER to be informative, and these were removed. For the *rbcLa* and combined *rbcLa* + *matK* matrices, 674 sequences were used in the matrices including singletons, and 463 and 461 sequences were included, respectively, in the *rbcLa* and combined datasets without singletons respectively.

The matrices with singletons included single sequences from four early diverging grass lineage species (not found in South Africa) (Appendix 1A) in order to enable rooting of trees for tree-based methods of measuring identification success. *Anomochloa marantoidea* Brongn. was used as the root for the datasets with singletons. Two *Streptochaeta angustifolia* Soderstr. sequences were included in the matrices without singletons as the root in the tree-based analyses in SPIDER.

The addition of singletons to a SPIDER analysis masks the overall performance of the data, as singletons will always be matched to a different species in the three distance metrics used to test the data. The rest of the results section focusses on the data without singletons as this data allows the best measure of identification potential. It should be noted that for actual DNA barcode identifications of unknown species in the future, the datasets with singletons should be used, as the singletons will increase the chances that an unknown species will find a match. The datasets with singletons contain 374 species for the *rbcla* and combined datasets, and 415 species for the *matK* dataset, in comparison to 155, 187 and 154 species for *rbcla*, *matK* and combined datasets without singletons, respectively (Table 4.1).

The mean and median number of species for all three datasets without singletons was three (Table 4.1). The number of genera were 85, 100 and 85 for *rbcLa*, *matK* and the combined datasets, respectively.

Table 4.1 SPIDER summary statistics for the r*bcLa*, *matK* and combined matrices, with and without singletons.

Statistic	Singletons	*rbcLa*	*matK*	Combined
No. Individuals	**Incl.**	674	779	674
	Excl.	463	576	461
Sequence alignment length	**Incl.**	555	811	1383
	Excl.	555	795	1383
No. species	**Incl.**	374	415	374
	Excl.	155	187	154
No. genera	**Incl.**	123	141	123
	Excl.	85	100	85
Mean individuals per spp.	**Incl.**	2	2	2
	Excl.	3	3	3
Median individuals per spp.	**Incl.**	1	1	1
	Excl.	3	3	3
No. barcodes < 500bp	**Incl.**	30	5	0
	Excl.	19	5	0
Mean seq. length bp	**Incl.**	543	747	1273
	Excl.	543	784	1278

4.2.2.2 Barcode gap analysis and genetic divergence

Genetic divergence and the existence of a “barcode gap” is examined in SPIDER by comparing the largest intraspecific distance within a species with the smallest interspecific distance between that species and other species, rather than the mean intra- and interspecific distances, as the use of mean intra- and interspecific distances artificially inflates the estimation of the barcode gap (Meier *et al.* 2008). These distances are calculated using a K2P distance model with pairwise distances. Only the data without singletons are presented here.

The *rbcLa* data showed lower genetic divergence than the *matK* and combined data, with a mean lowest interspecific distance of 0.38% for *rbcLa*, compared with 0.98% and 0.85% for the *matK* and combined datasets, respectively (Table 4.2).

For all three datasets, overall intraspecific variation is lower than interspecific variation, with *rbcLa* having the lowest intraspecific and interspecific variations overall. For *rbcLa*, 76% of the largest intraspecific distances were equal to 0, whereas for the smallest interspecific distances, only 33% were equal to 0. The mean largest intraspecific distance for *rbcLa* was 0.087%, and the mean smallest interspecific distance was 0.38%. For *matK* 42% of the largest intraspecific distances were equal to 0, and 20% of the smallest interspecific distances were = 0. The mean largest intraspecific distance for the *matK* data was 0.27%, and the mean smallest interspecific distance was 0.98%. For the combined data, 41% of the largest intraspecific distances were equal to 0, and only 10% of the smallest interspecific distances were = 0. The mean largest intraspecific distance for the combined data was 0.15%, and the mean smallest interspecific distance was 0.85% (Table 4.2). The mean smallest interspecific distances are likely to be slightly higher in the *matK* dataset than in the combined dataset because very short uninformative sequences were removed from the *matK* dataset, but in the combined dataset, R found all data informative enough to be included, therefore these very short *matK* sequences were included.

For all three datasets, few individuals had largest intraspecific distances > 1% K2P distance (which is the threshold for BOLD animal species identification (Ratnasingham & Hebert 2007), with values of 1% (lowest variation), 6% and 2% for *rbcLa*, *matK* and the combined datasets, respectively (Table 4.2). This means that some individuals will not have a species match based on intraspecific distances falling within the default BOLD threshold for the Threshold ID and Best Close Match criteria. In comparison, the proportion of smallest interspecific distances > 1% K2P distance was greater for all three datasets, with 10%, 39%, and 34% for *rbcLa*, *matK* and the combined datasets, respectively (Table 4.2). The fact that more than 50% of all individuals in all three datasets have smallest interspecific distances < 1% K2P distance is not necessarily of concern, as thresholds can be optimised in SPIDER for each dataset (Table 4.3) and the optimised threshold is considerably lower than 1% for all datasets tested.

Taxa are defined as having no barcode gap in SPIDER if the largest intraspecific distance within a species subtracted from the smallest interspecific distance from the closest con-specific is equal to zero. If the value is less than zero, a reverse barcode gap exists, where an individual is found to be more similar to the nearest con-specific than to members of the same species.

40% of individuals in the *rbcLa* data frame have no barcode gap, making it the worst performing barcode locus in terms of barcode gap. For *matK* with no singletons, 32% of individuals have no barcode gap, and the combined data frame contains only 19% of individuals with no barcode gap, making it the best performing barcode in terms of presence of a barcode gap (Table 4.2). p-values < 0.05 were calculated for all three datasets in the Wilcoxon signed rank test (Table 4.2), indicating that overall interspecific distances are significantly greater than intraspecific distances for all three datasets.

Figures 4.2 and 4.3 are visual representations of the barcoding gaps in the three datasets without singletons. Figure 4.2 shows line graphs of the barcoding gap in each individual dataset. Blue lines indicate that a barcoding gap is present, and yellow lines indicate that a reverse gap is present i.e. the largest intraspecific distance is greater than the smallest interspecific distance. In Fig. 4.2 B (*matK* dataset), two very long yellow lines are present, indicating very large reverse barcoding gaps. These two samples may have been incorrectly identified, which would result in a large intraspecific value and the small or non-existent interspecific gap if the individual was matching another species present in the dataset.

Figure 4.3 shows box-plots of the smallest interspecific and largest intraspecific distances for the three datasets. In each case, the median interspecific distance and the range of interspecific distances are greater than the median and range of intraspecific distances.

Table 4.2 Genetic divergence and barcode gap statistics, no-singleton data only.

Statistic	*matK*	*rbcLa*	Combined
Prop. largest intraspecific dist. >1%	6%	1%	2%
Prop. smallest interspecific dist. >1%	39%	10%	34%
Prop. largest intraspecific dist. = 0	42%	76%	41%
Prop. smallest interspecific dist. = 0	20%	33%	10%
Mean largest (%) intraspecific dist. (range)	0.27 (0 – 9.48)	0.09 (0 - 1.89)	0.15 (0 - 1.65)
Mean smallest (%) interspecific dist. (range)	0.98 (0 - 8.08)	0.38 (0 - 3.91)	0.85 (0 - 6.25)
% individuals with no barcode gap	32	40	19
Wilcoxon signed rank test	V = 105966.5, $p < 2.2e-16$	V = 49323, $p < 2.2e-16$	V = 85936, $p < 2.2e-16$

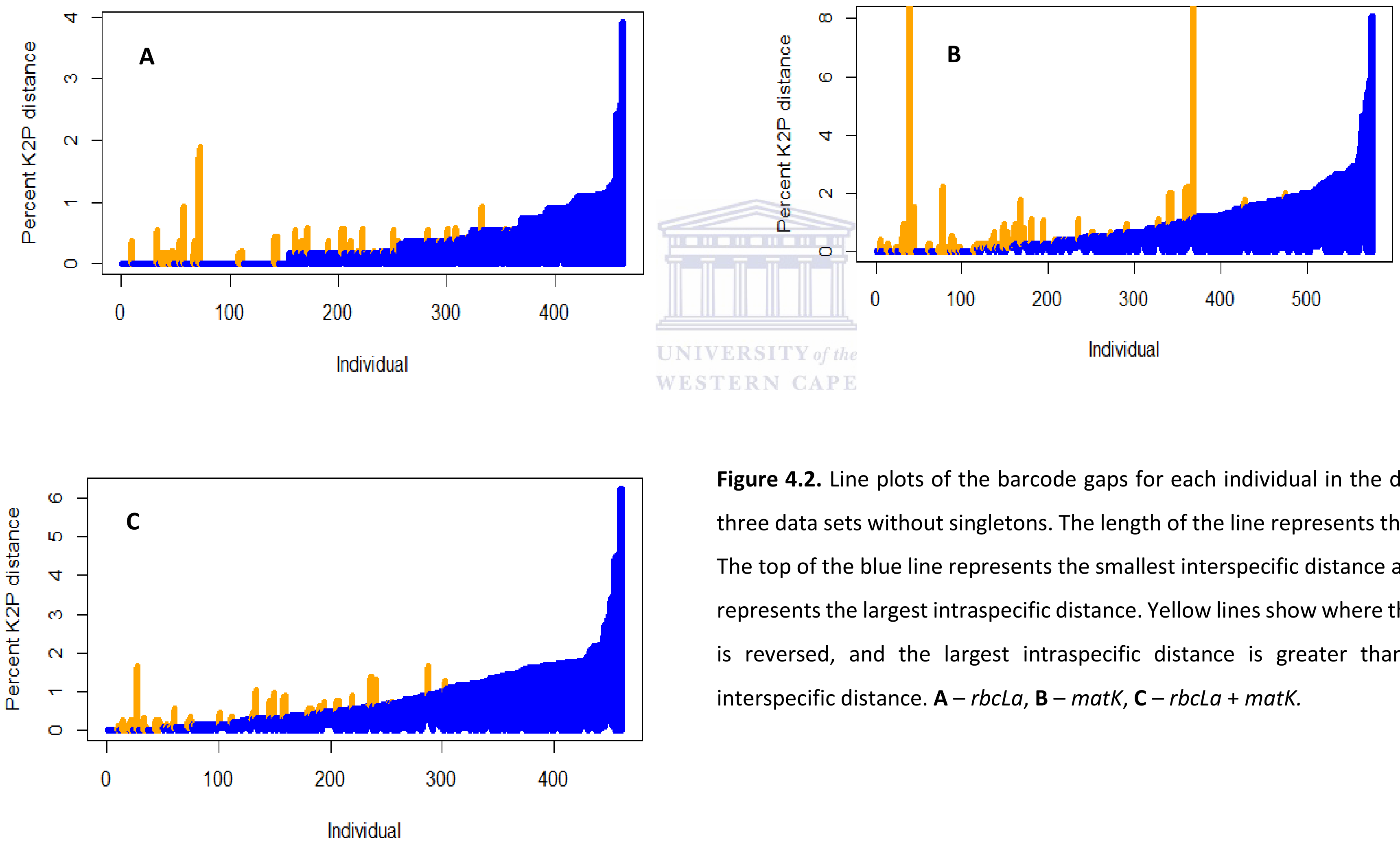


Figure 4.2. Line plots of the barcode gaps for each individual in the data set for the three data sets without singletons. The length of the line represents the barcode gap. The top of the blue line represents the smallest interspecific distance and the bottom represents the largest intraspecific distance. Yellow lines show where the barcode gap is reversed, and the largest intraspecific distance is greater than the smallest interspecific distance. **A** – *rbcLa*, **B** – *matK*, **C** – *rbcLa* + *matK.*

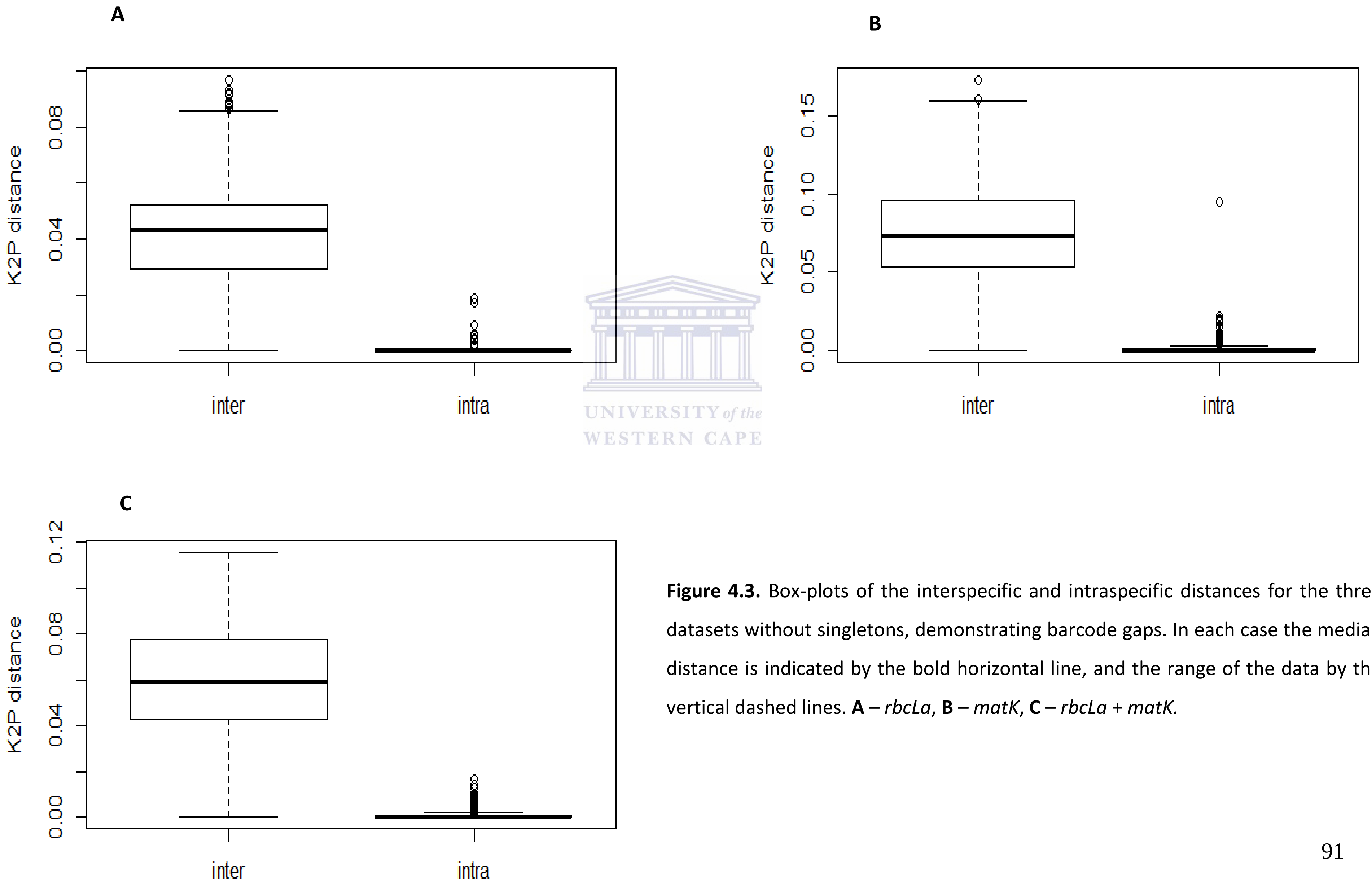


Figure 4.3. Box-plots of the interspecific and intraspecific distances for the three datasets without singletons, demonstrating barcode gaps. In each case the median distance is indicated by the bold horizontal line, and the range of the data by the vertical dashed lines. **A** – *rbcLa*, **B** – *matK*, **C** – *rbcLa* + *matK*.

4.2.2.3 Distance-based measures for evaluation of species identification success-rates

For all three data sets, analyses were carried out on data both with and without singletons included, but again, only the results of the data without singletons is reported, except where stated.

Of the three distance-based methods tested on the Poaceae data, the Nearest Neighbour criterion (k-NN), which finds the closest individual or species index to each target, gave the best overall species identification results for all three data partitions. It also performed better than the tree-based methods of identification. 76%, 80%, and 91% correct identifications were made for *rbcLa*, *matK* and the *matK+rbcLa* data sets without singletons, respectively (Table 4.4). For the k-NN criterion, if a target is matched with its own species index, it will give a result of TRUE in SPIDER, and if it is matched with a different species index it will give a FALSE result (Table 4.6). Singletons should then always give a FALSE result, and this will reduce the rate of successful identifications and negatively affect the impression of a dataset being useful for DNA barcoding. This can be seen in the results of the three datasets when singletons are included, where only 52%, 55%, and 59% correct identifications were made for *rbcLa*, *matK* and the *matK+rbcLa* data sets, respectively (Table 4.4 and Table 4.6).

Meier's Best Close Match (BCM) criterion was the next best performing criterion of the three distance-based measures for the Poaceae datasets, with 63%, 74% and 86% correct identifications for the *rbcLa*, *matK* and the *matK+rbcLa* data sets without singletons, respectively, with the distance threshold set at the default of 1% (Table 4.4).

The BCM criterion finds the closest species index to each target (similarly to k-NN) but only from within the set distance threshold (default of 1%). If there is an equally close match with more than one species, an "ambiguous" result is given. If there is no closest species index within the threshold then a "No ID" result is given (see Table 4.6 for detailed results for each of these categories).

Optimisation of the distance threshold in SPIDER using the ThreshOpt function (which finds the distance threshold that produces the lowest cumulative error) actually lowered the proportion of successful species identifications for this criterion to 57%, 65%, 73% for the *rbcLa*, *matK* and the *matK+rbcLa* datasets without singletons respectively (see Table 4.3 for optimised thresholds and Table 4.4 for identification success rates).

Lowering the threshold to the optimum (that with the lowest cumulative error of false positive and false negative identifications) for each dataset without singletons (0.1% for the *rbcLa* and combined datasets and 0.2% for the *matK* data set, see Table 4.3 for optimised thresholds and Fig 4.4 for a graphical representation of the threshold optimisation for each data set) increased the number of “No ID” results (Table 4.6) with the BCM criterion. It appears that the optimised thresholds, while selecting for the overall lowest cumulative error, decreases the ability of the BCM criterion to make a correct match, as it is less likely for there to be any match at all within such a small range of genetic distances.

Table 4.3 Optimised K2P distance thresholds for the BCM and BOLD threshold ID criteria at genus and species level, using the ThreshOpt function in SPIDER.

	Singletons	***rbcLa* (%)**	***matK* (%)**	**Combined (%)**
Species level identification	**Incl.**	0.1	0.1	0.1
	Excl.	0.1	0.2	0.1
Genus level identification	**Incl.**	0.1	0.2	0.4
	Excl.	0.1	0.4	0.4

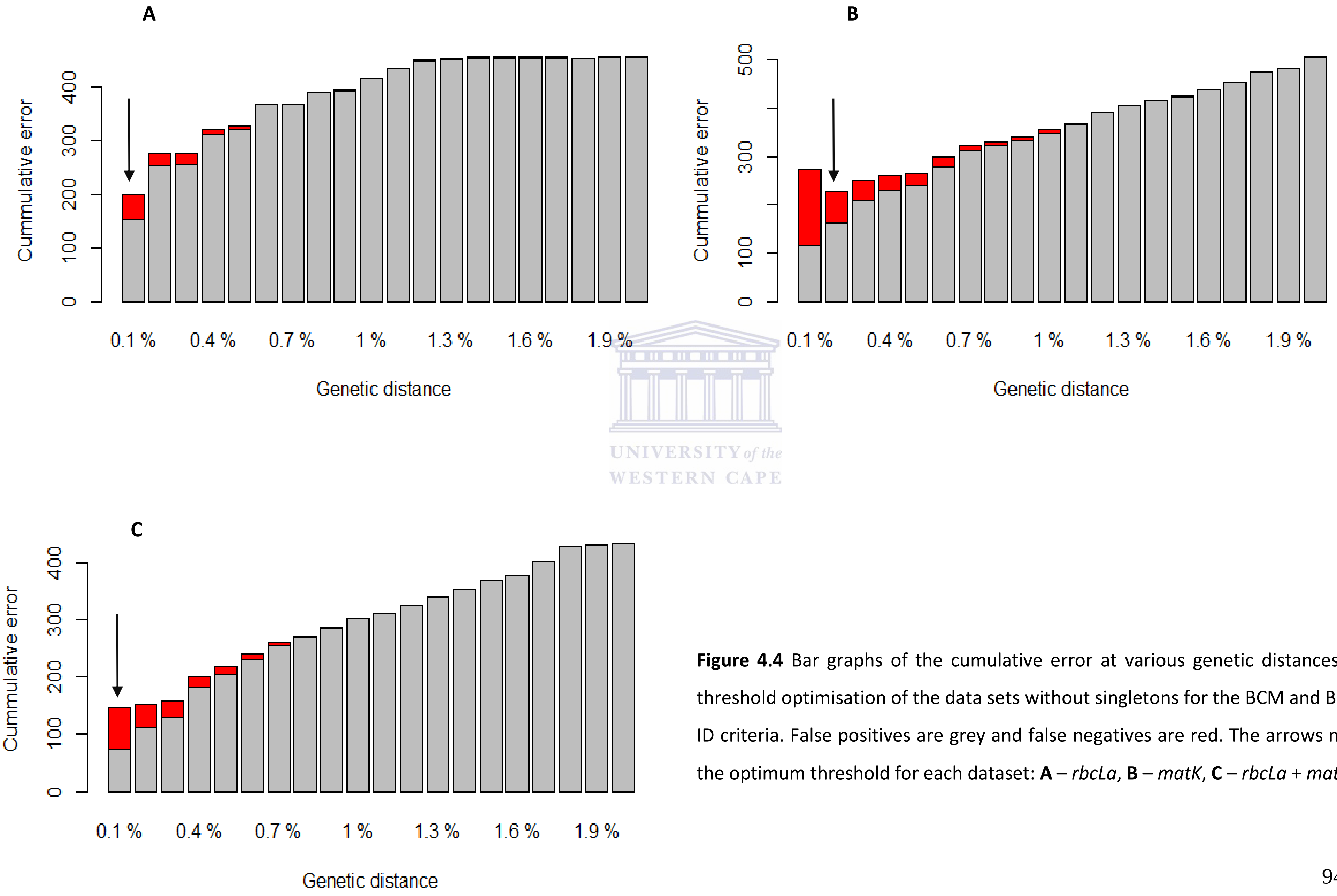


Figure 4.4 Bar graphs of the cumulative error at various genetic distances for threshold optimisation of the data sets without singletons for the BCM and BOLD ID criteria. False positives are grey and false negatives are red. The arrows mark the optimum threshold for each dataset: **A** – *rbcLa*, **B** – *matK*, **C** – *rbcLa* + *matK*.

The BOLD threshold identification criterion provided the worst level of correct species identifications of the distance-based methods for the Poaceae data for all three data sets. At the 1% default threshold, 10%, 38% and 34% correct identifications were made for the *rbcLa*, *matK* and the *matK+rbcLa* datasets without singletons respectively (Table 4.4 and Table 4.6).

However, optimising the threshold in SPIDER (see Table 4.3 and Fig. 4.3 for optimised thresholds for each data set) improved the proportion of correct identifications. Even at the optimised threshold, only 57%, 61%, and 68% correct identifications were achieved for *rbcLa*, *matK* and *matK+rbcLa* datasets without singletons respectively (Table 4.4). Similarly to the BCM criterion, the addition of singletons in the data greatly increased the number of incorrect identifications made by the BOLD threshold ID criterion (Tables 4.4 and 4.6), as any matches made within the threshold will be incorrect for singletons.

Table 4.4 Identification success rates for each of the three distance-based measures of species identification success for the three data sets, with and without singletons.

		Species level identification			Genus level identification		
Measure	**Singletons**	***rbcLa* (%)**	***matK* (%)**	**Combined (%)**	***rbcLa* (%)**	***matK* (%)**	**Combined (%)**
k-NN	**incl.**	52	56	59	86	89	90
	excl.	76	84	91	90	98	97
BOLD: 1% thresh.	**incl.**	2.9	17	15	20	52	55
	excl.	10	38	34	19	72	66
BOLD: opt. thresh.	**incl.**	25	32	58	62	64	69
	excl.	57	61	68	77	82	83
BCM: 1% thresh.	**incl.**	28	44	52	75	82	88
	excl.	63	74	86	85	94	97
BCM: opt. thresh.	**incl.**	25	32	44	62	67	82
	excl.	57	65	73	77	90	93

Abbreviations: BCM = best close match; excl. = excluded; incl. = included; k-NN = nearest neighbour; opt. = optimum; thresh. = threshold

4.2.2.4 Tree-based measures of DNA barcode identification success

The species monophyly criterion in SPIDER measures whether each species is monophyletic over a neighbour-joining (NJ) tree. For the Poaceae data, 60%, 62% and 75% of species were found to be monophyletic for *rbcLa*, *matK* and *matK+rbcLa* datasets without singletons, respectively (Table 4.5). This criterion gives more correct identifications than the BOLD threshold ID criterion, but does not perform as well as the k-NN and the BCM criteria (at default 1% threshold for BCM).

When singletons are included in the data, they are assumed by SPIDER to be either always monophyletic (setting = TRUE) or never monophyletic (setting = FALSE), depending on the criteria chosen (although neither of these assumptions are always true in reality). As the datasets with singletons included have many singletons, there would be a significant quantity of incorrect identities made if singletons were assumed to be not monophyletic, thus for this data singletons were assumed to be always monophyletic (setting = TRUE). 79%, 82% and 87% of species were found to be monophyletic for the *rbcLa*, *matK* and *matK+rbcLa* data sets with singletons, with singletons assumed to be monophyletic (Table 4.5). This is an increase over the datasets without singletons that is due entirely to the assumption that singletons are always monophyletic. The data is better tested for species monophyly without the singletons included.

While it is possible to calculate bootstrap support for the NJ trees as a more accurate measure of species monophyly, the large quantity of data and the intensive computational requirements of the bootstrap calculations caused R to crash on numerous occasions when processing some of the data sets. No bootstrap calculation could be performed for the *matK* data, and so the results of the bootstrap calculations are not reported here. The bootstrap results for the *rbcLa* and combined data sets are listed in Table 4.6.

Rosenberg's probability of reciprocal monophyly tests whether monophyly "is a chance outcome of random branching" or not across the NJ tree (Rosenberg 2007). For this study, all nodes significant to $\alpha = 0.05$ according to Rosenberg's random model were considered significantly monophyletic. Only 19%, 19% and 21% of the nodes in the NJ tree for *rbcLa*, *matK* and *matK+rbcLa* data sets, respectively (Table 4.5 and Table 4.6) were found to be significantly monophyletic, which means that the majority of nodes that were resolved as monophyletic in the NJ trees were done so by chance.

Table 4.5 Identification success rates the tree-based measures of species and genus identification success for the three data sets, with and without singletons. Singletons are assumed to be monophyletic for these results.

		Species identification success			**Genus identification success**		
Measure	**Singletons**	***rbcLa* (%)**	***matK* (%)**	**Combined (%)**	***rbcLa* (%)**	***matK* (%)**	**Combined (%)**
NJ mono	**incl.**	79	82	87	63	70	75
	excl.	60	65	75	66	82	85
Rosenberg	**incl.**	20	20	21	-	-	-
	excl.	19	19	21	-	-	-

Abbreviations: mono = monophyly; NJ = neighbour joining; Rosenberg = Rosenberg's probability of reciprocal monophyly, significant to α = 0.05.

4.2.2.5 Evaluation of genus-level identification success rates

Identification success rates were also measured at genus level, using the three distance-based measures and the NJ mono tree-based measure. Genus-level identification will be useful if a naturalised species cannot be correctly identified at species level, but happens to belong to genus for which all species are naturalised. Examples of such naturalised genera in South Africa that are represented in these data sets are *Avena* L., *Briza* L., *Lolium* L., *Paspalum* L., *Polypogon* Desf. and *Puccinellia* Parl.

The Nearest Neighbour criterion was again the most successful for genus-level identification, with 90%, 98% and 97% identification success for the *rbcLa*, *matK* and *matK*+*rbcLa* datasets without singletons respectively (Table 4.4 and Table 4.6).

Similarly to the species-level results, the second best performing metric was the BCM at the default threshold of 1%, with 85%, 94% and 97% identification accuracy for the *rbcLa*, *matK* and *matK*+*rbcLa* datasets without singletons, respectively (Table 4.4 and Table 4.6). Again, optimising the threshold actually decreased the performance of this metric at genus level.

The NJ mono tree-based measure of monophyly, which in this case measures the number of genera that are monophyletic, performed roughly as well as the BOLD ID metric at the optimised threshold. The BOLD ID metric was the worst-performing metric in the species-level analysis. 66%, 82% and 85% genus-level identification accuracy levels were obtained for the *rbcLa*, *matK* and *matK*+*rbcLa* datasets without singletons respectively using the NJ mono criterion (see Table 4.5.), and 77%, 82%, and 83% were obtained for the BOLD ID criterion at the optimised thresholds of 0.1%, 0.4% and 0.4% for the *rbcLa*, *matK* and *matK*+*rbcLa* datasets without singletons, respectively (see Table 4.4. for identification success rates, and Table 4.3. for optimised thresholds).

Table 4.6 Detailed species identification success rates for all parameters tested for the three datasets (*rbcLa*, *matK* and combined) with and without singletons.

		Near Neighbour (k-NN)				
		Species level identification			**Genus level identification**	
Locus	**Singletons**	**False**	**True**	**Total no. specimens**	**False**	**True**
rbcLa	**Incl.**	352	322	674	97	576
	Excl.	109	354	463	46	417
matK	**Incl.**	339	440	779	86	693
	Excl.	95	481	576	14	562
Combined	**Incl.**	276	398	674	68	606
	Excl.	40	421	461	12	449

		BOLD ID 1% threshold								
		Species level identification					**Genus level identification**			
Locus	**Singletons**	**Ambiguous**	**Correct**	**Incorrect**	**No ID**	**Total no. specimens**	**Ambiguous**	**Correct**	**Incorrect**	**No ID**
rbcLa	**Incl.**	431	20	214	9	674	502	134	28	9
	Excl.	416	46	0	1	463	372	90	0	1
matK	**Incl.**	402	132	197	48	779	292	405	34	48
	Excl.	345	221	4	6	576	155	414	1	6
Combined	**Incl.**	347	103	195	29	674	241	373	31	29
	Excl.	359	100	0	2	461	155	306	0	0

BOLD ID optimised threshold

		Species level identification					**Genus level identification**			
Locus	**Singletons**	**Ambiguous**	**Correct**	**Incorrect**	**No ID**	**Total no. specimens**	**Ambiguous**	**Correct**	**Incorrect**	**No ID**
rbcLa	**Incl.**	228	171	144	131	674	104	420	15	134
	Excl.	144	263	10	46	463	58	357	2	46
matK	**Incl.**	136	250	75	318	779	65	499	24	191
	Excl.	148	349	13	66	576	70	475	2	29
Combined	**Incl.**	104	268	88	214	674	81	465	16	112
	Excl.	66	313	8	74	461	59	383	1	18

BCM 1% threshold

		Species level identification					**Genus level identification**			
Locus	**Singletons**	**Ambiguous**	**Correct**	**Incorrect**	**No ID**	**Total no. specimens**	**Ambiguous**	**Correct**	**Incorrect**	**No ID**
rbcLa	**Incl.**	233	189	243	9	674	112	505	47	9
	Excl.	147	290	25	1	463	62	392	8	1
matK	**Incl.**	145	346	240	48	779	43	636	52	48
	Excl.	105	425	40	6	576	22	544	4	6
Combined	**Incl.**	68	348	229	29	674	9	592	44	29
	Excl.	40	395	26	0	461	7	449	5	0

BCM optimised threshold										
		Species level identification					Genus level identification			
Locus	**Singletons**	**Ambiguous**	**Correct**	**Incorrect**	**No ID**	**Total no. specimens**	**Ambiguous**	**Correct**	**Incorrect**	**No ID**
rbcLa	**Incl.**	228	171	144	131	674	104	420	15	134
	Excl.	144	263	10	46	463	58	357	2	46
matK	**Incl.**	136	250	75	318	779	42	520	26	191
	Excl.	103	375	32	66	576	22	521	4	29
Combined	**Incl.**	66	299	95	214	674	8	533	21	112
	Excl.	38	335	14	74	461	6	432	5	18

NJ Mono (singletons assumed to be monophyletic)						
		Species level identification			Genus level identification	
Locus	**Singletons**	**False**	**True**	**Total no. species**	**False**	**True**
rbcLa	**Incl.**	79	294	373	46	77
	Excl.	62	93	155	29	56
matK	**Incl.**	77	338	415	43	98
	Excl.	66	121	187	18	82
Combined	**Incl.**	48	326	374	31	91
	Excl.	39	115	154	13	72

NJ Mono (singletons assumed to be not monophyletic)

		Species level identification			Genus level identification	
Locus	**Singletons**	**False**	**True**	**Total no. species**	**False**	**True**
rbcLa	**Incl.**	300	73	373	77	46
matK	**Incl.**	313	102	415	79	62
Combined	**Incl.**	270	104	374	63	60

Rosenberg's probability of reciprocal monophyly

		Species level identification			Genus level identification	
Locus	**Singletons**	**Significant to α = 0.05**	**not significant**	**Number of nodes**	**Significant to α = 0.05**	**not significant**
rbcLa	**Incl.**	135	537	672	-	-
	Excl.	86	376	462	-	-
matK	**Incl.**	152	626	778	-	-
	Excl.	112	463	575	-	-
Combined	**Incl.**	137	536	663	-	-
	Excl.	98	362	460	-	-

NJ Monoboot						
		Species level identification			**Genus level identification**	
Locus	**Singletons**	**False**	**True**	**Total no. specimens**	**False**	**True**
rbcLa	**Excl.**	354	109	463	-	-
matK	**Excl.**	-	-	-	-	-
Combined	**Excl.**	357	104	461	-	-

Abbreviations: BCM = best close match; boot = bootstrap (>70%); excl. = excluded; incl. = included; k-NN = nearest neighbour; mono = monophyly; NJ = neighbour joining; opt. = optimum; Rosenberg = Rosenberg's probability of reciprocal monophyly, significant to α = 0.05; thresh. = threshold.

4.3 Discussion

4.3.1 PCR success rates

In this study, various obstacles were faced that prevented a full DNA barcode database representing all naturalised grasses in South Africa from being assembled. The available herbarium specimens were in some cases very old and produced degraded DNA which did not produce a PCR product with the primers commonly used for DNA barcoding. Fresh samples will produce better quality DNA for analysis. The commonly used *matK* primers were also not specific enough to successfully PCR the specimens in this study, and this was overcome by the selection of more specific primers. In a few cases, a herbarium specimen's DNA sequences matched a different grass than that as which it was identified (using BLAST and BOLD methods of matching closely related sequences), and this may indicate an issue of incorrect identification in some cases, or one of PCR contamination.

Another issue was the presence of polyphenolic compounds in many grass specimens, which are known to inhibit PCR. Based on preliminary tests in this study and on results of other studies (Koonjul *et al.* 1999, Nunes *et al.* 2011) the addition of PVP to the DNA extraction protocol or to the PCR protocol may overcome this obstacle (Figure 4.1). As most of the specimens collected for this study were sampled between 1990 – 2012, it is surmised that the majority of the PCR failures experienced were due to the presence of polyphenolic compounds rather than old, fragmented DNA.

Due to the difficulties experienced and the low PCR success rates achieved with both the *rbcLa* and *matK* PCRs due to the above-mentioned obstacles, it is not possible to make any conclusions about the ease of use and quality of sequences produced by the primers for the two loci used in this study, until the correct measures have been taken to overcome these obstacles.

4.3.2 The barcode gap and genetic divergence

As mentioned in the results, the inclusion of singletons in DNA barcoding data tends to lend to an underestimation of the performance of the data, when it is being tested for identification efficacy. All three distance-based methods of measuring identification success treat each individual sequence as a query and match it against the rest of the sequences in the database. A singleton will have no match to its own species, and so an accurate identification cannot be made. For the sake of testing the identification potential of the three barcoding data sets, singletons were removed in this study, and although the data both with and without singletons were tested, only the results for the data without singletons were considered important. However, these singletons are important to improve species representation in the data sets and should definitely be included in the grass DNA barcode database for South Africa, as this will increase the chances that an accurate match will be made to an unknown grass in the future.

When each data set is considered as a whole, as represented in Fig. 4.3 A-C, there is an overall barcode gap present in all three loci or sets of loci tested (*rbcLa*, *matK* and the combined data set). However, when each individual is considered within a data set (as seen in Fig. 4.2 and Table 4.2 under the percentage of individuals with no barcode gap) there are individuals within each data set for which no barcode gap or an inverse gap exists. This means that some species will potentially not be correctly identified based on the differences between intra- and interspecific distances. The *rbcLa* + *matK* combined data set has 19% of individuals with no barcode gap, which means that theoretically 81% (Table 4.2) of the individuals within the data set would be correctly identified based on the existence of a barcode gap.

4.3.3 Performance of the tested markers as DNA barcodes

In terms of identification success based on all of the metrics tested as well as on the presence of a barcode gap, *rbcLa* is always the worst performing locus (Tables 4.2, 4.4 and 4.6), as was expected, based on the results of previous studies (De Vere *et al.* 2012 , Gere *et al.* 2013, Hoveka

2014, Kabongo 2014). Only 60% of individuals in the *rbcLa* data without singletons had a barcode gap, and the highest identification success rate was 79% for the NJ species monophyly criterion. The lowest success rate for *rbcLa* without singletons was 10% with the BOLD ID metric at 1% default threshold.

matK performs considerably better than *rbcLa* as a single locus barcode, with a highest successful identification rate of 84% for the k-NN metric in the data without singletons (Table 4.4), although only 68% of individuals in this *matK* data set were found to have a measurable barcode gap (Table 4.2). In one instance, using the BOLD ID metric with 1% default threshold, *matK* identification success (38%) actually outperformed that of the combined dataset (34%) (Table 4.4). It is possible that this result is due to the fact that SPIDER could not use some of the very short partial *matK* fragments and so they were removed from the data, but were not removed from the combined data as they were informative enough when combined with *rbcLa* data to be analysed (Table 2.4). The individuals with the most uninformative sequences have been filtered out of the *matK* data.

The combined *rbcLa* + *matK* dataset is the best-performing barcode of the three overall. In every case other than the BOLD ID with 1% threshold (as mentioned above) and the bootstrap tree based analysis (Table 4.5) it gave the best identification results, with a highest success rate of 91% without singletons for the k-NN metric (Table 4.4). 81% of individuals in the combined data without singletons were found to have a barcode gap greater than 0 (Table 4.2).

Based on the results of this study, the recommended barcode for grasses in southern Africa and for naturalised grasses in South Africa would be *rbcLa* + *matK*, of the three loci or combinations tested. However, in other studies, the plastid intergenic spacer *trnH-psbA* has been found to be more variable and a better individual DNA barcode than *matK* (Kress & Erickson 2007, CBOL 2009, Hoveka 2014). A single marker would reduce the time and costs involved in sequencing two regions, and *trnH-psbA* reportedly has a higher PCR success rate than *matK* (Kress & Erickson 2007, CBOL 2009, Gere *et al.* 2013, Hoveka 2014). Hoveka (2014) achieved a 100% identification success rate with *trnH-psbA* as a single marker, in a group of invasive aquatic plants, although as the species used in that study were from different plant families and were not as closely related as the grasses in this study (all from family Poaceae), such a high success rate is not necessarily to be expected in the data used in this study. It could be worth investigating this marker in the future for the identification of grasses in southern Africa, and finding out if more individuals would have a barcode gap and could be identified using this marker either on its own or in combination with the other two.

4.3.4 Performance of the metrics used to test the data for identification efficacy

The Nearest Neighbour criterion (k-NN) gave the best identification accuracy results of all the metrics tested, both the distances-based and tree-based metrics. 91% identification accuracy was achieved for the *rbcLa* + *matK* data set (Table 4.4). This metric is not currently used in making actual identifications, it is only used by SPIDER as a means to assess a data set.

The second-best performing distance-based measure of identification success was the Best Close Match, which performs in a similar manner to the k-NN, but within a threshold distance. Optimisation of the threshold for each data set actually decreased identification success for the three data sets for this metric, so for family Poaceae it seems that the 1% default threshold is optimal for the BCM metric.

The BOLD threshold identification criterion performs worse than any of the other distance-based methods and worse than the tree-based NJ species monophyly criterion. This metric is similar to that used to make identifications on the BOLD database for animal species. The highest identification success rate for this metric was with the optimised threshold of 0.1% for the combined data set without singletons; 68% of individuals were correctly identified (Table 4.4). The BOLD database currently uses a BLAST alignment search to make identifications with plant sequences, so it is difficult to predict how the Poaceae data from southern Africa will perform with actual identifications until tested further.

The tree-based NJ species monophyly criterion has commonly been used for DNA barcoding, but its usage has caused some controversy (Collins & Cruikshank 2013). In this study it was the second-best performing metric overall, with a highest identification success rate of 87% for the combined data set without singletons (Table 4.5). But when stringency is imposed on the tree topology with the Rosenberg's probability of reciprocal monophyly metric, the identification success rates decrease dramatically (Table 4.5).

Although good identification success rates were achieved with the NJ species monophyly criterion in this study, the use of a neighbour-joining tree in DNA barcoding has been criticised, as this type of tree has been shown to perform poorly in species identification, and is also not a favoured tree-drawing method for phylogenetic studies (Will & Rubinoff 2004, Meier *et al.* 2008). Tree-based methods of identification in general are not able to provide a "no identification" result, and position in a clade is not always sufficient information to assign a specimen to a particular species. While tree based methods are useful for providing a visual representation of the clade in which an unknown specimen lies and can give an idea of the genus and sister species, some researchers feel that it should not be relied upon as the sole method of species identification in a DNA barcoding database (Collins & Cruikshank 2013).

It has in fact been argued that the use of a K2P distance matrix (which forms the base of all the distance-based methods of species identification and the NJ species monophyly criterion in this and many other DNA barcoding studies and is used in the BOLD database) is not the best measure for barcoding closely related species with small interspecific distances, and that uncorrected p-distances are recommended instead (Srivathsan & Meier 2012). Future work on this DNA barcoding database for Poaceae in southern Africa could include an investigation into the use of uncorrected p-distances in identification of grass species.

4.3.5 Genus-level identification success

Genus-level identification success rates were high for the Poaceae data tested in this study, with correct identifications of up to 97% for the combined *rbcLa* + *matK* data without singletons using the k-NN and the BCM metrics (1% threshold, see Table 4.4). Even the *rbcLa* data had high success rates of up to 90% for the k-NN metric (Table 4.4). Although correct identification at species level is preferred over an identification to genus level only, some genera contain very closely related species that were not distinguishable at species level. For example in this study, SPIDER was unable to distinguish between some *Avena* and *Lolium* species, even using the most successful k-NN metric with the most successful data set, the *rbcLa* + *matK* data without singletons. However when these were assessed at genus level, all *Avena* and *Lolium* species were assigned to the correct genus (see Chapter 5 for phylogenetic trees including these genera). As both of these genera are alien to South Africa as a whole, a genus-level identification would suffice for these species in order to know that an alien grass is present.

Even at genus level, 100% identification accuracy was not achieved for these data sets, and it is suspected that in some cases at least, incorrect identification may play a role. It is therefore vital that accurate taxonomic identification works hand in hand with DNA barcode database assembly. The BOLD database aims to link these two important aspects of species identification by including scans of each herbarium specimen from which DNA was extracted, so that identification can be checked if needed.

Chapter 5. Phylogeny of family Poaceae in southern Africa, based on the two DNA-barcoding markers

5.1 Introduction

Grasses are one of the most important plant families for human economy and agriculture. Poeaceae is the fifth-largest of all plant families (Clayton & Renvoize 1986). As they are such an important group, the Poaceae family tree has been fairly well-studied, and most major groupings are well-resolved. There are 12 monophyletic sub-families which form three major groupings, the early-diverging grass lineages (sub-families Anomochlooideae, Pharoideae, and Puelioideae), the BEP clade (sub-families Bambusoideae, Ehrhartoideae and Pooideae) and the most recently-diverged PACMAD clade (sub-families Panicoideae, Aristidoideae, Chloridoideae, Micrairoideae, Arundinoideae, and Danthonioideae). While many grass tribes have been well-resolved, some require more in-depth sampling and further examination and there are many unresolved grass genera that require further study (Bouchenak-Khelladi *et al.* 2008).

Tree-based species monophyly is currently a method used for identification in DNA barcoding, although there is some criticism of this method (Will & Rubinoff 2004, Meier *et al.* 2008). For this reason, it is useful to test the performance of the DNA barcoding markers as phylogenetic markers. Both *rbcLa* and *matK* are plastid markers used in phylogenetics studies in plants, and have been used in grass studies, often in combination with other molecular markers and/or morphological characters. As the Poaceae family tree has already been fairly extensively characterised with more in-depth systematics studies involving multiple characters, the present study does not aim to discover new relationships within the family, but rather to compare the performance of the two DNA barcoding to those used in other studies performed on the family, and to assess the potential usefulness of this data when utilised in tree-based methods of DNA barcoding (discussed in Chapter 4) with hope that the data can be used in the identification of naturalised grasses in South Africa. It is also hoped that these data may be useful in further studies on grass tribes and genera in the future.

5.2 Results and Discussion

5.2.1 Summary statistics

Both Maximum Parsimony (MP) and Bayesian Inference (BI) trees were reconstructed for each of the three data sets, *rbcLa*, *matK* and *rbcLa + matK*. As the MP trees were generally congruent with those of the BI, especially for the *matK* and combined data sets at tribal and sub-familial levels, the BI 50% majority-rule consensus trees are presented with both posterior probabilities (PP) and MP bootstrap percentages (BP) plotted onto the branches. Where no bootstrap support is present, a hyphen (–) is indicated. Where the MP and BI trees are not congruent (more common in the *rbcLa* data), an asterisk (*) is indicated instead of BP below the branches. The MP strict consensus trees with bootstrap support are presented in Appendix 2 A-C for comparison to the BI trees.

The *rbcLa + matK* data contained the most parsimony-informative sites (596 out of 1383 included characters, 43%, Table 5.1), but the *matK* data had a higher proportion of parsimony informative sites within the 811 included characters (57% for *matK* vs 43% for the combined data). The *rbcLa* data had the smallest number of variable and parsimony-informative sites (35% variable sites and, which is to be expected, considering it is a highly conserved gene sequence). The Consistency and Retention indices (CI & RI) measuring homoplasy were fairly uniform across all three data sets, (0.331, 0.338, 0.332 CI values for *rbcLa*, *matK*, and combined data sets respectively, and 0.944, 0.930, 0.924 RI values for *rbcLa*, *matK*, and combined data sets, respectively, Table 5.1).

Table 5.1. Summary statistics estimated from maximum parsimony (MP) analyses of individual and combined marker data sets. CI = Consistency Index and RI = Retention Index.

Parameter	***rbcLa***	***matK***	***rbcLa + matK***
No. of taxa included	679	839	679
No. of included characters	555	828	1383
No. of constant characters	362	265	672
No. of variable sites	193 (35%)	546 (67%)	711 (51%)
No. of parsimony-informative sites	152 (27%)	460 (57%)	596 (43%)
No. of steps (Tree length)	786	2776	3445
No. of trees (Fitch)	270	60	2520
CI	0.331	0.338	0.332
RI	0.944	0.930	0.924
Average number of changes per variable site(number of steps/ number of variable sites)	4.1	5.1	4.8

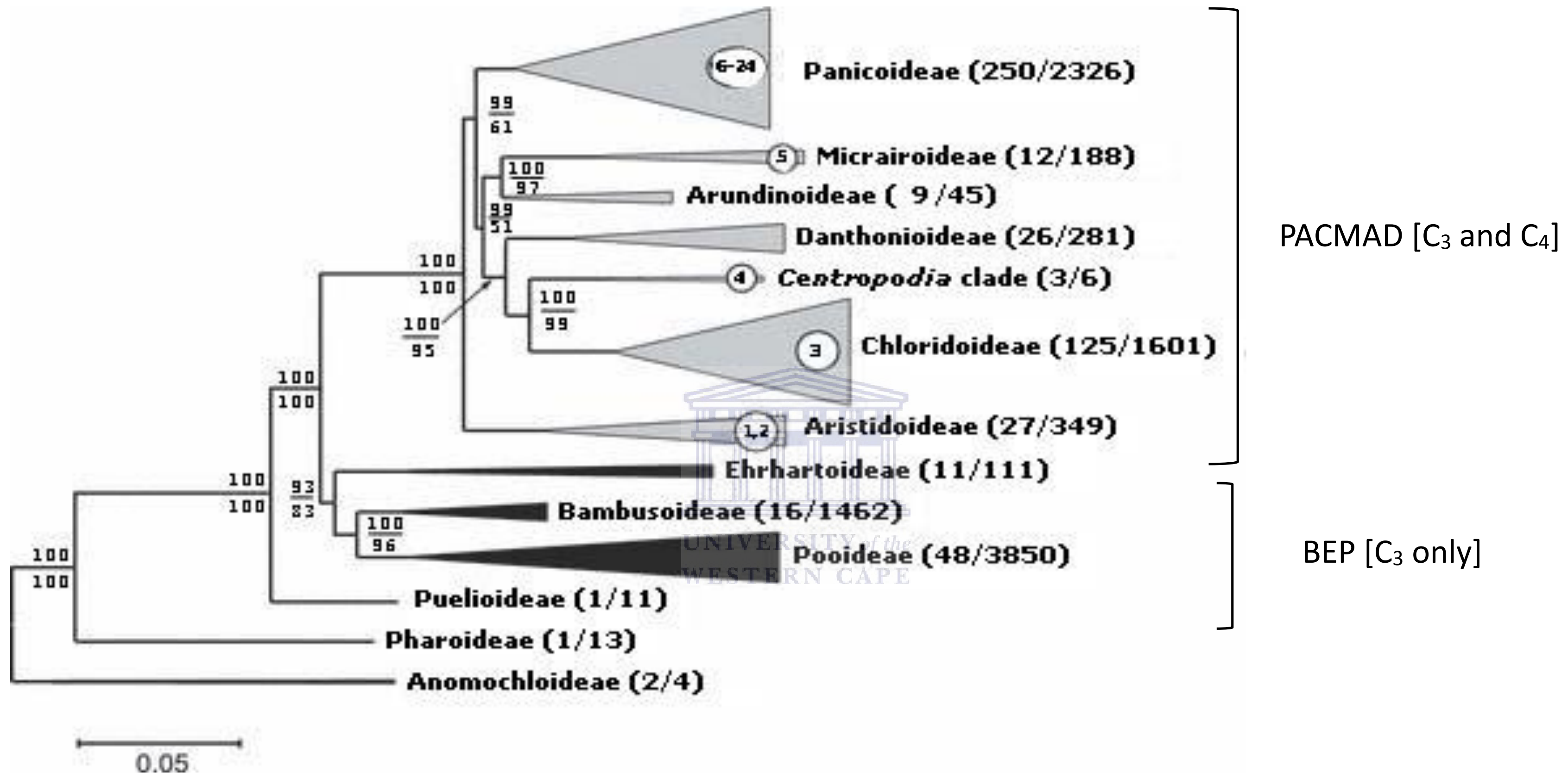


Figure 5.1 Phylogenetic tree of family Poaceae, showing the early diverging lineages Anomochloideae, Pharoideae and Puelioideae, as well as the BEP and PACMAD clades, based on Bayesian inference from *rbcL*, *ndhF* and *trnK/matK* data (from GPWG II 2012). The numbers within the sub-family clades in the PACMAD clade represent the instances of the evolution of C_4 photosynthesis. BI posterior probability percentages (PP) are above branches and Maximum Likelihood (ML) bootstrap percentages (BP) are below branches. Numbers in brackets are the number of representatives from each sub-family included in the study out of the total for that sub-family.

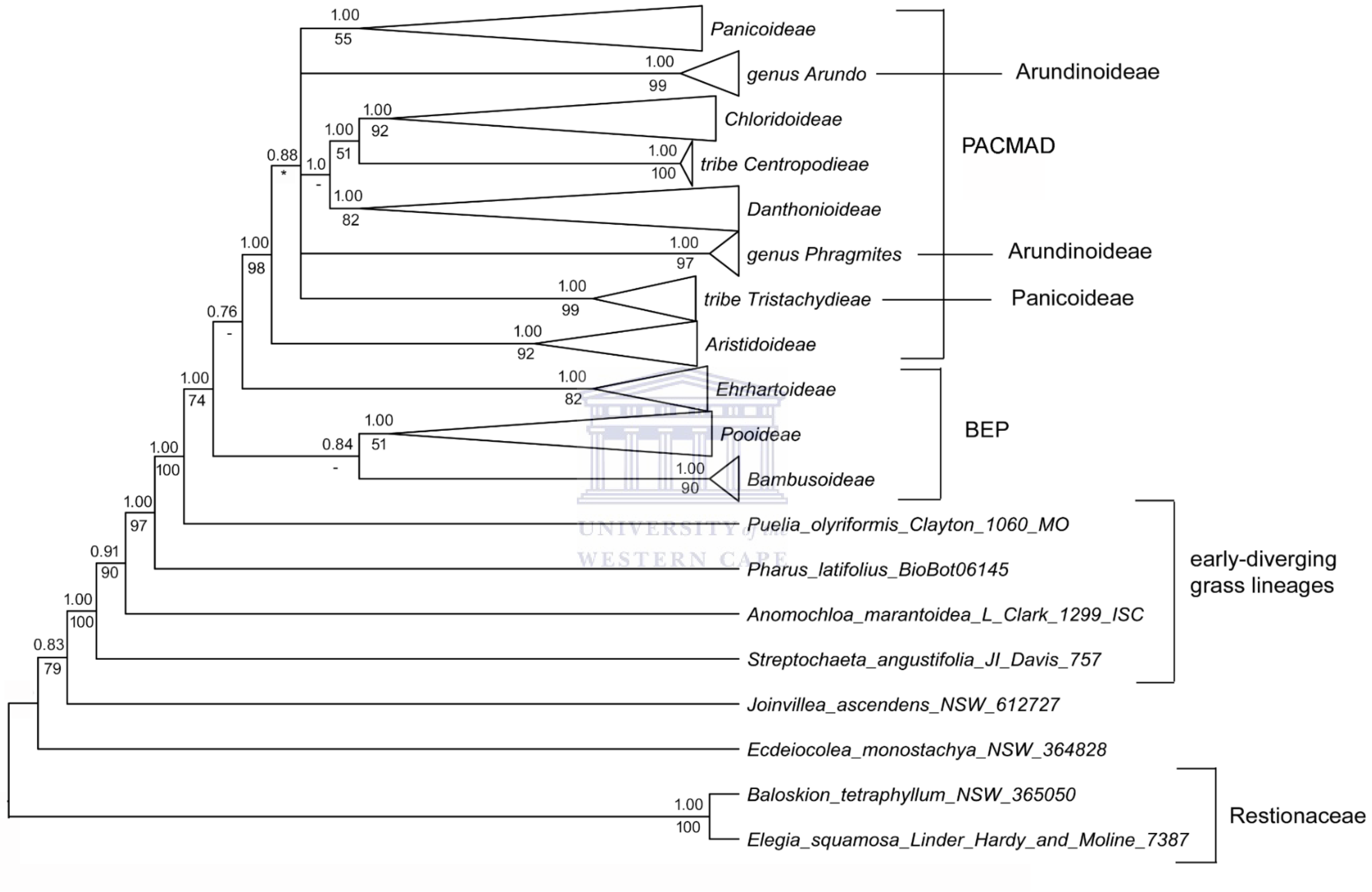


Figure 5.2 Collapsed topology of the 50% majority-rule consensus tree obtained from Bayesian inference (BI) analyses from this study based on *matK* data, showing grass sub-families and sister species. BI posterior probabilities (PP) are above branches and MP bootstrap percentages (BP) are below branches. , - indicates no MP bootstrap support, * indicates a clade is not present or resolved in the MP analysis.

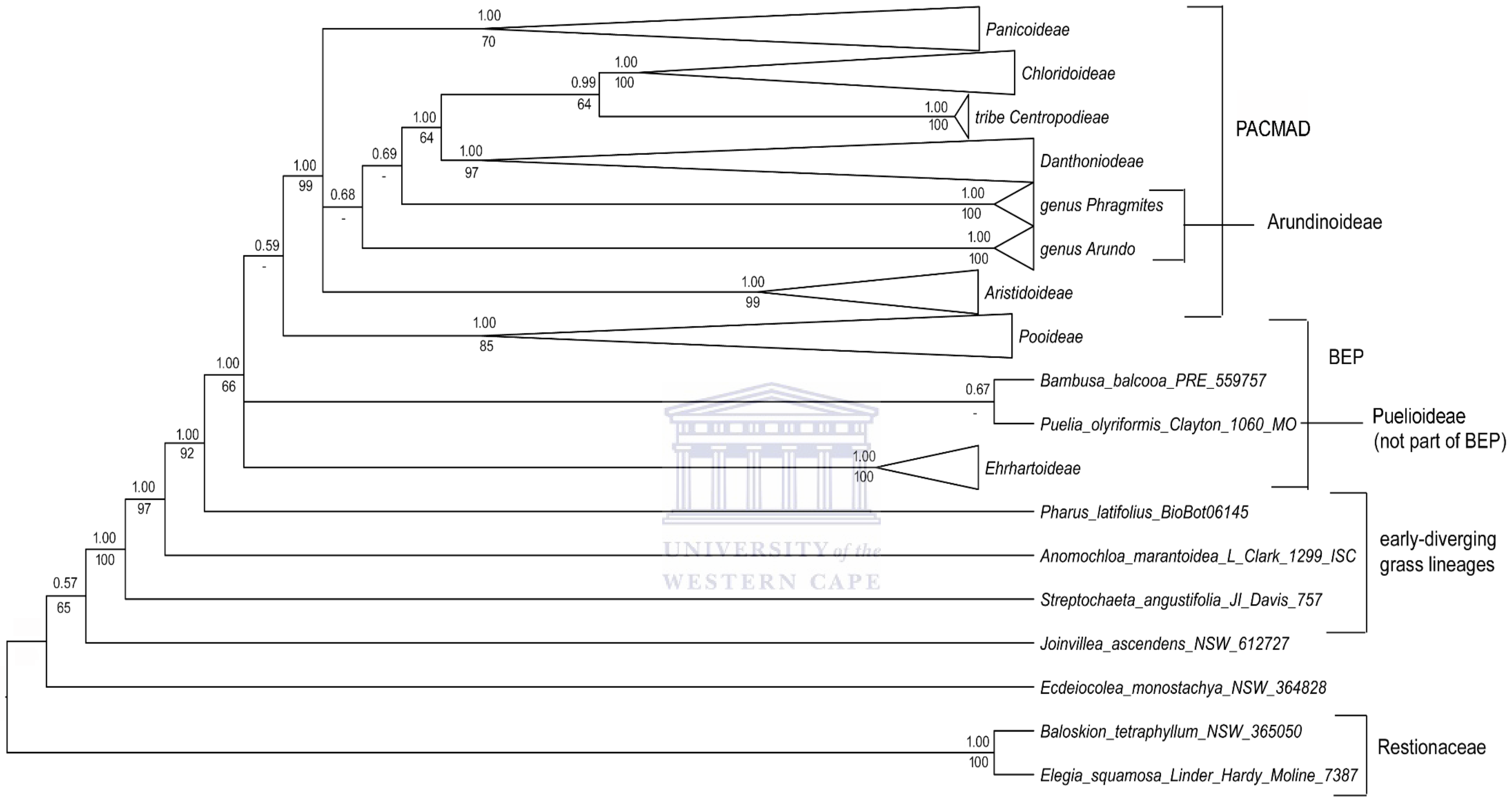


Figure 5.3 Collapsed topology of the 50% majority-rule consensus tree obtained from Bayesian inference (BI) analyses from this study based on *rbcLa* + *matK* data, showing grass sub-families and sister species. BI posterior probabilities (PP) are above branches and MP bootstrap percentages (BP) are below branches. , - indicates no MP bootstrap support.

5.2.2 *matK* and combined data trees

5.2.2.1 PACMAD clade and sub-family Aristidoideae

The PACMAD clade has strong support in the *rbcLa* + *matK* tree (Figure 5.3) with a PP value of 1.00 and a BP value of 99, and in the *matK* tree (Figure 5.2), with a PP of 1.00 and a BP value of 98. Aristidoideae, which is sister to the rest of the PACMAD clade in the recently published tree reproduced in Figure 5.1 (GPWG II 2012), is also resolved as sister to the rest of the PACMAD clade in the *matK* BI tree, although this relationship is only weakly supported with PP = 0.88 (Figure 5.2) and the relationship between Aristidoideae and the rest of the PACMAD clade is not present in the *matK* MP tree (Appendix 2B). In the *rbcLa + matK* tree (Figure 5.3) Panicoideae and Aristidoideae are sister to the rest of the PACMAD clade. Sub-family Aristidoideae itself forms a strongly supported clade in both the *matK* and the *rbcLa* + *matK* trees (PP = 1.00 for both trees and BP = 92 and 99 for *matK* and the combined data respectively). There is only one tribe in sub-family Aristidoideae, Aristideae. In this study the Aristidoideae are represented by two genera, namely *Aristida* L. and *Stipagrostis* Nees. In both the *matK* and the combined data BI trees these two genera form two separate monophyletic clades within Aristidoideae, with both clades being strongly supported (PP = 1.00, BP = 100 for both clades in both trees, see Figures 5.4 and 5.5). Species within genus *Aristida* are generally fairly well-resolved in the *matK* and combined trees, with some exceptions, but *Stipagrostis* species are less resolved, particularly in the *matK* tree where they form one large polytomy (Figure 5.4).

There are no known naturalised species of either of these two genera in South Africa, although as there are many native representatives of these genera in South Africa, it is possible that an introduced species has been missed. *Aristida* is a large genus with four centres of diversity, in Africa, Central and South America, North America and Australia. *Stipagrostis* is limited to Africa, the Middle East and central Asia (De Winter 1965). Neither are found in the Mediterranean or in northern Europe, which is where many naturalised grass species in South Africa are from, due to colonial imports for forage grasses, so it is possible that no alien species from these genera have been introduced into the country.

5.2.2.2 Sub-family Panicoideae

Panicoideae is resolved as sister to the remaining four sub-families within the PACMAD clade (Arundinoideae, Micrairoideae, Danthonioideae and Chloridoideae) in the published tree in Figure 5.1, but in this study, none of the markers or combinations used resolved the Panicoideae in this position. In the *matK* tree (Figure 5.2), the sub-family Panicoideae is not resolved, with tribe Tristachyideae (represented by the three native genera *Danthoniopsis*, *Loudetia* and *Tristachya* in this study) appearing in a separate but strongly supported clade (PP = 1.00, BP = 99). In the combined data tree, Panicoideae itself forms a single clade with moderate bootstrap support of BP = 70 and strong support on the BI tree (PP = 1.00, Figure 5.3). Tribe Tristachyideae forms a clade that is sister to the rest of the Panicoideae in the combined data tree with strong support in the BI of 1.00 PP but weak MP support of 62 BP (Figure 5.5). This is consistent with the findings of GPWG II (2012), in which a monophyletic tribe Tristachyideae (PP = 1.00, ML BP = 100) nests within a weakly supported clade of six tribes (PP = 0.53, BP = 65), many of which used to be placed in sub-family Centothecoideae (GPWG I 2001), and this clade is sister to the rest of Panicoideae (PP = 1.00 BP = 100, detailed tree in supplementary data in GPWG II 2012, data not presented in this study).

All of the tribes in sub-family Panicoideae that are represented in this study (four out of eleven) are found to be monophyletic in both the *matK* and *rbcLa* + *matK* BI trees. Tribe Paniceae is monophyletic in both BI trees with low support of PP = 0.94 and 0.88 for *matK* and *rbcLa* + *matK,* respectively (Figures 5.4 & 5.5, respectively). However, there is no support for the monophyly of tribe Paniceae in the *matK* and combined data MP trees (see Appendix 2B & C). Tribe Andropogoneae is monophyletic in both data sets with strong support (PP = 1.00 for both trees and BP = 93 and 100 for *matK* and the combined data, respectively). The two small tribes Paspaleae (represented in this study by genera *Paspalum* L., *Axonopus* (Steud.) Chase ex P.Beauv. and *Steinchisma* Raf.) and Tristachyidieae are monophyletic with strong support of PP = 1.00 and BP = 99 for the *matk* data set, and PP = 1.00 and BP = 100 for the combined data set for both tribes.

Tribe Paspaleae was previously placed in tribe Paniceae, but Paniceae was shown to be paraphyletic and was split into two separate clades with a chromosome base number of x =

10 in tribe Paspaleae, and a chromosome base number of x = 9 in tribe Paniceae (Morrone *et al.* 2012). Further studies have also resolved these two tribes (GPWG II 2012, Lizarazu *et. al* 2014). In the present study, these two separate clades are also resolved and found to be monophyletic in both the *matK* and combined data analyses, as mentioned above. Tribe Paspaleae has been found to form a clade with tribe Andropogoneae (GPWG II 2012, Morrone *et al.* 2012) and in the present study, the clade Paspaleae + Andropogoneae has been resolved as well, although it has no bootstrap support, and moderate to strong support of PP of 0.74 and PP of 0.97 in BI of the *matK* and combined data sets, respectively (Figures 5.4 and 5.5).

Across sub-family Panicoideae, genera are generally monophyletic and species well-resolved in both data sets, although with numerous exceptions. For example, there are polytomies present in genus *Digitaria* Haller, although this genus has slightly better resolution in the combined data than in the *matK* data (Figures 5.4 & 5.5). *Sorghum* Moench is paraphyletic in the *matK* tree (Figure 5.4), but in the combined data tree the genus is better resolved, forming a monophyletic clade except for the presence of a single *Imperata cylindrica* (L.) P.Beauv. specimen (Figure 5.5). *Imperata* Cirillo and *Sorghum* are closely related (GPWG II 2012, detailed tree not shown in this study).

The genera *Cenchrus* and *Pennisetum* (proposed to be combined into one genus, *Cenchrus* L., as mentioned in Chapter 3, Chemisquy *et al.* 2010) do not form one clade in the *matK* tree, but five separate clades, with polytomies within some of the clades (Figure 5.4). The combined tree has better resolution of species and one monophyletic *Cenchrus*/*Pennisetum* clade is resolved, with strong BI support of PP = 1.00 but weak MP support of BP = 55 (Figure 5.5). This clade contains numerous naturalised species, so it would be advantageous to attempt to improve the resolution in this clade with better sampling and the addition of more molecular markers.

Panicum L. is not considered a monophyletic genus at present (Aliscioni *et al.* 2003, Sede *et al.* 2008, Morrone *et al*. 2012). In the tree published by GPWG II (2012), this genus is found in both tribes Paspaleae and Paniceae and is polyphyletic within both tribes (detailed supplementary data not shown). In the present study, genus *Panicum* is polyphyletic within tribe Paniceae in both the *matK* and combined data sets (Figures 5.4 & 5.5).

5.2.2.3 Sub-family Micrairoideae

The sub-family Micrairoideae is not represented in this study, as mentioned in Chapter 3. A single specimen of *Isachne rigens*, which belongs to a genus placed in Micrairoideae, is nested with members of genus *Panicum* in sub-family Panicoideae in both the *matK* and combined analyses in this study (Figures 5.4 and 5.5). This species is thought to be synonymous with *Panicum arbusculum,* and both names are listed on the trees.

5.2.2.4 Sub-family Arundinoideae

Sub-family Arundinoideae is unresolved in the *matK* BI tree (Figures 5.2 & 5.4) and also in the *rbcLa* + *matK* BI tree, where genus *Phragmites* Adans is sister to sub-family Danthoniodieae with weak support (PP = 0.69, no BP support) and *Arundo* Tourn. ex L. as sister to *Phragmites* with weak support (PP = 0.68, no BP support) (Figures 5.3 & 5.6). A monophyletic Arundinoideae clade is weakly supported in the data from GPWG II (2012, detailed tree not shown, PP = 0.52, ML BP = 65). The monophyly of this sub-family is supported in other studies (GPWG I 2001, Duvall *et al*. 2007) although there has been dispute over the monophyly of this clade in the literature, and earlier studies have found Arundinoideae to be polyphyletic or paraphyletic (Barker *et al*. 1995, Clark *et al.* 1995, Barker *et al.* 1999). Recently, Bouchenak-Khelladi *et al.* (2008) found that in one of their two data sets Arundinoideae were not monophyletic and *Phragmites* and *Arundo* were in different lineages, but in their second data set Arundinoideae was monophyletic.

As this sub-family is only represented in the present study by three species and two genera (*Arundo* and *Phragmites*) which are known to be fairly distantly related within the sub-family (Hilu *et al.* 1999, GPWG I 2001, Bouchenak-Khelladi *et al.* 2008), it is not possible to make any conclusions as to the monophyly of this sub-family.

5.2.2.5 Sub-family Danthonioideae

The Danthoniodeae + Chloridoideae clade, a relationship that is well-documented in the literature (Bouchenak-Khelladi 2008, GPWG II 2012: PP = 1.00, ML BP = 95, see Figure 5.1

above), with Danthonioideae as sister to Chloridoideae, is present in the trees from both the *matK* and the combined data sets with strong PP support in the BI analyses in both trees (PP = 1.00 for both) but only weak support from the MP analysis in the combined data set (BP = 65, Figure 5.3) and no support in the *matK* data set (Figure 5.2). Sub-family Danthonioideae is monophyletic and well-supported in the trees from both data sets, with PP values of 1.00 for both trees and BP values of 82 and 97 for *matK* and the combined data sets, respectively.

Most genera in sub-family Danthonioideae are well-resolved and monophyletic in the *matK* and combined data trees, with *Geochloa* H.P. Linder & N.P. Barker and *Tribolium* Desv. being exceptions in the *matK* and combined trees and *Capeochloa* HP Linder & N.P. Barker in the combined tree (Figures 5.4 & 5.5).

It was recently proposed by Linder *et al.* (2010) that all members of genus *Pentaschistis* Stapf. be combined with members of genus *Pentameris* P. Beauv into one clade which would be named *Pentameris*, as this is historically the oldest name. These two genera are difficult to distinguish morphologically, and their monophyly in respect to each other has not been well-supported. These two genera have many native representatives in southern Africa.

Many of these species are currently listed with *Pentaschistis* as the accepted name and *Pentameris* as an unresolved name on The Plant List (2013). In this study, the currently listed accepted name on the Plant List was the name preferably selected. Also in South African herbaria many of the species collected for this study are still listed under the name *Pentaschistis*, as are they also in the GenBank and BOLD databases from which samples were also collected. For these reasons, in the present study, many of the species in this clade are still listed under the genus *Pentaschistis*, but it should be noted that all *Pentaschistis* species will likely be referred to as *Pentameris* species in the future, and are currently being listed under *Pentameris* in some recent literature (Manning & Goldblatt 2012, Snijman 2013).

Data from the present study indicate that the *Pentaschistis*/*Pentameris* clade is not monophyletic in the *matK* tree as there is one outlier, *Pentaschistis tysonii* Stapf voucher Linder HP 6812 BOL (which is not included in the combined data). When the *matK* sequence of this specimen is subjected to a BLAST search it has a 99% match with *Cortaderia* species, so it is possible that this specimen has been incorrectly labelled or identified. The *Pentaschistis*/*Pentameris* clade is monophyletic in the combined data tree with strong support of PP = 1.00 and BP = 100 (Figure 5.5). There are polytomies within this clade in both data sets, although there is better resolution in the combined data tree.

5.2.2.6 Sub-family Chloridoideae

Sub-family Chloridoideae has been resolved monophyletic with strong support (PP = 1.00, ML BP = 99, Figure 5.1, GPWG II 2012). The sub-family is often sub-divided into tribe Centropodieae or the *Centropodia* clade, and the rest of the Chloridoideae (Figure 5.1). Tribe Centropodieae comprises of two genera, *Centropodia* Reichb. and *Ellisochloa* P.M. Peterson & N.P. Barker (Peterson *et al.* 2011). In this study, tribe Centropodieae is represented by genus *Ellisochloa* only, but this genus does form a monophyletic clade within Chloridoideae in the trees from both the *matK* and combined data sets, which is in agreement with the recently published Poaceae family tree (GPWG II 2012), with strong support of 1.00 PP and 100 BP for the tribe in both (Figures 5.2 & 5.3). The rest of the Chloridoideae form a well-supported clade in both data sets as well, with PP values of 1.00 for both trees and BP values of 100 and 92 for *matK* and the combined data, respectively.

All of the tribes in sub-family Chloridoideae that are represented in this study are resolved as monophyletic in the *matK* and combined data BI trees (Figures 5.4 and 5.5 respectively). Tribe Eragrostideae has support of PP = 1.00/1.00 and BP = 81/100 (*matK*/combined data set); tribe Cynodonteae has support of PP = 1.00/1.00 and BP = 67/68 100 (*matK*/combined data set); tribe Zoysieae (represented by the genus *Sporobolus* R.Br. in this study) has support of PP =1.00/1.00 and BP = 77/84, and tribes Triraphideae (represented in this study by genus *Triraphis* R.Br.) and Centropideae both have strong support of PP = 1.00 and BP = 100 for both the *matK* and combined data sets.

Most genera are monophyletic in the sub-family Chloridoideae in the *matK* and combined trees. *Eragrostis* Wolf, a large genus which is well-represented in South Africa, is not monophyletic in either the *matK* or combined data. In the tree published by GPWG II (2012, detailed tree in supplementary data, not shown in this study) this genus is not monophyletic and appears in at least two different tribes in sub-family Chloridoideae, with one species in sub-family Arundinoideae. Ingram & Doyle (2004, 2007) noted that the monophyly of *Eragrostis* is in question and suggested the inclusion of genus *Pogonarthria* Stapf. among others into genus *Eragrostis*. If *Pogonarthria* were to be included in *Eragrostis* in this study then *Eragrostis* does form a monophyletic clade with strong BI support of PP = 1.00/1.00 for the *matK* and combined data respectively, but with no bootstrap support (Figures 5.4 & 5.5). The genus is fairly well-resolved at species level, but with a few polytomies in the combined data tree (Figure 5.5).

Genus *Sporobolus* R.Br., another large and well-represented genus in South Africa, and the only representative of tribe Zoysieae in this study, is quite well resolved at species level with few polytomies. The genus is resolved as monophyletic with strong BI support of PP = 1.00/1.00 and moderate bootstrap support of BP = 79/84 for *matK* and the combined data sets, respectively (Figures 5.4 & 5.5). There are no listed naturalised species in this genus.

Eleusine Gaertn. is an example of a genus that is not well-resolved at species level. This genus contains species known to be naturalised as well as species thought to be native or of uncertain origin. The addition of more taxa and other molecular markers to the data may be required to distinguish between these species. The genus is monophyletic with strong support (PP = 1.00/1.00, BP = 93/100 for the *matK* and combined data sets, respectively).

5.2.2.7 BEP clade and sub-family Ehrhartoideae

The BEP clade diverged earlier than the PACMAD clade and consists of three sub-families, the Bambusoideae, the Ehrhrartoideae and the Pooideae. This clade is resolved as monophyletic in recent literature (Bouchenkak-Khelladi *et al*. 2008, Saarela & Graham 2010, GPWG II 2012). In the tree from GPWG II (2012), the BEP clade has low BI support (PP = 93) and moderate bootstrap support (BP = 83, Figure 5.1).

In this study, neither the *matK* nor the combined data resolve the BEP as a separate clade. In the *rbcLa* + *matK* tree, Pooideae is sister to the PACMAD clade, although this relationship is only weakly supported (PP = 0.59, no bootstrap support, Figure 5.3). In the *matK* tree, Pooideae and Bambusoideae form a clade with weak support (PP = 0.84, no bootstrap support), but Ehrhartoideae is sister to the PACMAD clade, with moderate BI support (PP = 0.76) but no bootstrap support (Figure 5.2). Sub-family Ehrhartoideae is only represented by two genera from two different tribes (*Ehrharta* Thunb. in tribe Ehrharteae and *Leersia* S.W. in tribe Oryzeae) in the *matK* data and only by genus *Ehrharta* in the combined data. In both data sets, sub-family Ehrhartoideae is monophyletic, with moderate to strong support (PP = 1.00/1.00, BP = 82/100 for the *matK* and combined data respectively, Figures 5.4 & 5.5).

5.2.2.8 Sub-family Pooideae

All of the seven out of ten tribes from sub-family Pooideae represented in this study are found to be monophyletic in both the *matK* and combined data trees (Figures 5.4 & 5.5). Tribes Bromeae (PP = 1.00/1.00, BP = 94/99 for *matk* and combined data, respectively), Meliceae (PP = 1.00/1.00, BP = 99/100 for *matk* and combined data, respectively) and Brachypodieae (PP = 1.00, BP = 100 for *matK* data only, this tribe is only represented by one specimen in the combined data) all have strong support. Tribes Triticeae (PP = 0.99/0.97 for *matK* and combined data sets, respectively) and Stipeae (PP = 1.00/1.00 for *matK* and combined data sets, respectively) both have strong BI support, but Triticeae has weak to moderate MP bootstrap support (BP = 78/71 for *matK* and combined data, respectively) and Stipeae has strong to moderate bootstrap support (BP = 100/78 for *matK* and combined data, respectively).

Originally tribe Poaeae and tribe Aveneae were described as two separate tribes in sub-family Pooideae (Clayton & Renvoize 1986), but more recent studies have found that neither of these groups are monophyletic, and that one larger tribe incorporating both Poaeae and Aveneae should be sub-divided into two clades, currently named Poaeae clade 1 and clade 2 (Soreng & Davis 2000, Davis & Soreng 2007). In this study Poaeae clades 1 and 2 are found to be monophyletic in both the *matK* and combined data BI trees (Figures 5.4 & 5.5). Clade 1 has strong support (PP = 1.00/1.00, BP = 95/98), and Clade 2 has generally strong support (PP = 0.93/1.00, BP = 99/100).

One unusual result is that an *Eragrostis pilosa* (L.) P.Beauv. specimen (voucher number 07 PMP 20912, downloaded from the BOLD database) is nested within the sub-family Pooideae in the *matK* BI tree in a clade with *Koeleria capensis* Nees (Figure 5.4). When this *matK* sequence is subjected to a BLAST search, it has 100% matches to some *Koeleria* Pers. and *Trisetum* Pers. species – both of which belong to sub-family Pooideae. It is likely that this specimen was incorrectly identified or incorrectly labelled, or that DNA cross contamination occurred.

As was noted in Chapter 3, the majority of naturalised species in South Africa (~64%) come from sub-family Pooideae, as can be seen in Figures 5.3 - 5.5 (naturalised species highlighted in green). Many of these are from Mediterranean Europe and are successful in the winter rainfall regions in South Africa. Therefore in terms of the identification of naturalised species in the country, it is useful to have good resolution at species level for this sub-family.

In the DNA-barcoding study (Chapter 4), it was not possible to identify all members of genus *Avena* at species level. In the *matK* and combined trees, again *Avena* species are not well-resolved at species level and the genus forms polytomies, although the genus is monophyletic with strong support (PP = 1.00/1.00, BP = 98/100 for the *matK* and combined data, respectively). As was also noted in Chapter 4, for the purposes of DNA-barcoding and identification of naturalised grasses in South Africa, an identification at genus level is sufficient for *Avena*, as the genus as a whole is not native to the country. However for the purposes of taxonomic and phylogenetic understanding of the species, the *rbcLa* and *matK* markers do not provide sufficient data, and more in depth morphological and molecular studies are required, although these can be complicated by the varying ploidy levels of species within this genus (Peng *et al.* 2010 a&b).

Lolium L. is another example of a genus that is not well-resolved at the species level in this study, especially in the *matK* data, where polytomies are retrieved (Figure 5.4). *Lolium* and *Festuca* L. are known to form a complex. Members of these two genera have been reported to hybridise to form festulolium grasses (Guo *et al.* 2005). The two genera are easy to distinguish using inflorescence morphology, but not using molecular markers (Gaut *et al.* 2010).

In this study in the *matK* and combined data trees, some *Festuca* species are resolved within the genus *Lolium* (Figures 5.4 & 5.5). Some *Festuca* species are currently considered as native in South Africa and others are listed as naturalised (see Appendix 1A), but all *Lolium* species are considered to be naturalised in the country. It appears that the two DNA-barcoding markers are not sufficient for distinguishing these species either for identification using DNA-barcoding or at a phylogenetic level. Hand *et al.* (2010) had some success at resolving more species in this complex by using two nuclear genes *Acc1* and *CEN*, and more recently, next-generation sequencing techniques have been employed to better understand the *Festuca-Lolium* complex at the molecular level (Hand *et al.* 2013).

5.2.2.9 Sub-families Bambusoideae and Puelioideae

In the combined data tree, the Bambusoideae are poorly represented with only one *Bambusa* specimen (see Appendix 1A), which forms a weakly supported clade (PP = 0.67, no bootstrap support) with *Puelia olyriformis* (Franch.) Clayton of sub-family Puelioideae, which is considered an early diverging grass lineage and not part of the BEP clade. Puelioideae have been resolved as sister to the BEP + PACMAD clades (GPWG I 2001, GPWG II 2012), thus in this case there are not enough specimens representing either clade to provide clear resolution on their relationship.

As there are three *Bambusa* specimens represented in the *matK* tree, the relationships within the BEP are slightly better resolved. Pooideae and Bambusoideae form a clade, similar to that published in 2012 by the GPWG II, with strong support from the BI (PP = 084) but no bootstrap support. However, the Ehrhartoideae are not resolved as sister to this Pooideae + Bambusoideae, as in the published tree, but rather as sister to the PACMAD clade (as mentioned above). In the *matK* tree, *Puelia olyriformis,* is sister to the BEP + PACMAD clades*,* as in the published literature (GPWG I 2001, GPWG II 2012), a relationship that is strongly supported, with PP = 1.00 and BP = 100 (Figure 5.2).

5.2.2.10 Early-diverging grass lineages

When examining the early-diverging grass lineages in this study in both the *matK* and combined data trees, sub-family Pharoideae is sister to Puelioideae + BEP + PACMAD, with strong support of PP = 1.00 for both trees and BP support of 92/97 for the combined and *matK* trees respectively (Figs. 5.2 & 5.3). This relationship is well-documented in the literature (Hilu *et al.* 1999, GPWG I 2001, Bouchenak-Khelladi *et al.* 2008, GPWG II 2012).

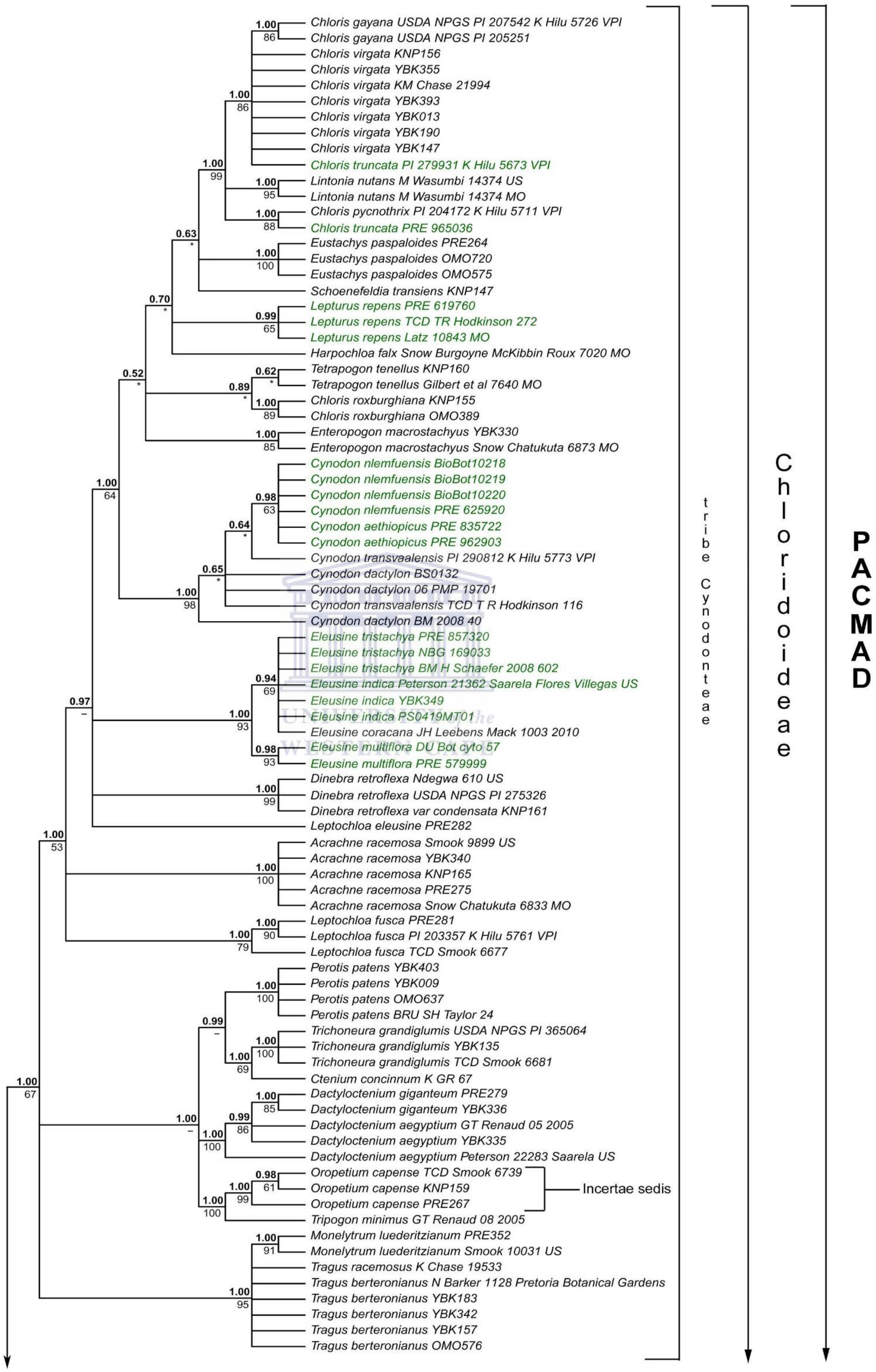
The most basal of the early-diverging grass sub-families is known to be Anomochlooideae, which contains two genera, *Anomochloa* Brongn. and *Streptochaeta* Schrad. ex Nees (Hilu *et al.* 1999, GPWG I 2001, Bouchenak-Khelladi *et al.* 2008, GPWG II 2012). Both the *matK* and combined data trees in this study resolve the Anomochlooideae as the most basal grass lineage, with *Streptochaeta* as sister to the rest of Poaceae (strong support in both trees with PP = 1.00 and BP = 100 for both). This is similar to the tree using *matK* data published by Hilu *et al*. in 1999. In most of the recently published trees, however, *Anomochloa* and *Streptochaeta* form a separate clade, rather than having *Streptochaeta* as sister to the rest of the family (GPWG I 2001, Bouchenak-Khelladi *et al.* 2008, GPWG II 2012).

5.2.2.11 Families sister and closely related to Poaceae

The *matK* and combined data trees in this study are in agreement with the literature published on order Poales in finding Joinvilleaceae as the sister family to Poaceae and Ecdeiocoleaceae as sister to Joinvilleaceae + Poaceae family (Hilu *et al.* 1999, GPWG I 2001, Bouchenak-Khelladi *et al.* 2008). The relationship of Joinvilleaceae to Poaceae is only weakly supported in the combined *rbcLa* + *matK* data (PP = 0.57, BP = 65, Figure 5.3) and moderately supported in the *matK* data (PP = 0.83, BP = 79, Figure 5.2). This family is only represented by one species in each dataset, and more sampling and increased representation may better resolve this relationship in these data sets.

The relationship of Ecdeiocoleaceae as sister to Joinvilleaceae + Poaceae is well-supported in both the *matK* and combined data sets presented here (PP = 1.00 for both trees and BP = 99 and 100 for *matK* and the combined trees respectively, Figures 5.2 & 5.3). While most studies agree with this relationship, some studies have found Ecdeiocoleaceae to be sister to Poaceae, instead of Joinvilleaceae (Bremer 2002, Michelangeli *et al.* 2003, Givnish *et al*. 2010) and others have found Ecdeiocoleaceae + Joinvilleaceae to be sister to Poaceae (Briggs *et al.* 2000, Bouchenak-Khelladi *et al.* 2014). As there is only one species from each of Joinvilleaceae and Ecdeiocoleaceae represented in each data set in this study, no conclusive comment can be made on the family that is sister to Poaceae within the graminid clade of order Poales.

Finally, Restionaceae is known to be a family in order Poales that is closely related to the graminid clade of order Poales (GPWG 2001, Michelangeli *et al.* 2003, Givnish *et al.* 2010, Bouchenak-Khelladi *et al.* 2014). In this study, family Restionaceae was represented by two species, *Baloskion tetraphyllum* (Labill.) B.G. Briggs & L.A.S. Johnson and *Elegia squamosa* Mast, which were used to root the trees.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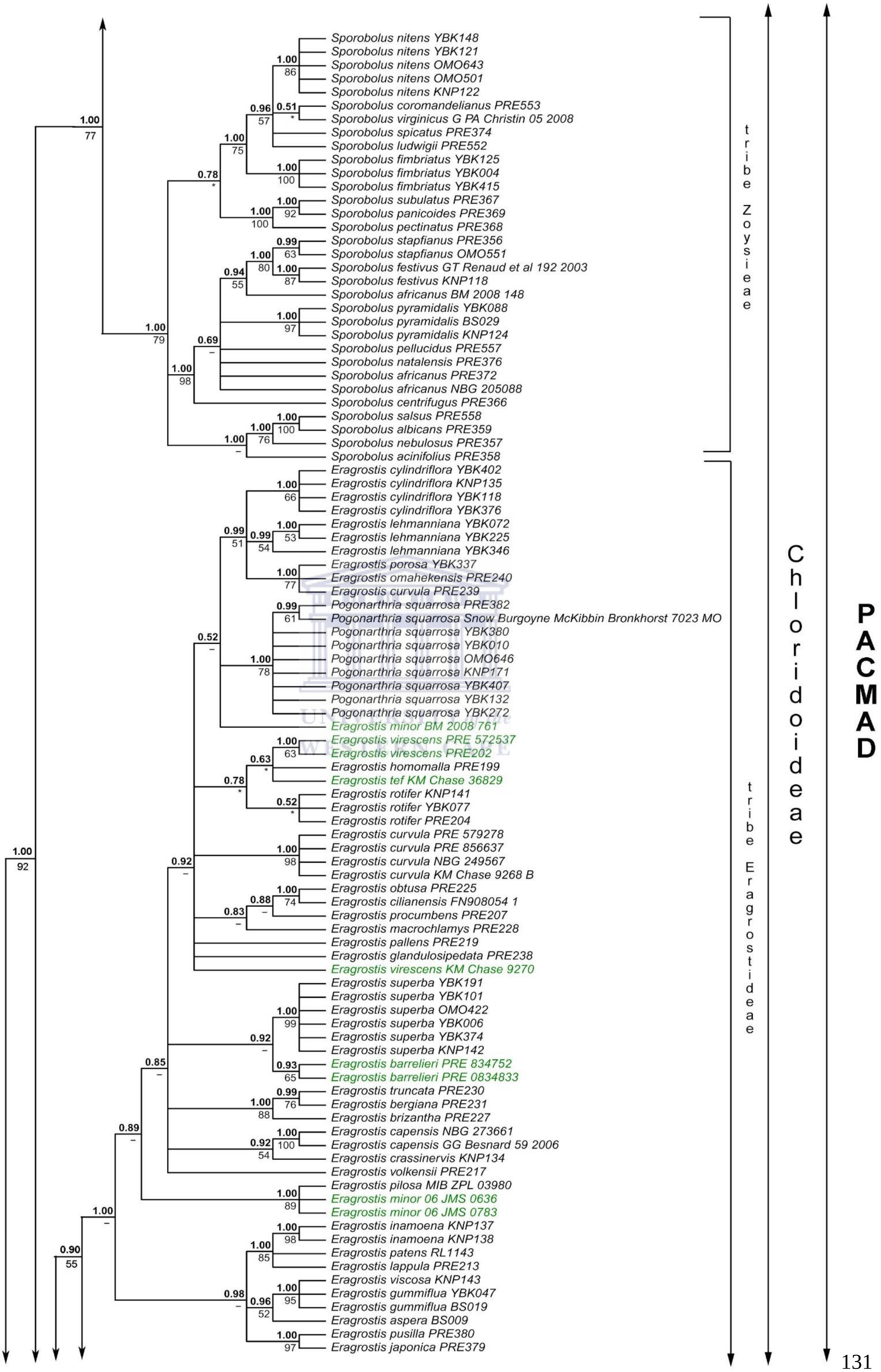
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Chloridoideae
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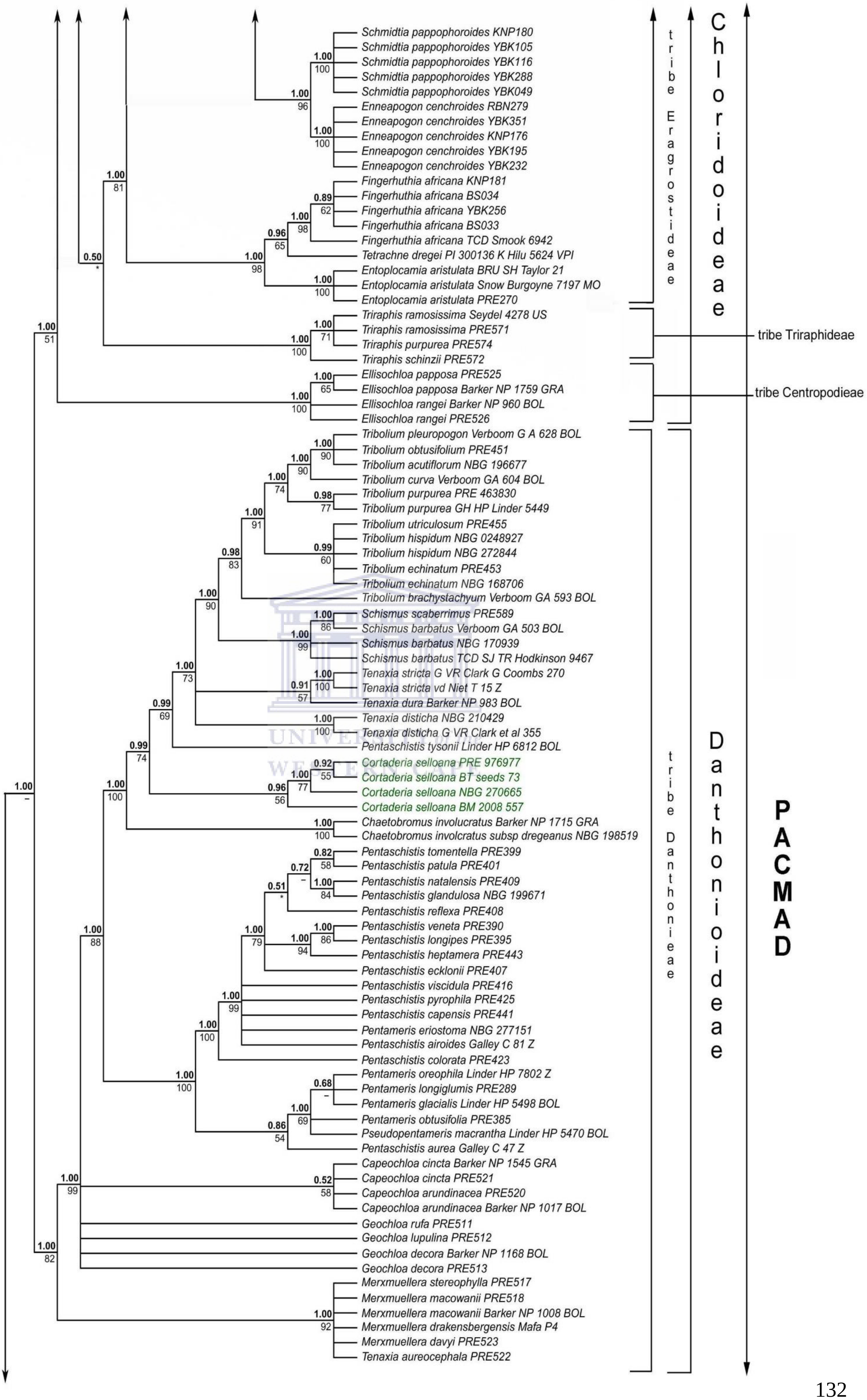
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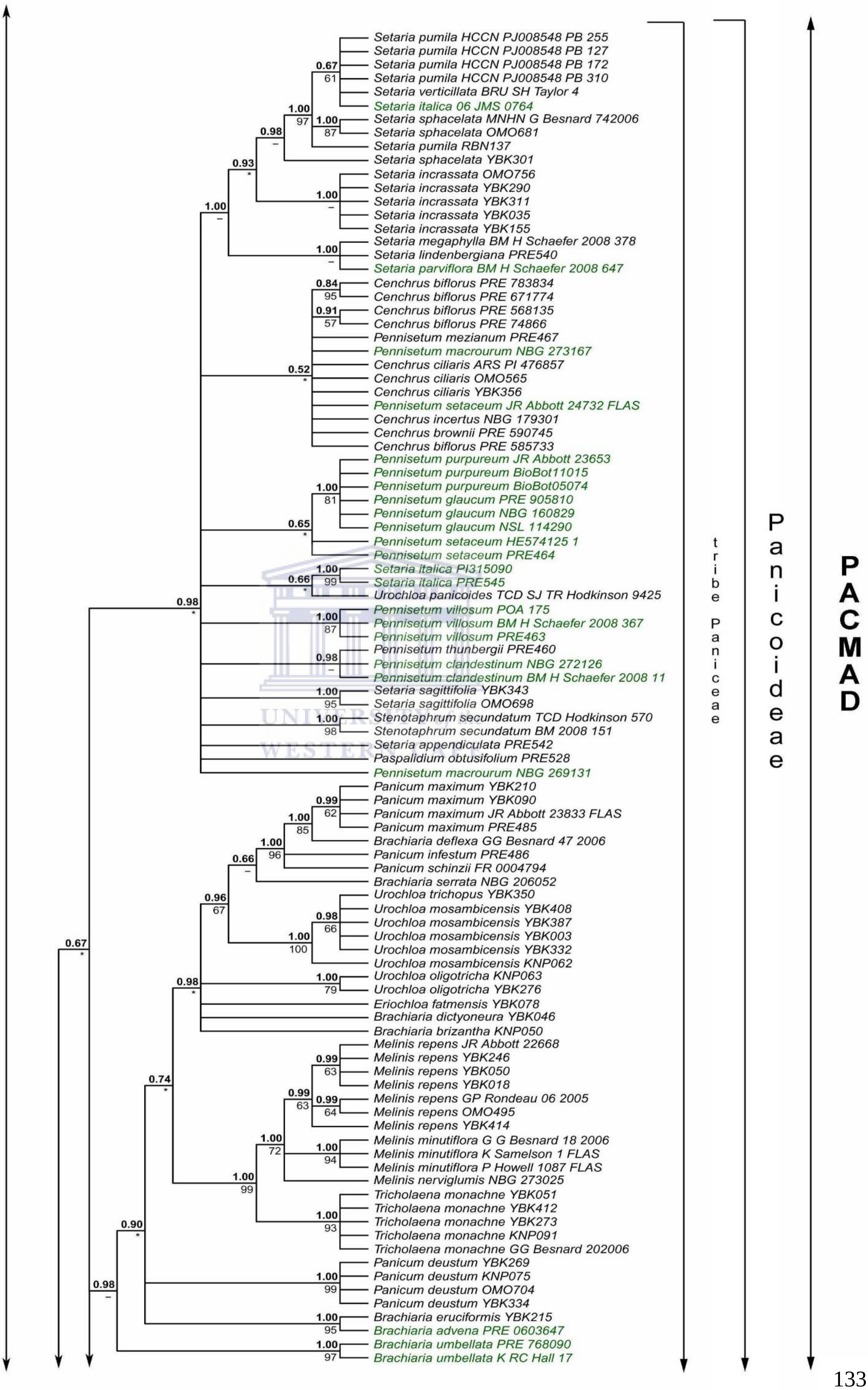
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*
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tribe Paniceae
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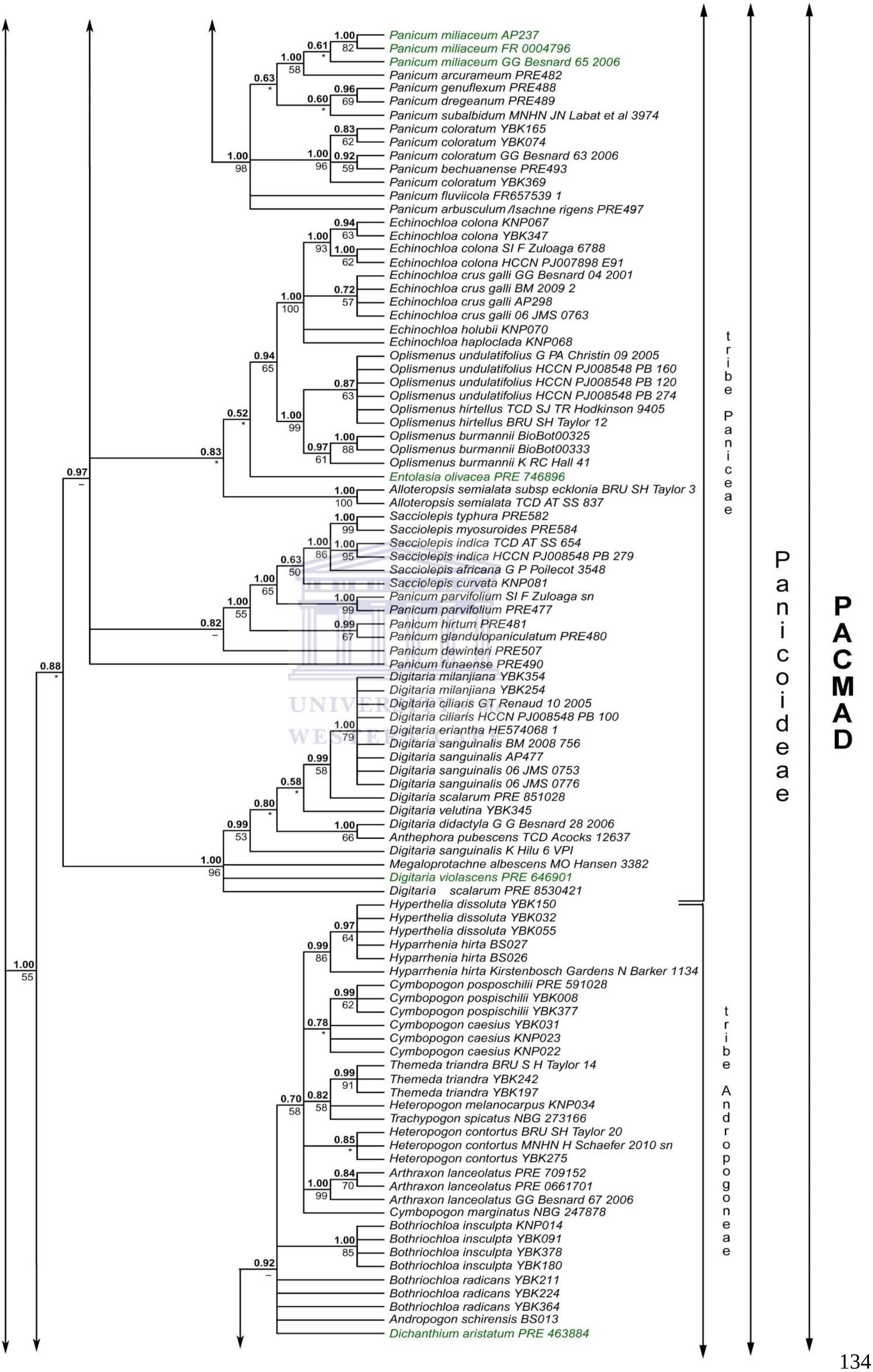
PACMAD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Panicum dewinteri PRE507
Panicum funaense PRE490
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Bothriochloa radicans YBK211
Bothriochloa radicans YBK224
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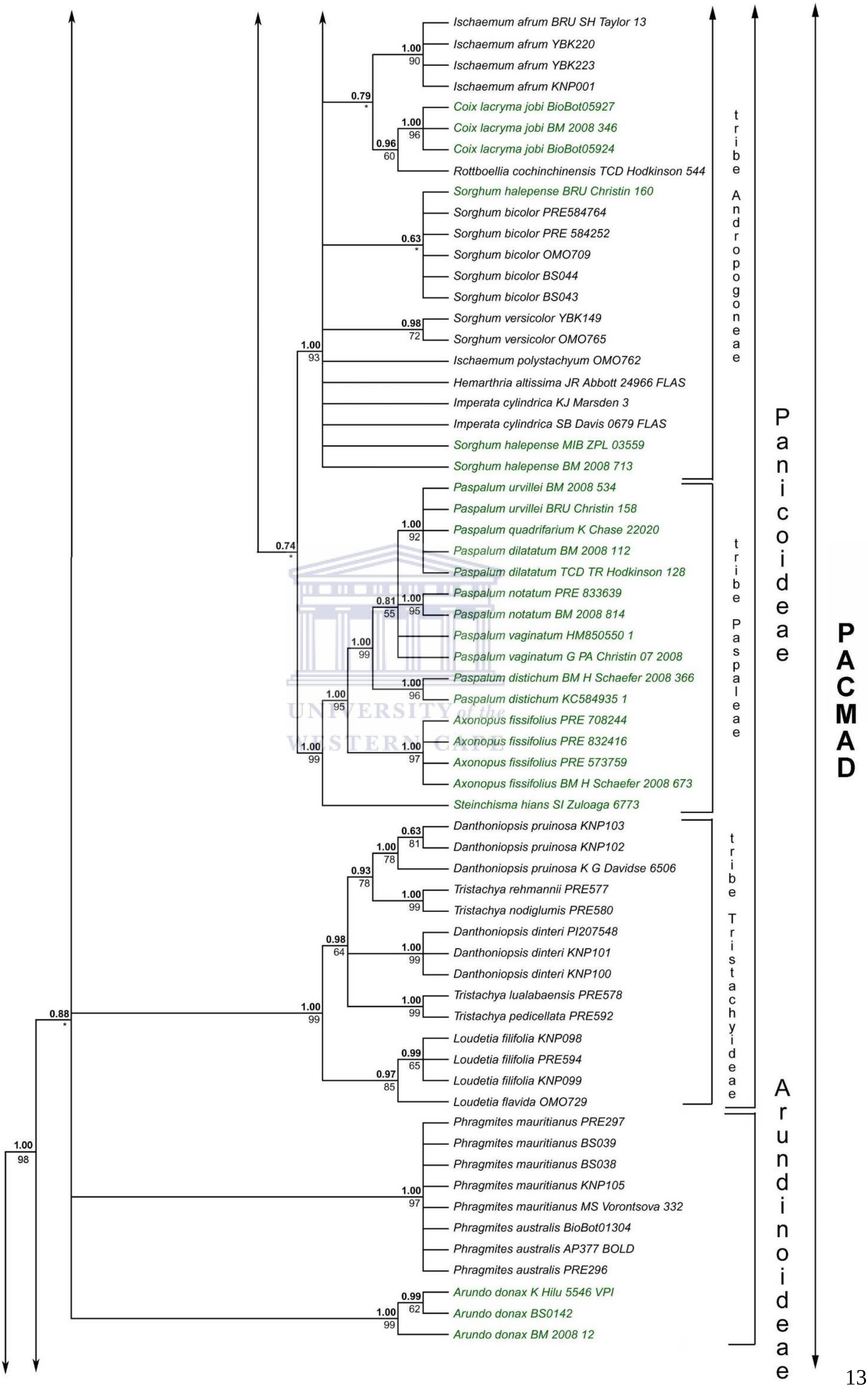
tribe Andropogoneae
Panicoideae
PACMAD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Sorghum halepense BRU Christin 160
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Paspalum notatum PRE 833639
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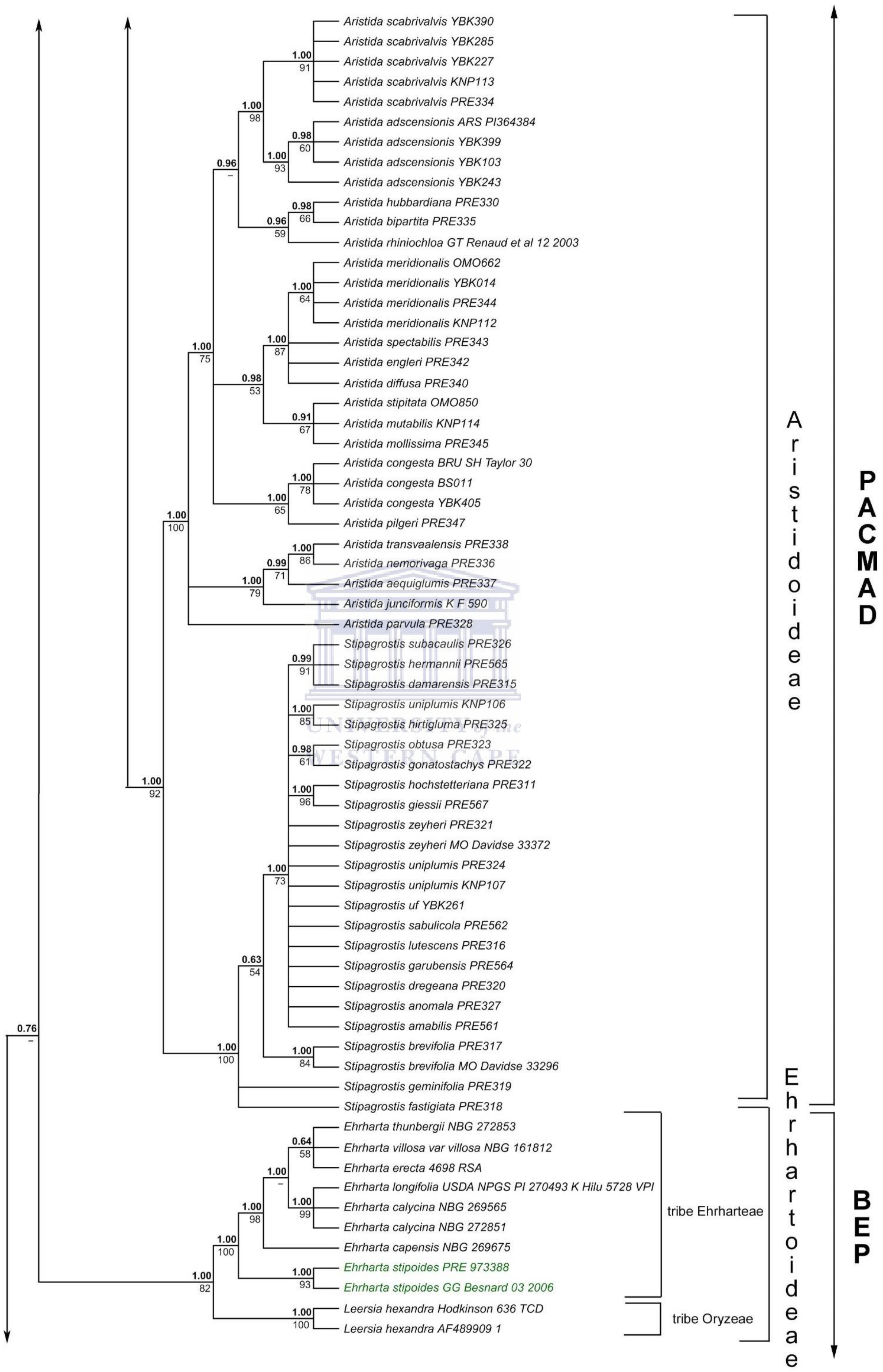
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Leersia hexandra Hodkinson 636 TCD
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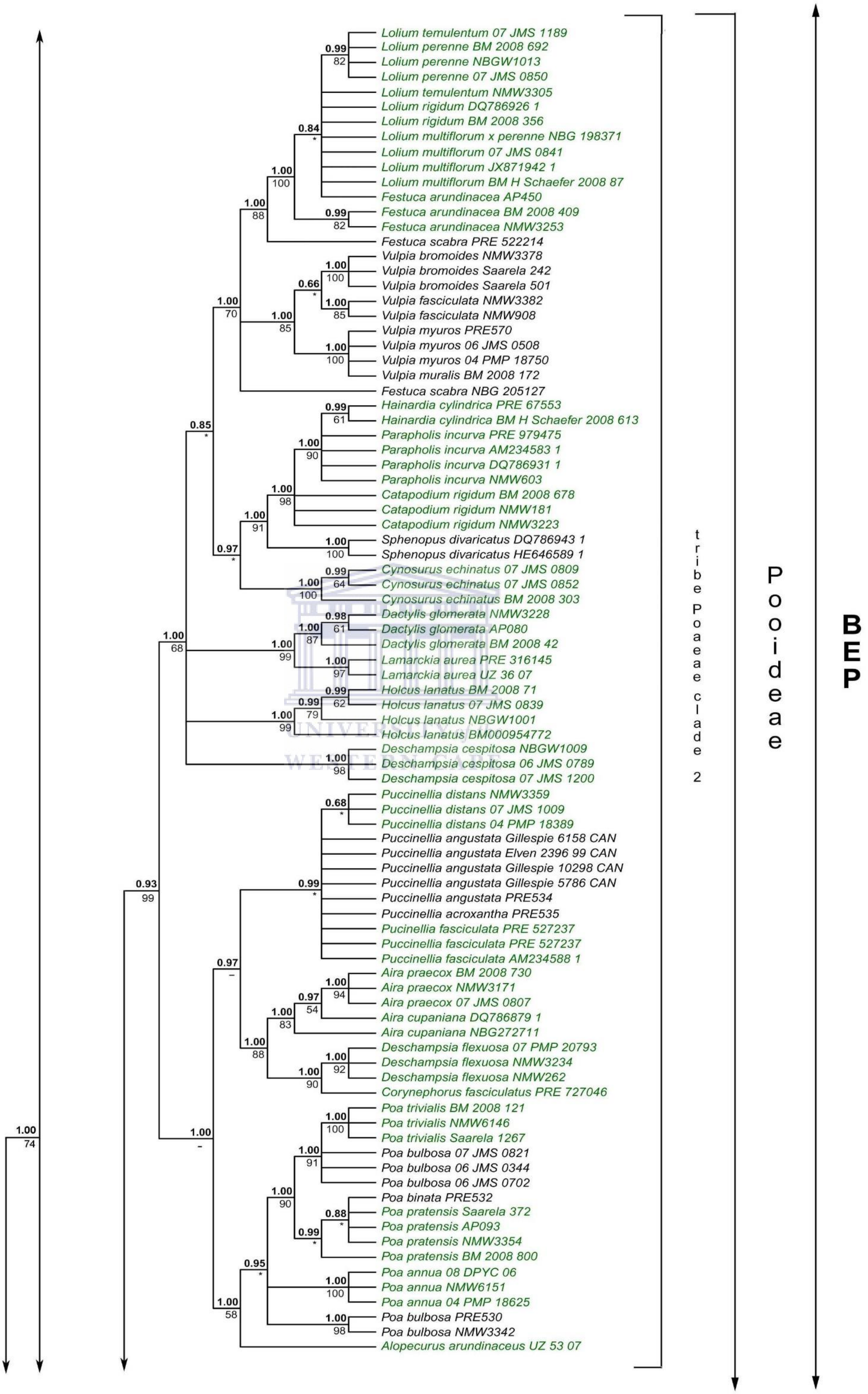
Ehrhartoideae
BEP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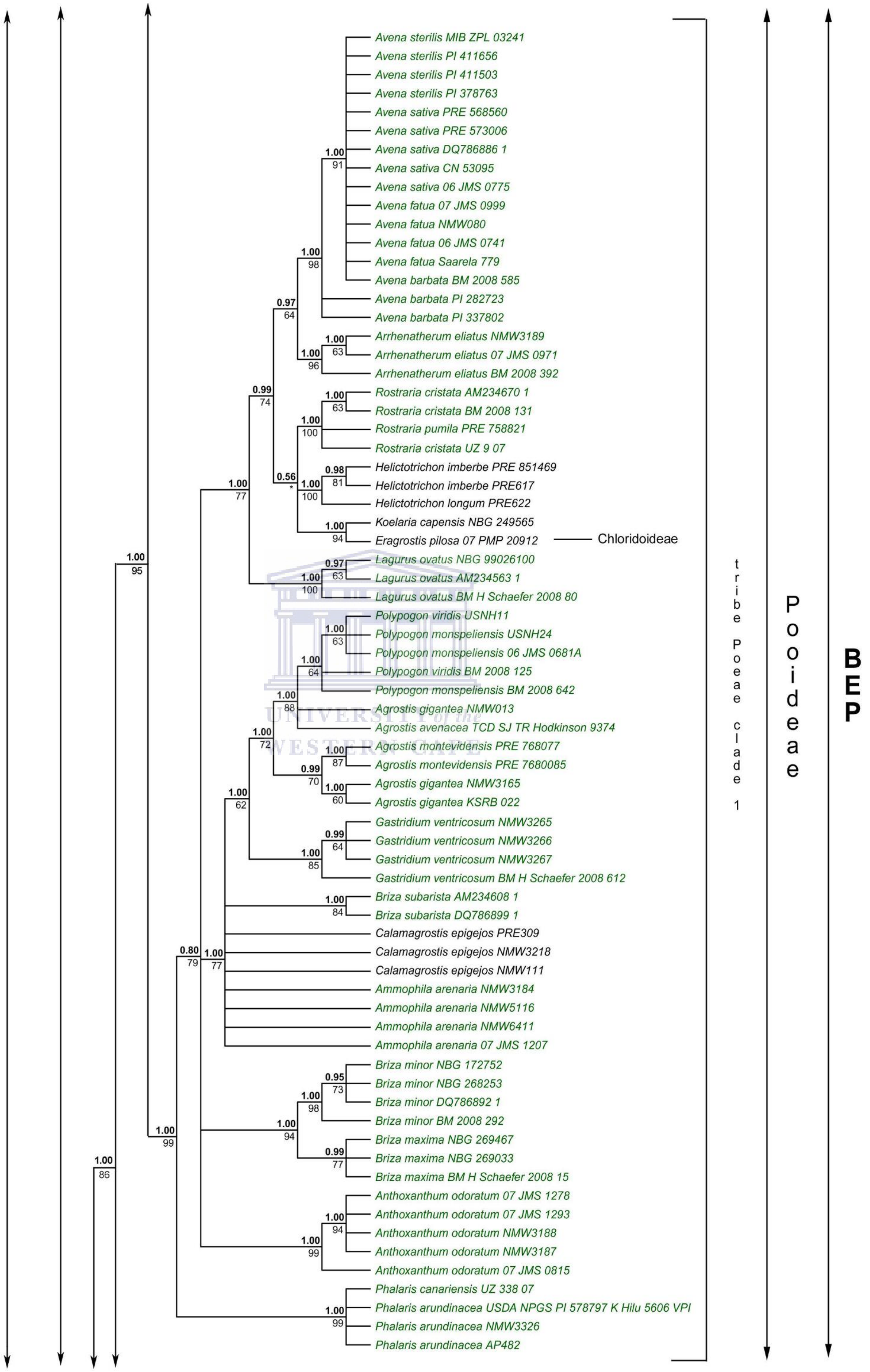
Pooideae
BEP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Avena fatua 07 JMS 0999
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Lagurus ovatus NBG 99026100
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Polypogon monspeliensis USNH24
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Gastridium ventricosum NMW3265
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Gastridium ventricosum NMW3267
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Briza subarista DQ786899 1
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Briza maxima BM H Schaefer 2008 15
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Pooideae
BEP
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1.00 98
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1.00 63
1.00 96
0.99 74
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0.98 81
0.56 *
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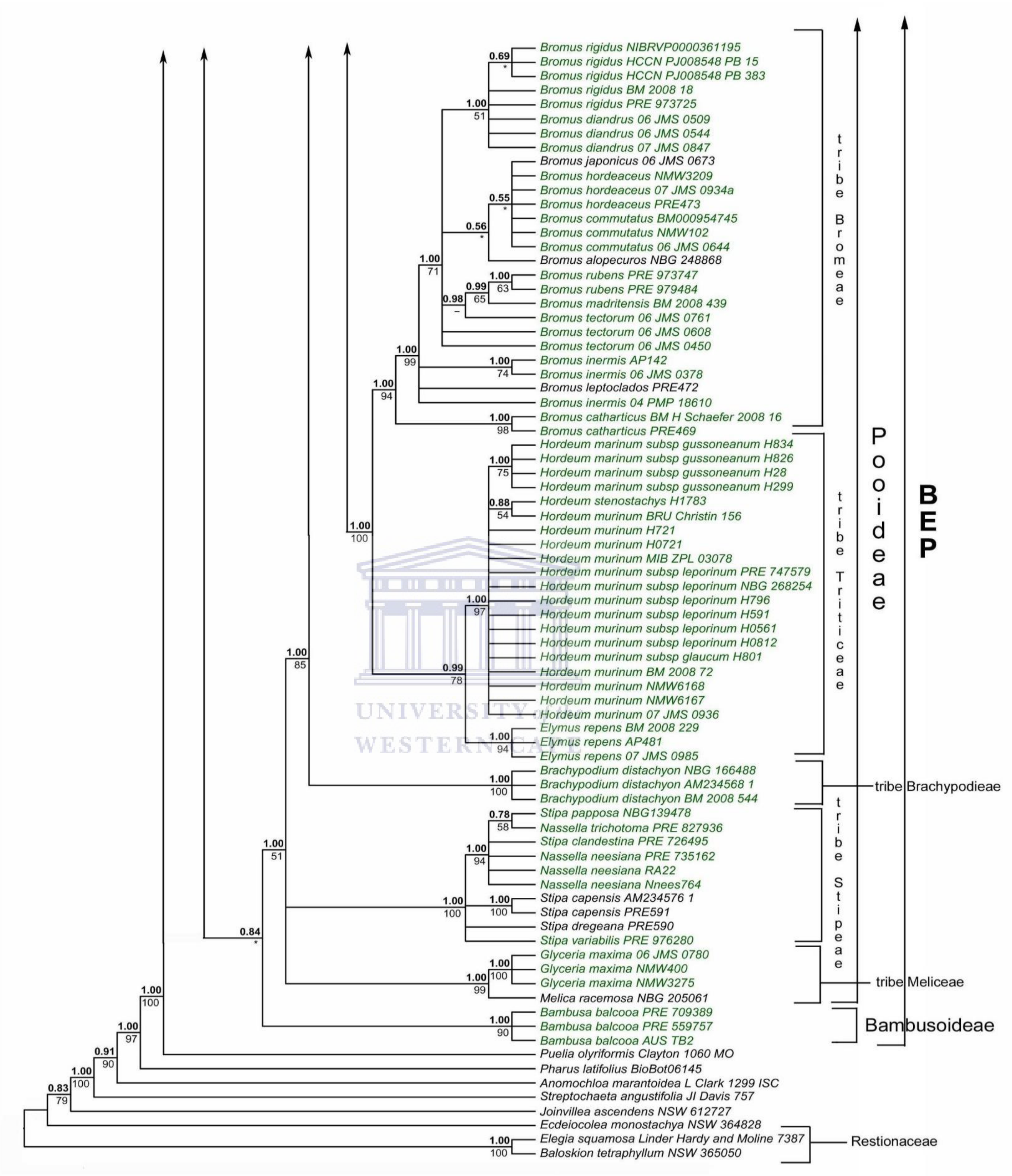
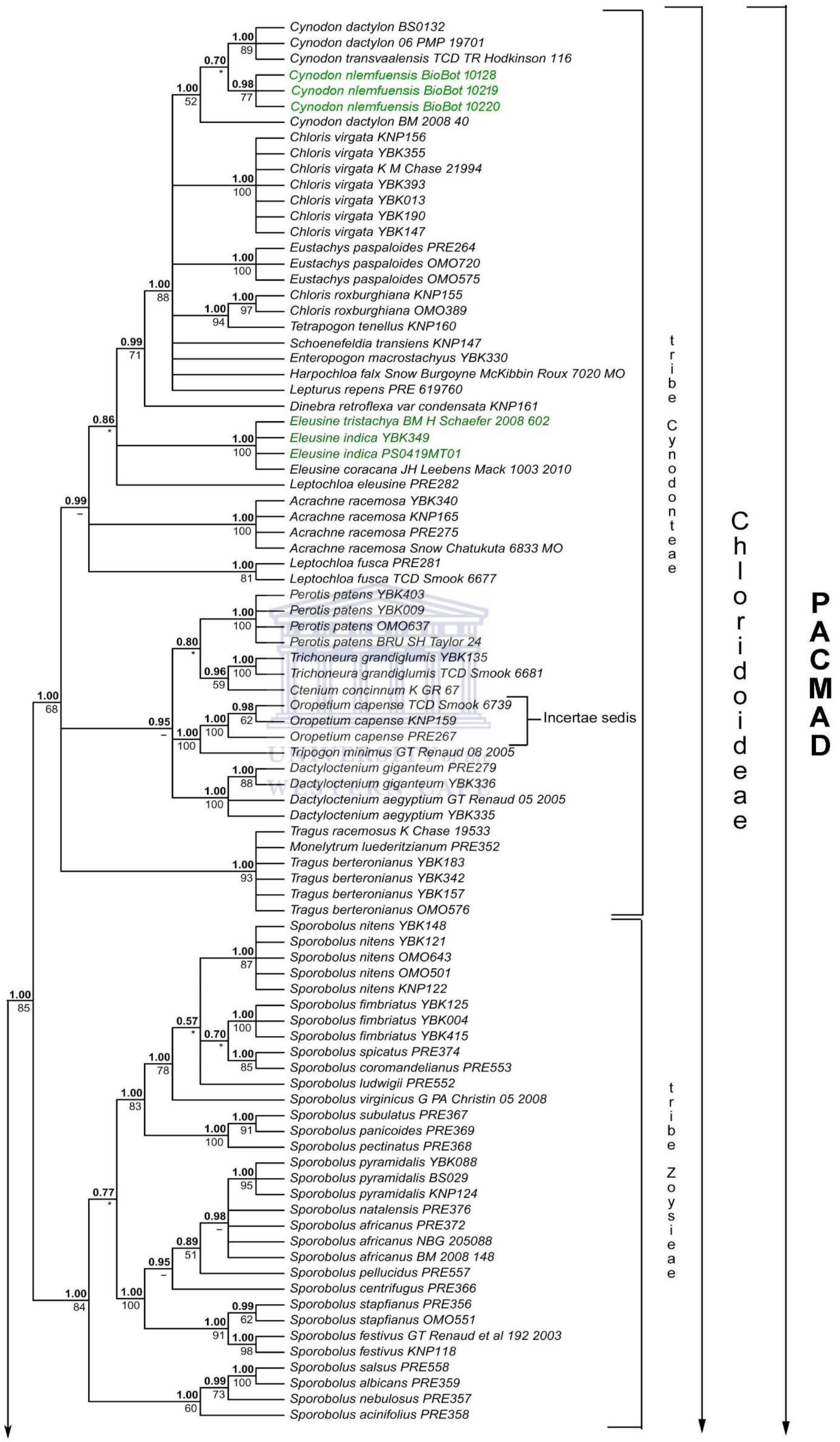


Figure 5.4 50% majority-rule consensus tree of southern African Poaceae obtained from Bayesian inference (BI) analyses based on *matK* data, showing grass tribes and sub-families, and some sister species. BI posterior probabilities (PP) are above branches in bold type and MP bootstrap percentages (BP) >50% are below branches. Species listed as naturalised in Chapter 2 are highlighted in green, - indicates no MP bootstrap support, * indicates a clade is not present or resolved in the MP analysis.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Schoenefeldia transiens KNP147
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Harpochloa falx Snow Burgoyne McKibbin Roux 7020 MO
Lepturus repens PRE 619760
Dinebra retroflexa var condensata KNP161
Eleusine tristachya BM H Schaefer 2008 602
Eleusine indica YBK349
Eleusine indica PS0419MT01
Eleusine coracana JH Leebens Mack 1003 2010
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Acrachne racemosa KNP165
Acrachne racemosa PRE275
Acrachne racemosa Snow Chatukuta 6833 MO
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Sporobolus fimbriatus YBK415
Sporobolus spicatus PRE374
Sporobolus coromandelianus PRE553
Sporobolus ludwigii PRE552
Sporobolus virginicus G PA Christin 05 2008
Sporobolus subulatus PRE367
Sporobolus panicoides PRE369
Sporobolus pectinatus PRE368
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Sporobolus africanus BM 2008 148
Sporobolus pellucidus PRE557
Sporobolus centrifugus PRE366
Sporobolus stapfianus PRE356
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Sporobolus festivus GT Renaud et al 192 2003
Sporobolus festivus KNP118
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Sporobolus nebulosus PRE357
Sporobolus acinifolius PRE358
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tribe Zoysieae
Chloridoideae
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1.00 87
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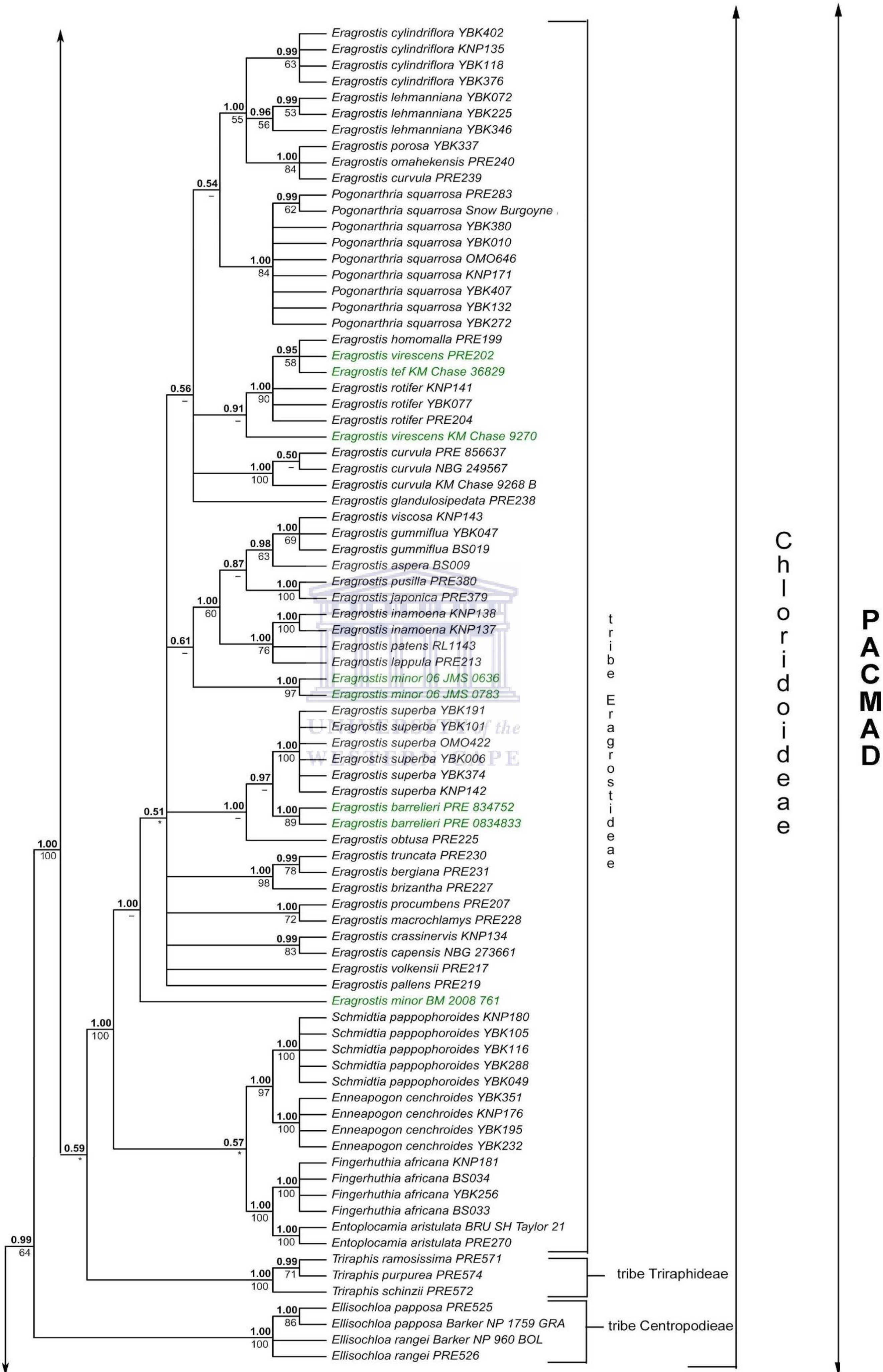
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Eragrostis omahekensis PRE240
Eragrostis curvula PRE239
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Pogonarthria squarrosa OMO646
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Eragrostis pusilla PRE380
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Triraphis ramosissima PRE571
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Ellisochloa papposa PRE525
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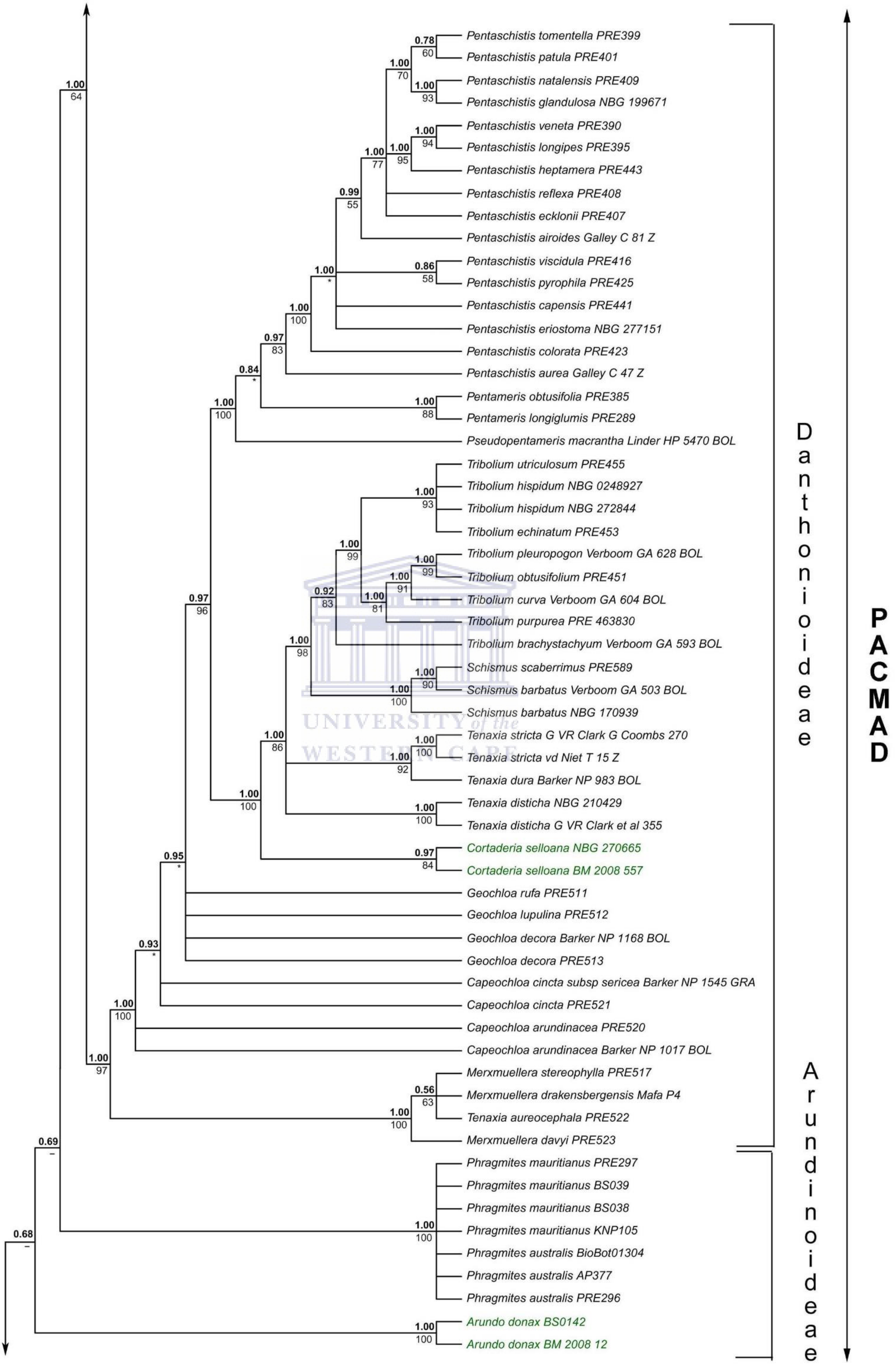
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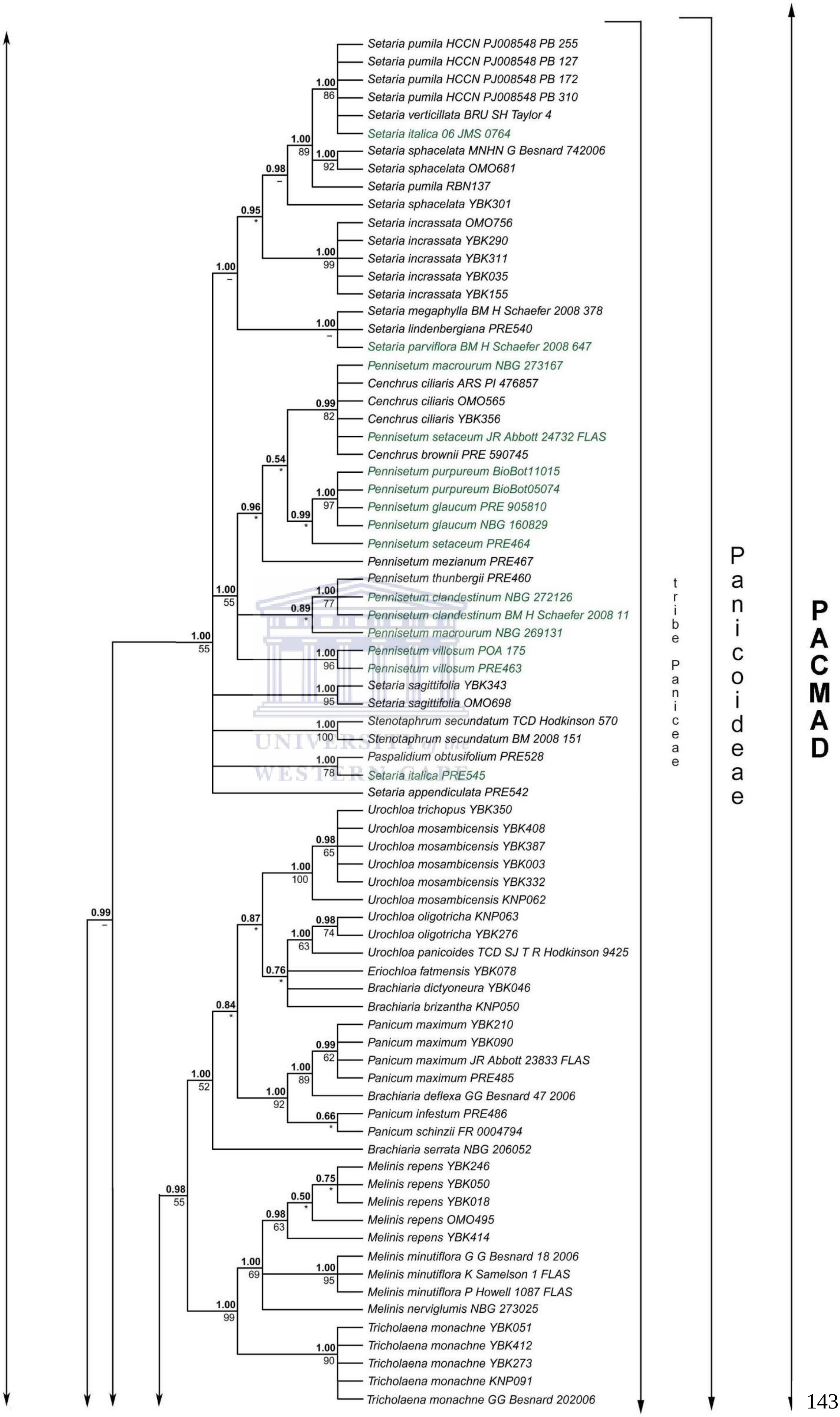
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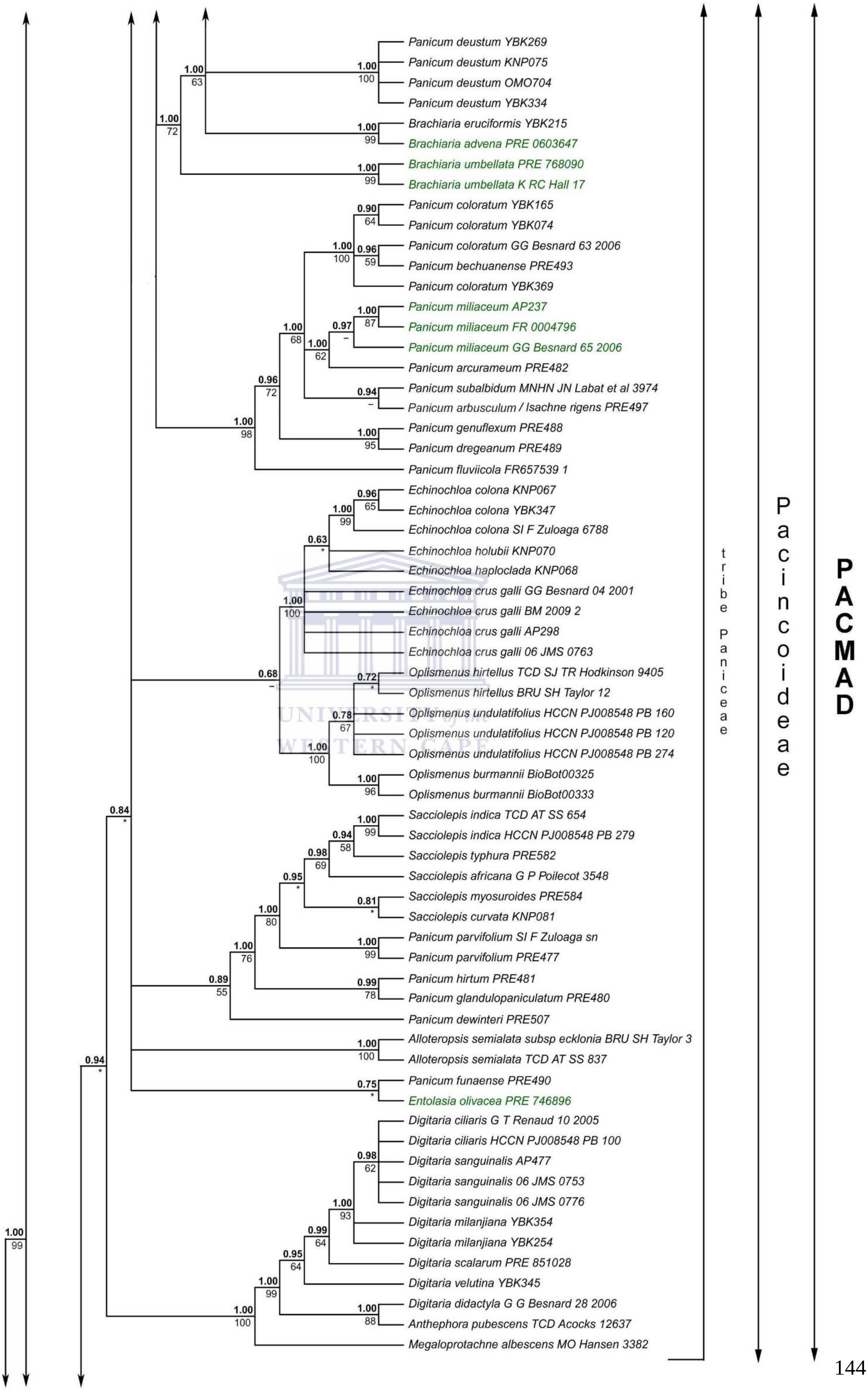
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Panicum arbusculum / Isachne rigens PRE497
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Sacciolepis indica TCD AT SS 654
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Sacciolepis typhura PRE582
Sacciolepis africana G P Poilecot 3548
Sacciolepis myosuroides PRE584
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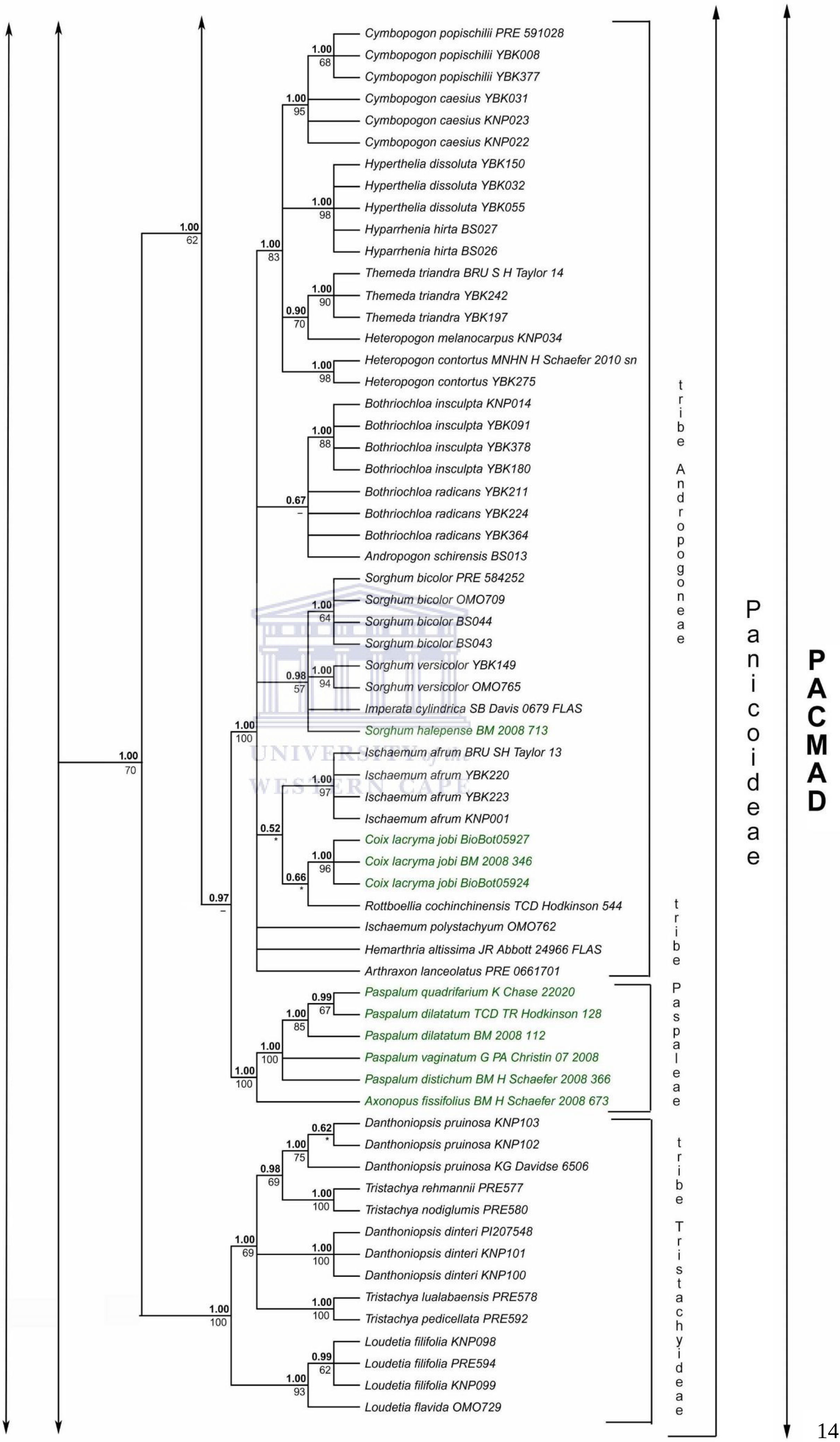
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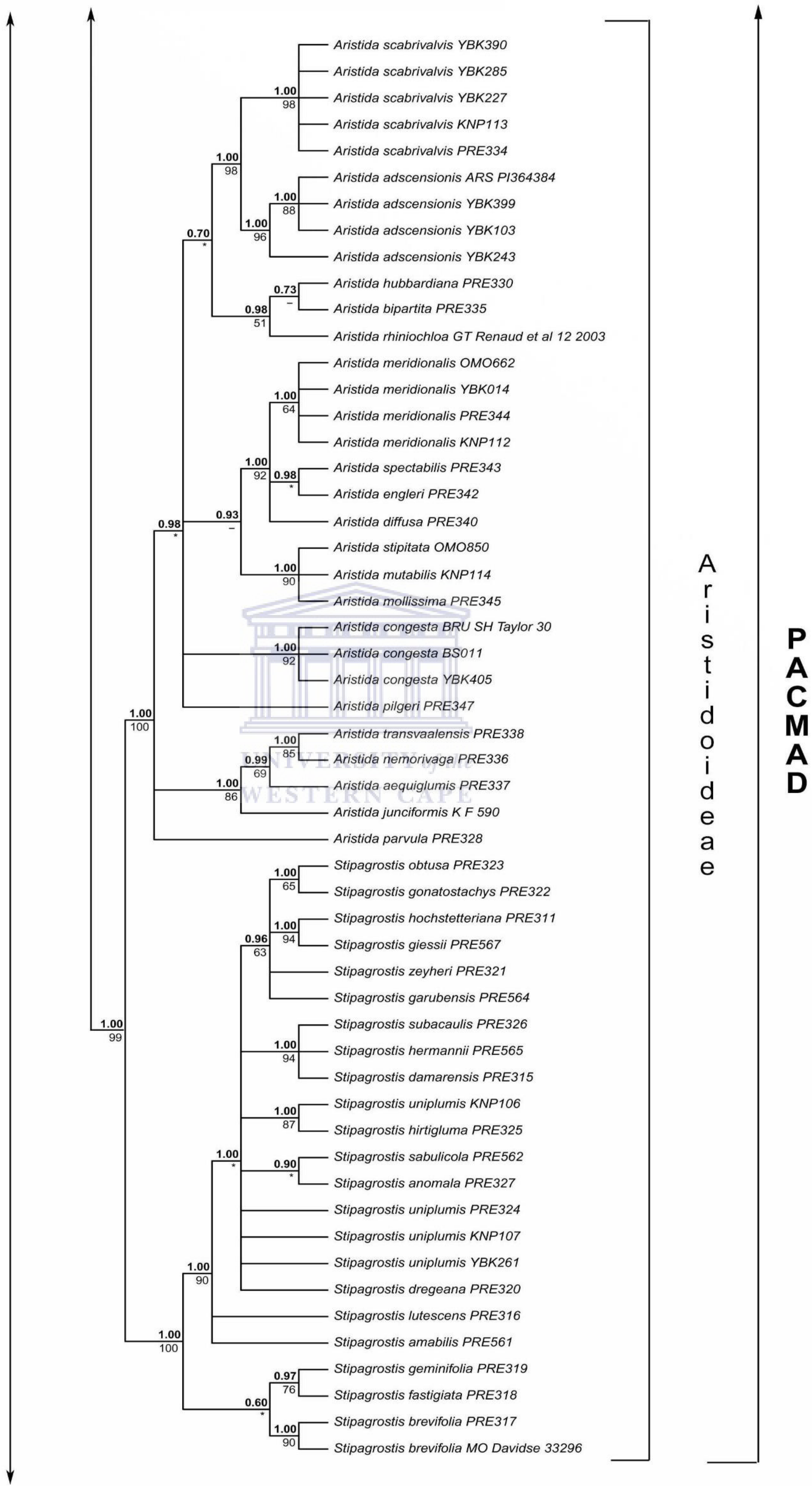
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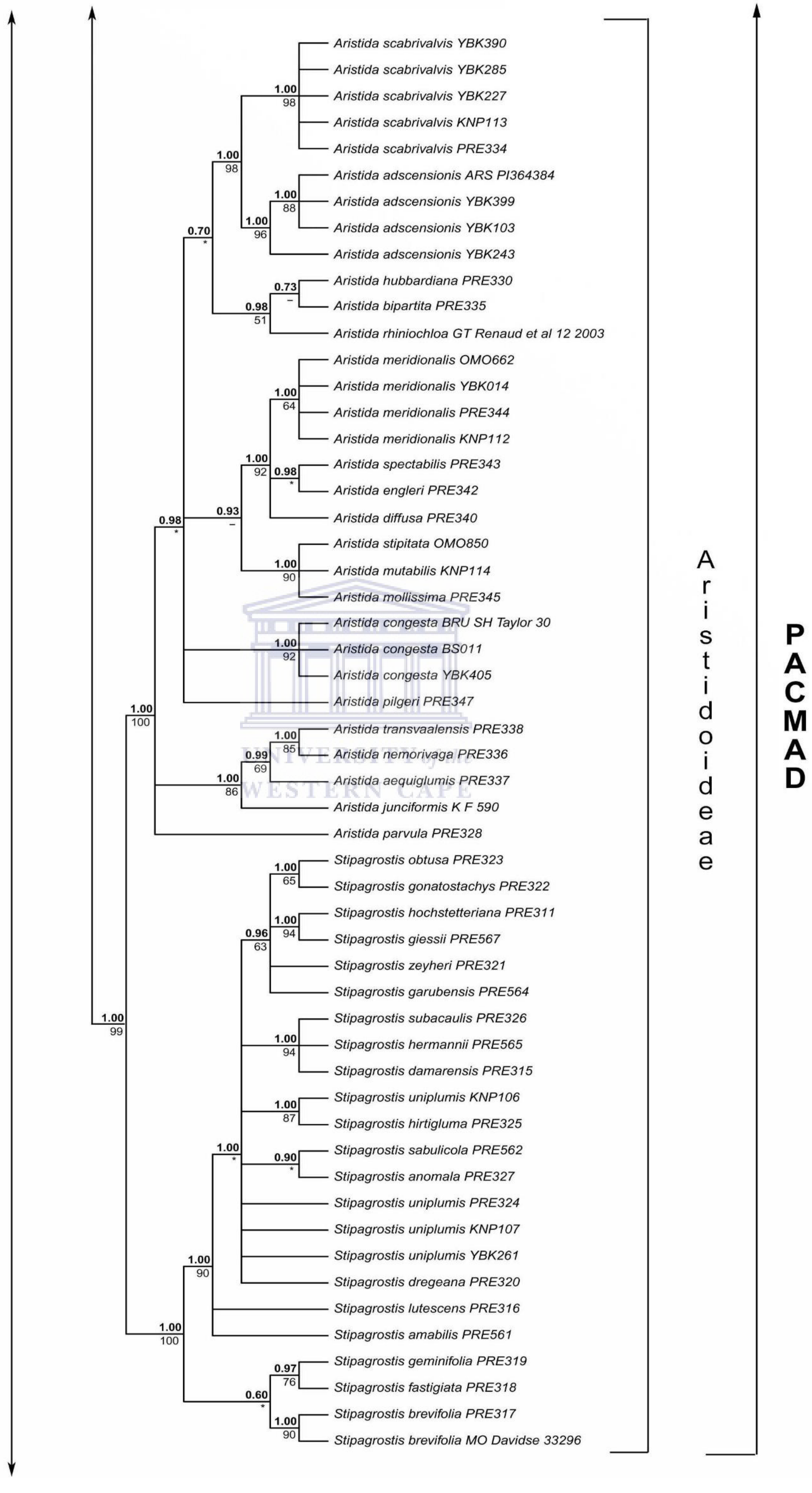
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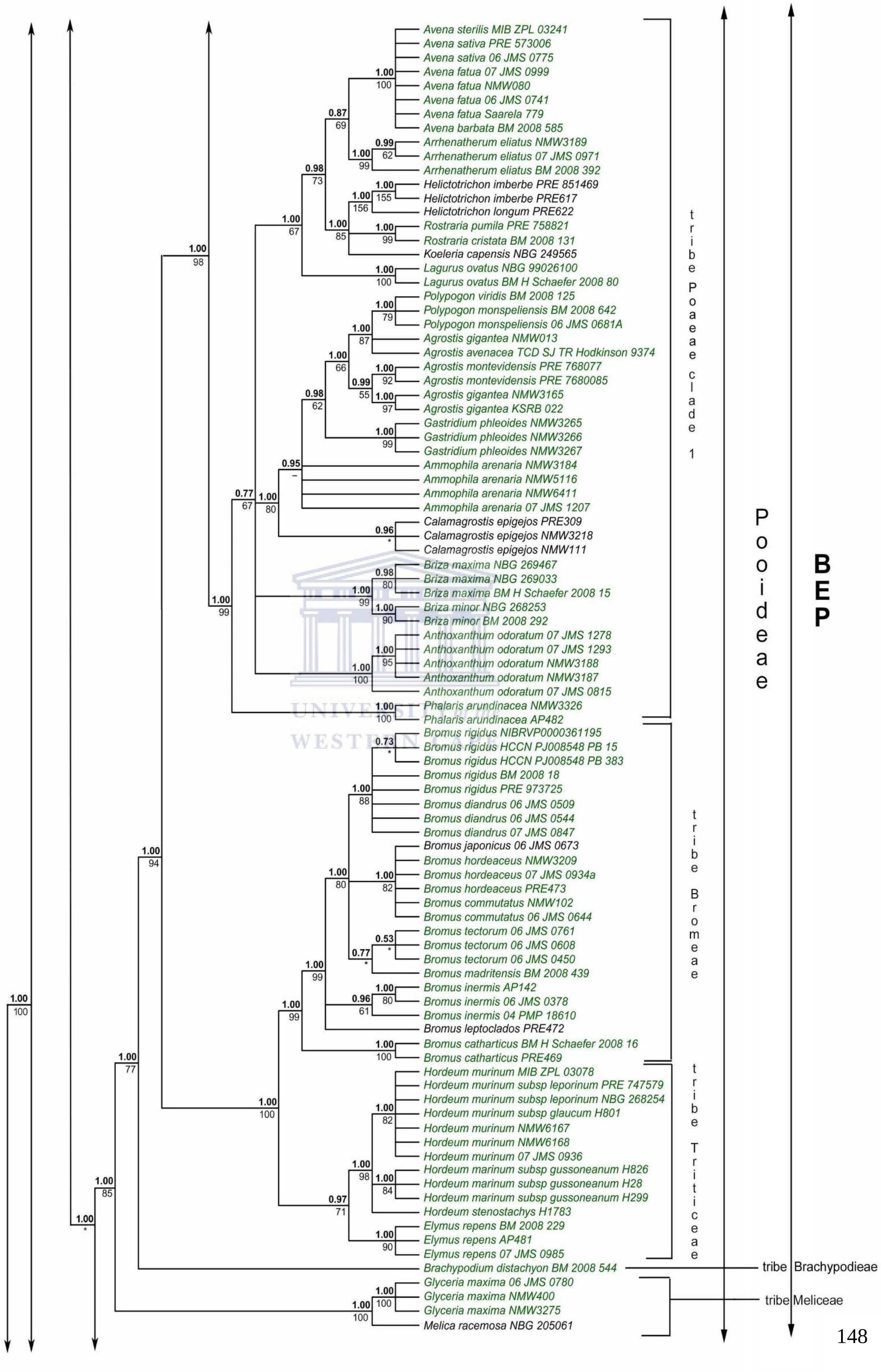
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Bromus catharticus PRE469
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Hordeum marinum subsp gussoneanum H826
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tribe Poaeae clade 1
tribe Bromeae
tribe Triticeae
tribe Brachypodieae
tribe Meliceae
Pooideae
BEP
1.00 100
0.87 69
0.99 62
1.00 99
0.98 73
1.00 155
1.00 156
1.00 67
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1.00 79
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0.95 –
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1.00 100
0.73 *
1.00 88
1.00 94
1.00 80
1.00 82
0.53 *
0.77 *
1.00 99
1.00 80
0.96 61
1.00 100
1.00 99
1.00 100
1.00 100
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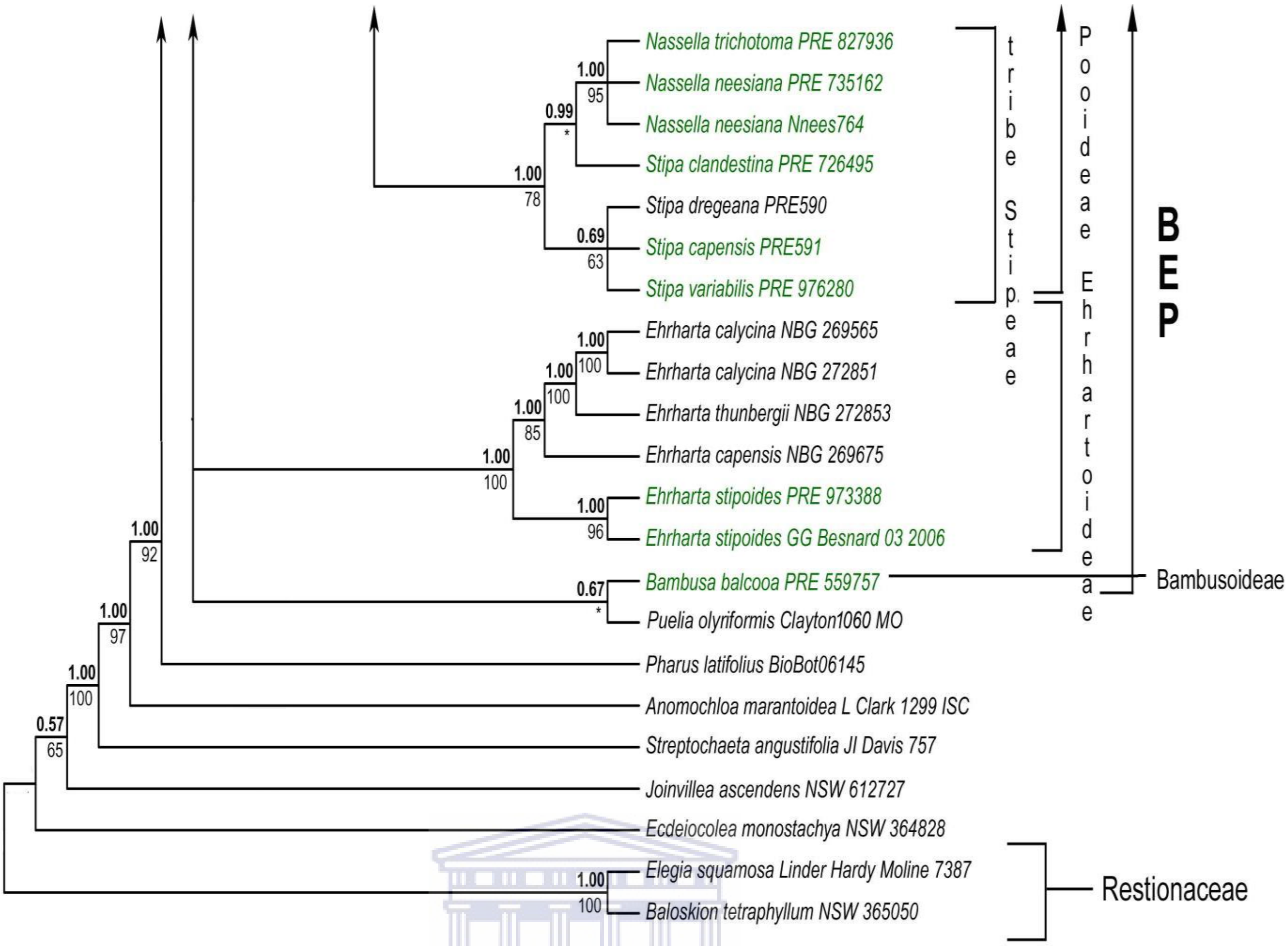


Figure 5.5 50% majority-rule consensus tree of southern African Poaceae obtained from Bayesian inference (BI) analyses based on *rbcLa + matK* data, showing grass tribes and sub-families, and some sister species. BI posterior probabilities (PP) are above branches in bold type and MP bootstrap percentages (BP) >50% are below branches. Species listed as naturalised in Chapter 2 are highlighted in green, - indicates no MP bootstrap support, * indicates a clade is not present or resolved in the MP analysis.

5.2.3 *rbcLa* trees

Both the MP and BI trees constructed with *rbcLa* data were not well-resolved compared to the *matK* and combined data sets. *rbcLa* is known to be a conserved chloroplast gene, and is thus not as useful as a marker for resolving taxa at species level.

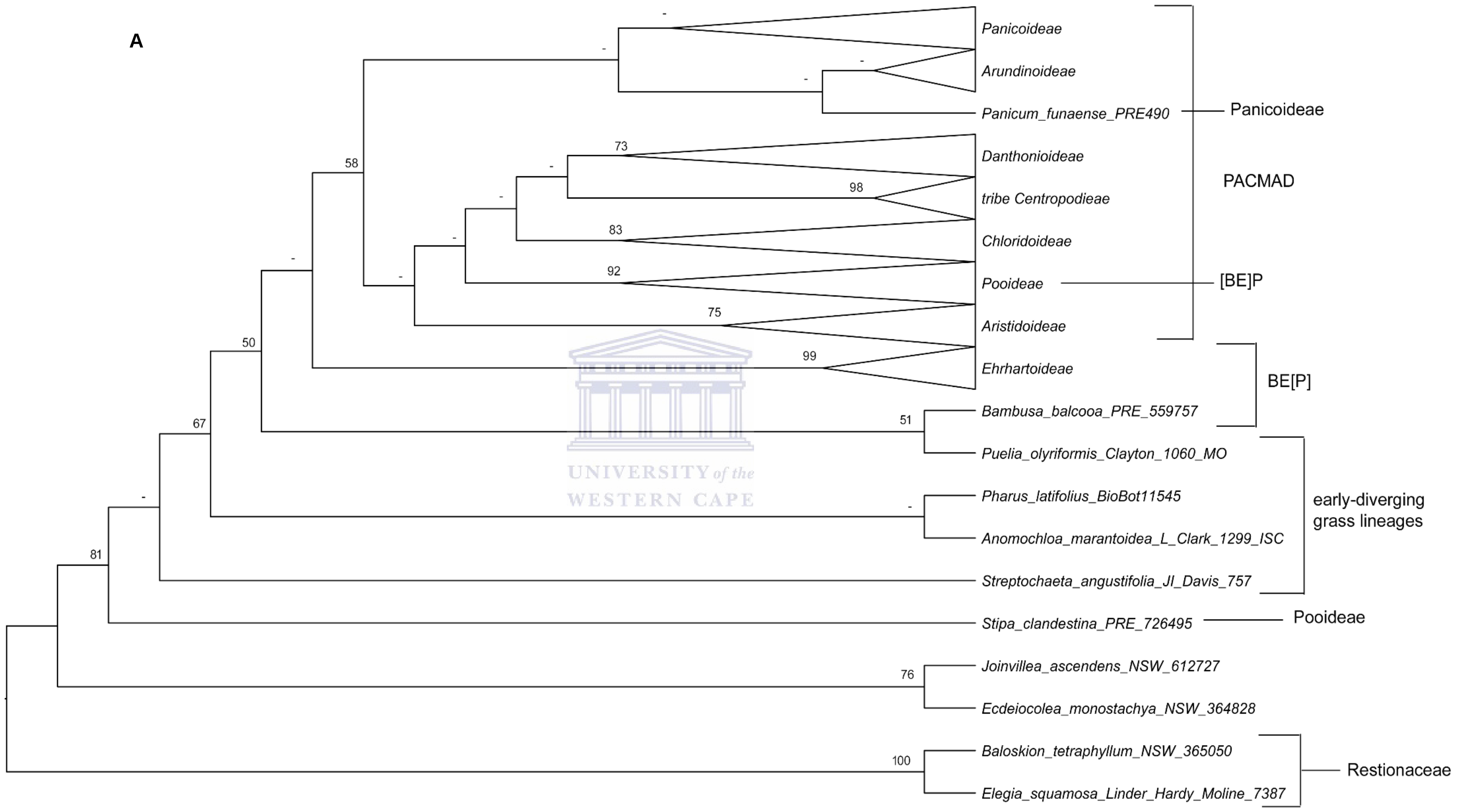
The PACMAD and BEP clades are not well-resolved in either BI or MP analysis of *rbcLa* data. Sub-family Pooideae (apart from *Stipa clandestina*) is nested within the PACMAD clade in both the MP and BI trees (Figure 5.6A & B). Ehrhartoideae is sister to the PACMAD clade in both trees, with *Bambusa* + *Puelia* forming a clade which is sister to Ehrhartoideae and PACMAD + Pooideae in both trees. *Puelia* is from sub-family Puelioideae and not Bambusoideae.

According to the tree published by GPWG II (2012), Aristidoideae should be the most basal sub-family of the PACMAD clade, but in the MP *rbcLa* tree, Panicoideae + Arundinoideae are the most basal of the PACMAD clade (Figure 5.6A), and in the BI *rbcLa* tree, Aristidoideae is nested within sub-family Panicoideae (Figure 5.6B).

In the *rbcLa* MP tree, most sub-families are monophyletic, except for Panicoideae and Pooideae (Figure 5.6A and Appendix 2A). *Stipa clandestina* Hack. belongs to sub-family Pooideae but in the MP tree it is resolved as the earliest diverging grass lineage. The sequence for this taxon matches *Nassella* E. Desv.and *Austrostipa* S.W.L.Jacobs & J.Everett species at 99% in a BLAST search, which indicates that it should nest within sub-family Pooideae. This taxon is in the same position in the BI tree (Figures 5.6B & 5.7). The *rbcLa* sequence for the species in this study does have a few missing bases at the end which may affect its position in the tree.

This *rbcLa* MP tree is the only tree in which sub-family Arundinoideae is resolved as monophyletic, although this clade is not supported. Sub-family Danthonioideae is weakly supported as monophyletic (BP = 74). Tribe Centropodieae of the sub-family Choloridoideae forms a clade with sub-family Danthonioideae in this tree (albeit without support), but the rest of the Chloridoideae are monophyletic with moderate bootstrap support (BP= 83, Figure 5.6A). Sub-family Aristidoideae is monophyletic with moderate support (BP= 75), and the monophyly of Ehrhartoideae and Pooideae (other than *Stipa clandestina*) are well-supported (BP= 99 and 92, respectively, Figure 5.6A).

In the collapsed *rbcLa* BI tree in Figure 5.6B it can be seen that sub-family Aristidoideae is nested within sub-family Panicoideae, although the clade Aristidoideae itself is monophyletic and well-supported (PP = 1.00). Sub-family Arundinoideae is not monophyletic in the *rbcLa* BI tree, and sub-family Pooideae is monophyletic and well-supported (PP = 1.00) except for the species *Stipa clandestina*, which as in the MP tree, resolves as the most early-diverging grass. In both the *matK* and *rbcLa* + *matK* BI trees (Figures 5.4 & 5.5), *Stipa clandestina* is placed within sub-family Pooideae and within tribe Stipeae, as would be expected. Sub-family Chloridoideae is also monophyletic (PP = 1.00) with the exclusion of tribe Centropideae, which is sister to Chloridoideae + Danthoniodeae (although this relationship is only weakly supported, PP = 0.58). Sub-families Danthonioideae and Ehrhartoideae are both monophyletic and well-supported (PP = 1.00 for both, Figure 5.6B).


A
Panicoideae
Arundinoideae
Panicum_funaense_PRE490
Danthonioideae
tribe Centropodieae
Chloridoideae
Pooideae
Aristidoideae
Ehrhartoideae
Bambusa_balcooa_PRE_559757
Puelia_olyriformis_Clayton_1060_MO
Pharus_latifolius_BioBot11545
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Joinvillea_ascendens_NSW_612727
Ecdeiocolea_monostachya_NSW_364828
Baloskion_tetraphyllum_NSW_365050
Elegia_squamosa_Linder_Hardy_Moline_7387
Panicoideae
PACMAD
[BE]P
BE[P]
early-diverging
grass lineages
Pooideae
Restionaceae
-
-
-
-
58
73
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98
-
83
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75
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99
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51
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-
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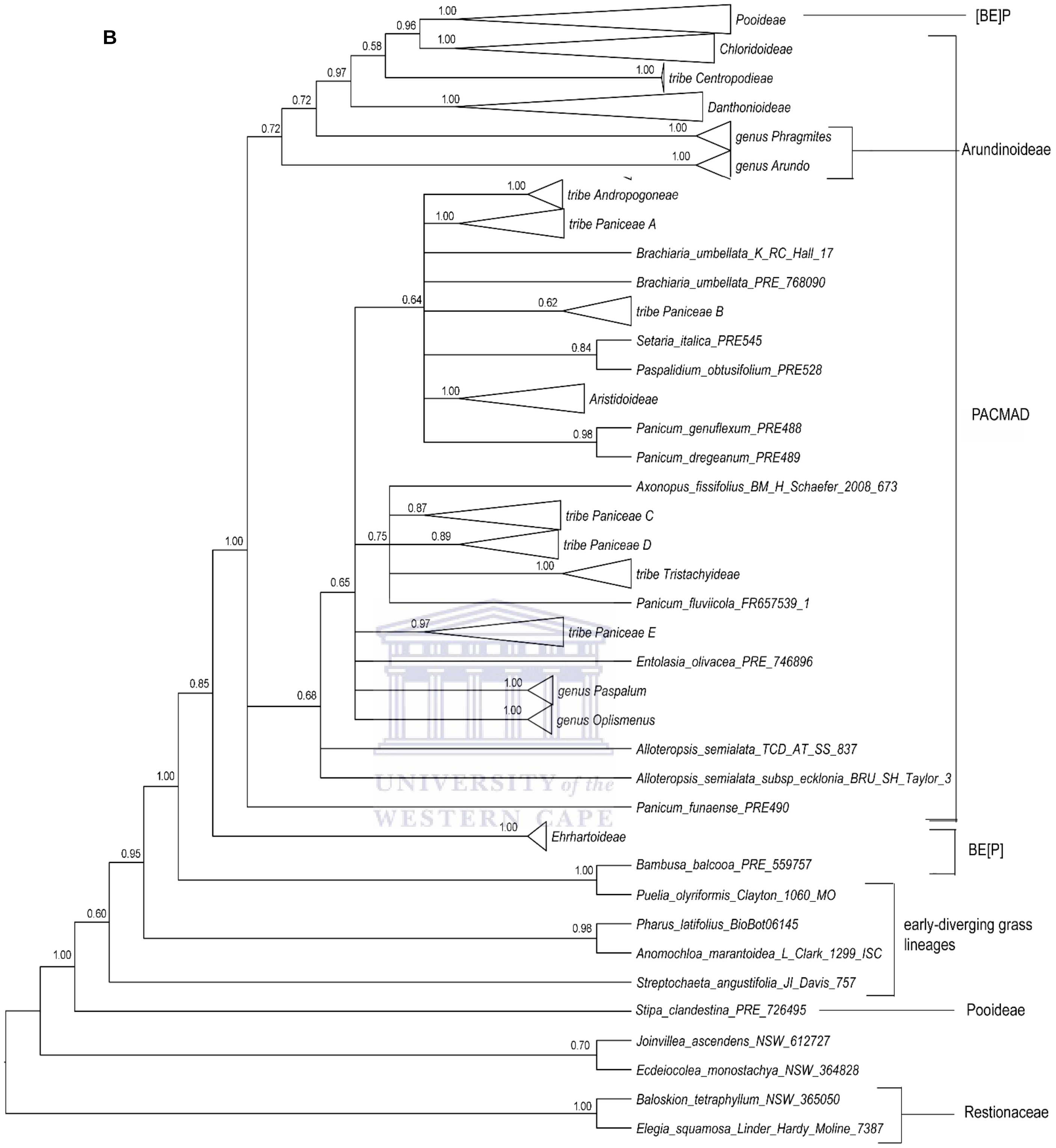
81
76
100



Figures 5.6A & B. Collapsed cladograms constructed from *rbcLa* data, showing grass sub-families and sister species. **A -** strict consensus tree obtained from Maximum Parsimony (MP) analysis. MP bootstrap percentages (BP) are above branches, – indicates no bootstrap support. **B** - 50% majority-rule consensus tree obtained from Bayesian inference (BI) analysis. BI posterior probabilities (PP) are above branches.

Many of the grass tribes are not monophyletic in the *rbcLa* BI tree (Figure 5.7). In sub-family Pooideae, Poaeae clades 1 and 2 are not monophyletic, nor is Triticeae (represented by *Hordeum* and *Elymus* in this study) nor Bromeae (represented by the genus *Bromus* in this study), although some of the tribes that are represented by fewer taxa, namely Meliceae (represented by the genera *Glyceria* and *Melica* in this study, PP= 1.00, BP = 86, Figure 5.7) and Stipeae (represented by the genera *Stipa* and *Nassella* in this study, support of PP = 1.00, BP = 69) are monophyletic.

Within sub-family Panicoideae some tribes are found to be monophyletic in the *rbcLa* BI tree. Tribes Andropogoneae and Tristachyideae are monophyletic and well-supported with PP = 1.00 for both. Tribes Paniceae and Paspaleae are not monophyletic in the BI tree (Figures 5.6B and 5.7). Tribe Paniceae is highly fragmented into multiple clades (Figure 5.6B).

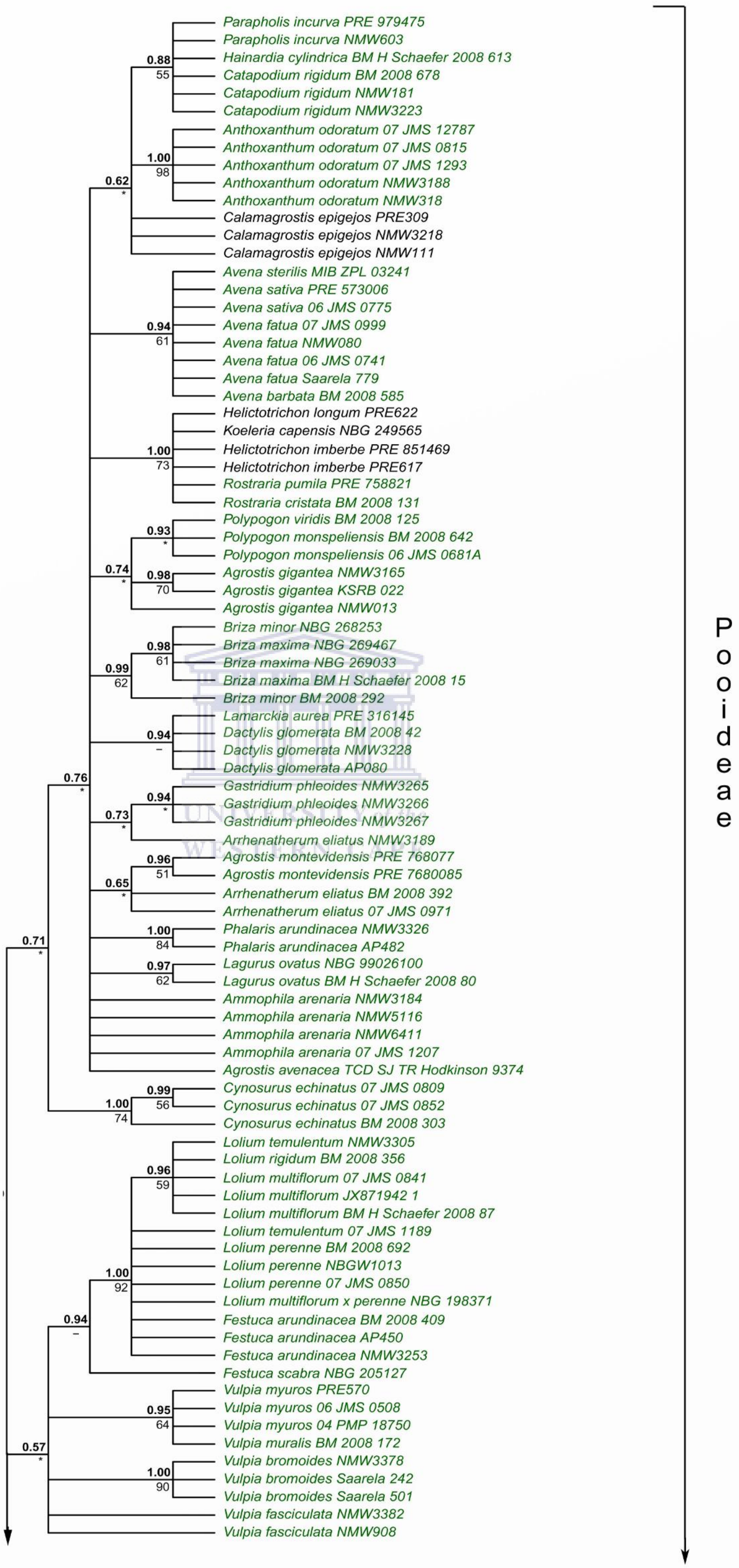
In sub-family Chloridoideae only one tribe, Centropodieae (represented by one genus, *Ellisochloa*) is monophyletic, with strong support (PP= 1.00, BP = 98, Figure 5.7). The other sub-families (Aristidoideae, Bambusoideae, Danthonioideae, and Ehrhartoideae) are all only represented by one tribe in the *rbcLa* data in this study.

In both *rbcLa* trees, *Pharus latifolius* and *Anomochloa marantoidea* form a clade, although they are in different grass sub-families. The sister clade to Poaceae in the two *rbcLa* trees is Ecdeiocoleaceae + Joinvilleaceae (Figures 5.6A & B), with strong support in both trees (PP = 1.00, BP = 100), unlike in the *matK* and combined data trees, where the sister clade to Poaceae is resolved as Joinvilleaceae, as mentioned above.

In general for the BI *rbcLa* tree (Figure 5.7), genera do tend to cluster together, although there are numerous exceptions. Many polytomies are present in the BI *rbcLa* tree, particularly in the sub-family Chloridoideae in the genera *Pogonrarthria* and *Eragrostis.* Another example is in sub-family Panicoideae amongst the genera *Ischaemum* L., *Heteropogon* Pers., *Hemarthria* R.Br., *Coix* L., and *Cymbopogon* Spreng.

According to the SPIDER analysis of a neighbour-joining tree in Chapter 4, about 66% of genera are monophyletic when singletons are excluded for the *rbcLa* data. Genus *Sporobolus* in sub-family Chloridoideae is an example of an unresolved genus in the *rbcLa* BI tree (Figure 5.7). This genus is monophyletic in the *matK* and combined data trees, as mentioned above. At species level only 60% of taxa were found to be monophyletic (without bootstrap support) in the SPIDER analysis of an NJ tree of *rbcLa* data without singletons (Chapter 4).

As so many species are not monophyletic, not well-supported, and as there are numerous polytomies present, *rbcLa* alone is not a useful marker on its own for examining phylogenetic relationships between the grass species of southern Africa. The *rbcLa* marker also did not fully resolve relationships at the tribal or sub-familial level.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Calamagrostis epigejos NMW111
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Avena sativa 06 JMS 0775
Avena fatua 07 JMS 0999
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Avena barbata BM 2008 585
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Agrostis gigantea NMW013
Briza minor NBG 268253
Briza maxima NBG 269467
Briza maxima NBG 269033
Briza maxima BM H Schaefer 2008 15
Briza minor BM 2008 292
Lamarckia aurea PRE 316145
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Dactylis glomerata NMW3228
Dactylis glomerata AP080
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Gastridium phleoides NMW3266
Gastridium phleoides NMW3267
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Festuca arundinacea AP450
Festuca arundinacea NMW3253
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1.00
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*
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61
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0.94
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0.76
*
0.94
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0.73
*
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0.65
*
1.00
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*
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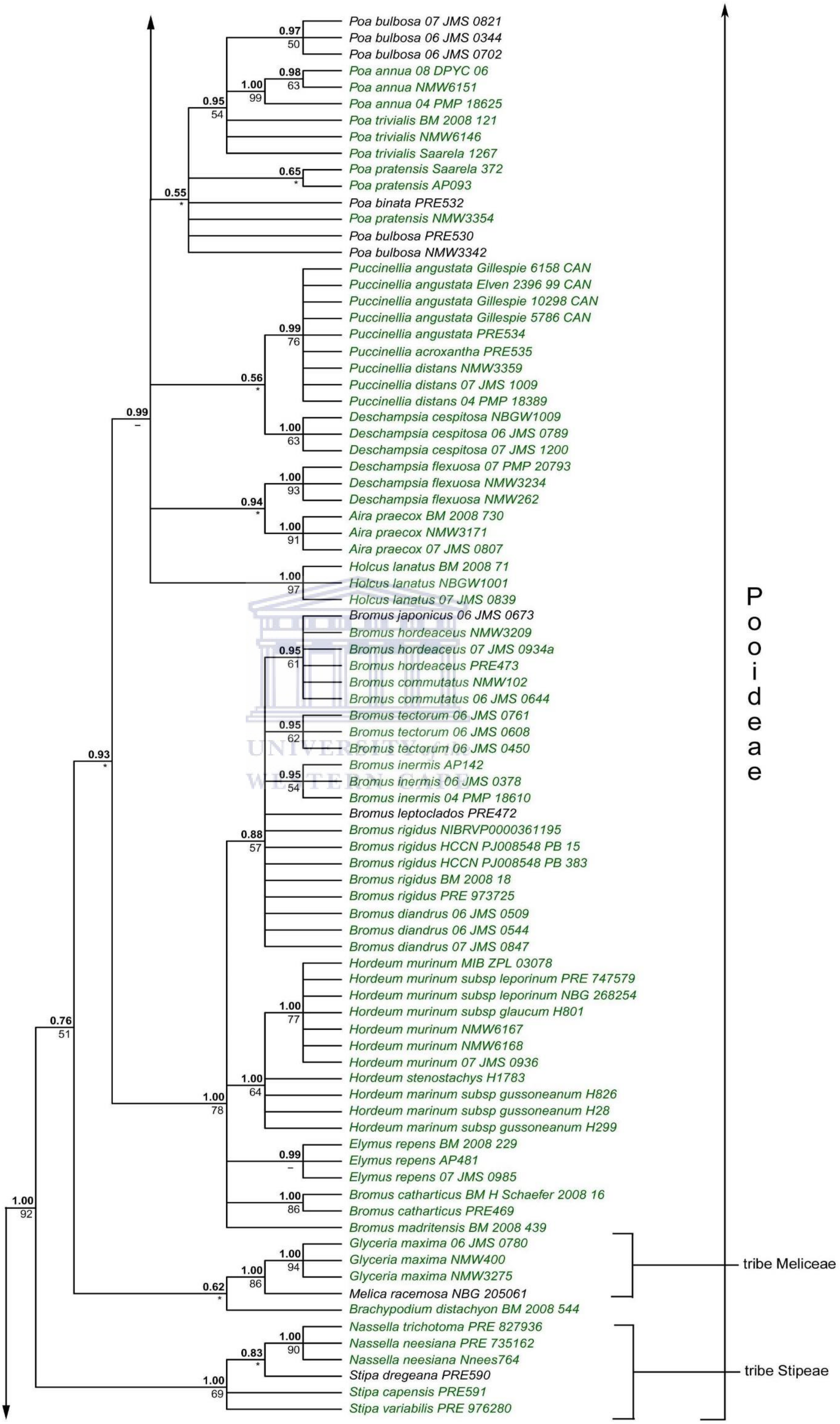
Pooideae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tribe Stipeae
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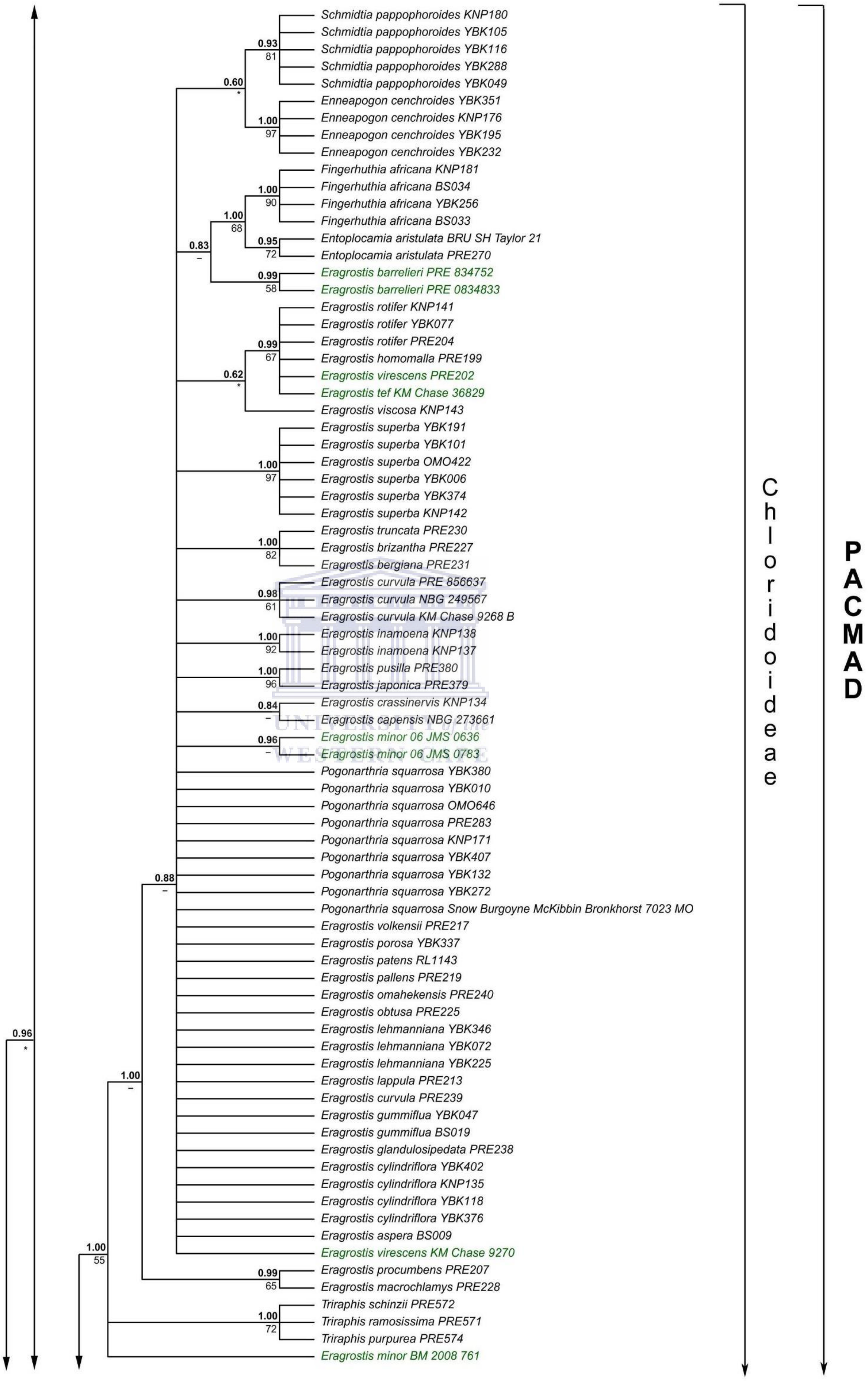
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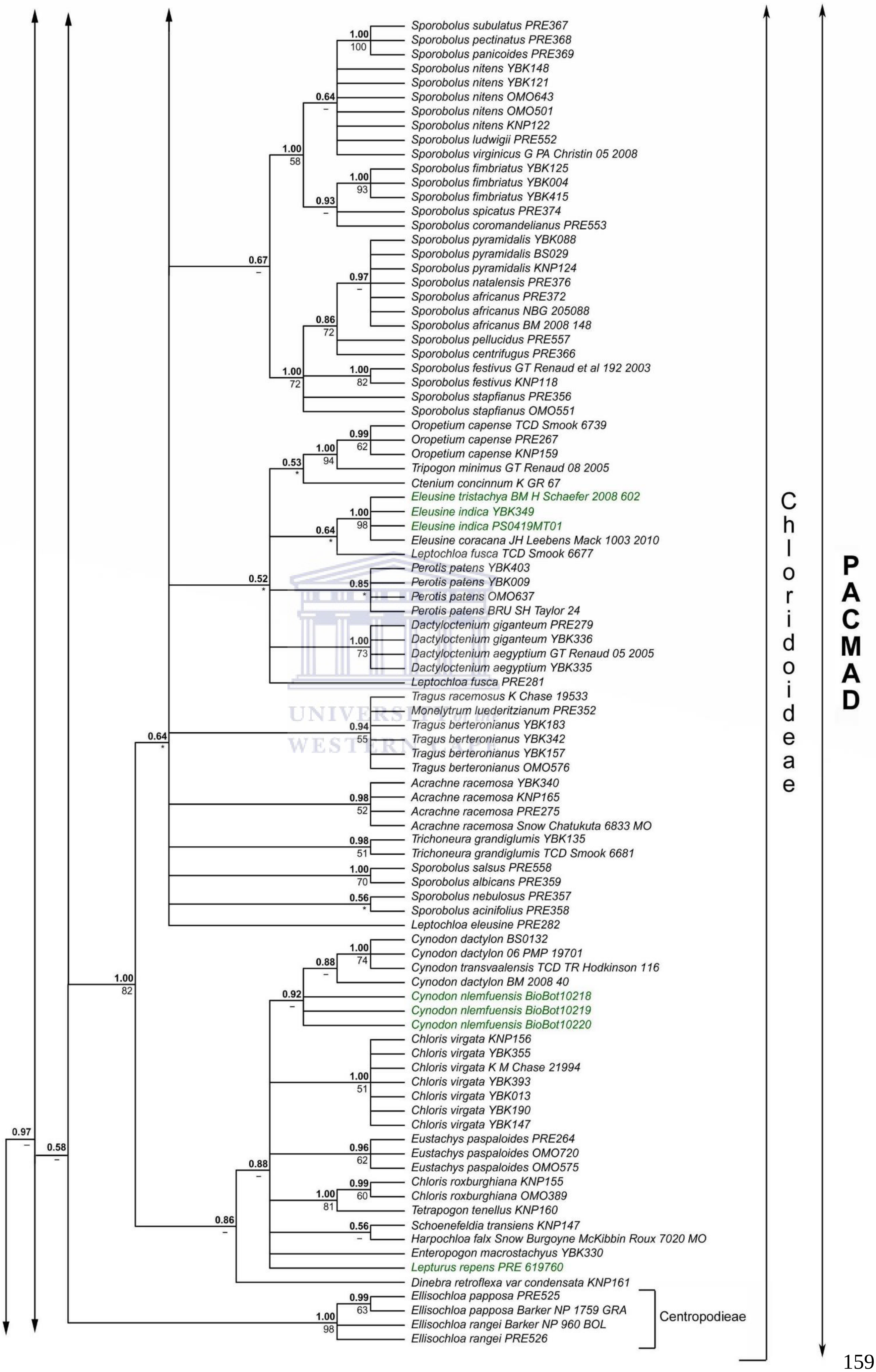
PACMAD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Sporobolus africanus PRE372
Sporobolus africanus NBG 205088
Sporobolus africanus BM 2008 148
Sporobolus pellucidus PRE557
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Sporobolus festivus KNP118
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Chloridoideae
PACMAD
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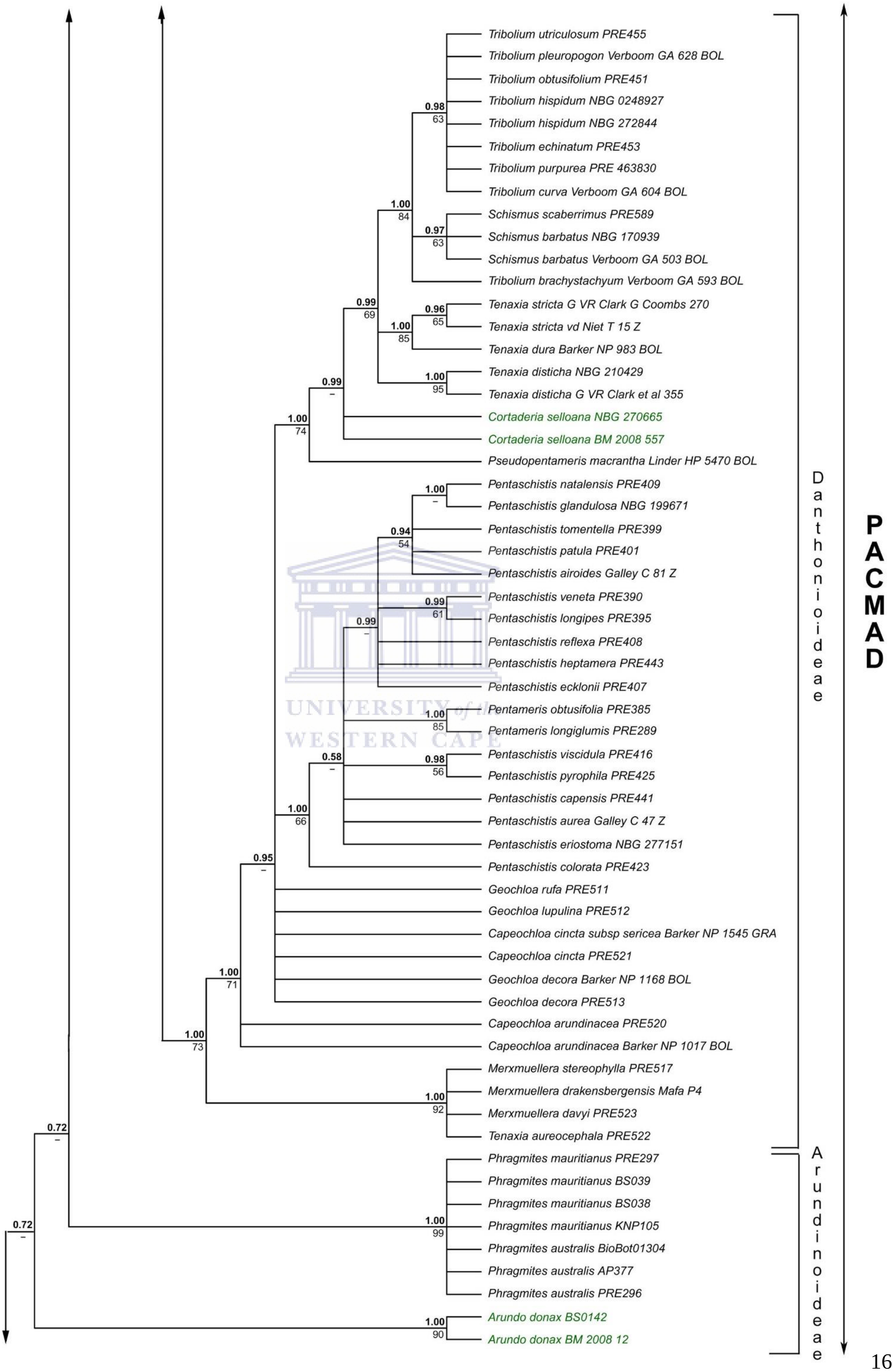
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Pentaschistis reflexa PRE408
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1.00 66
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Arundinoideae
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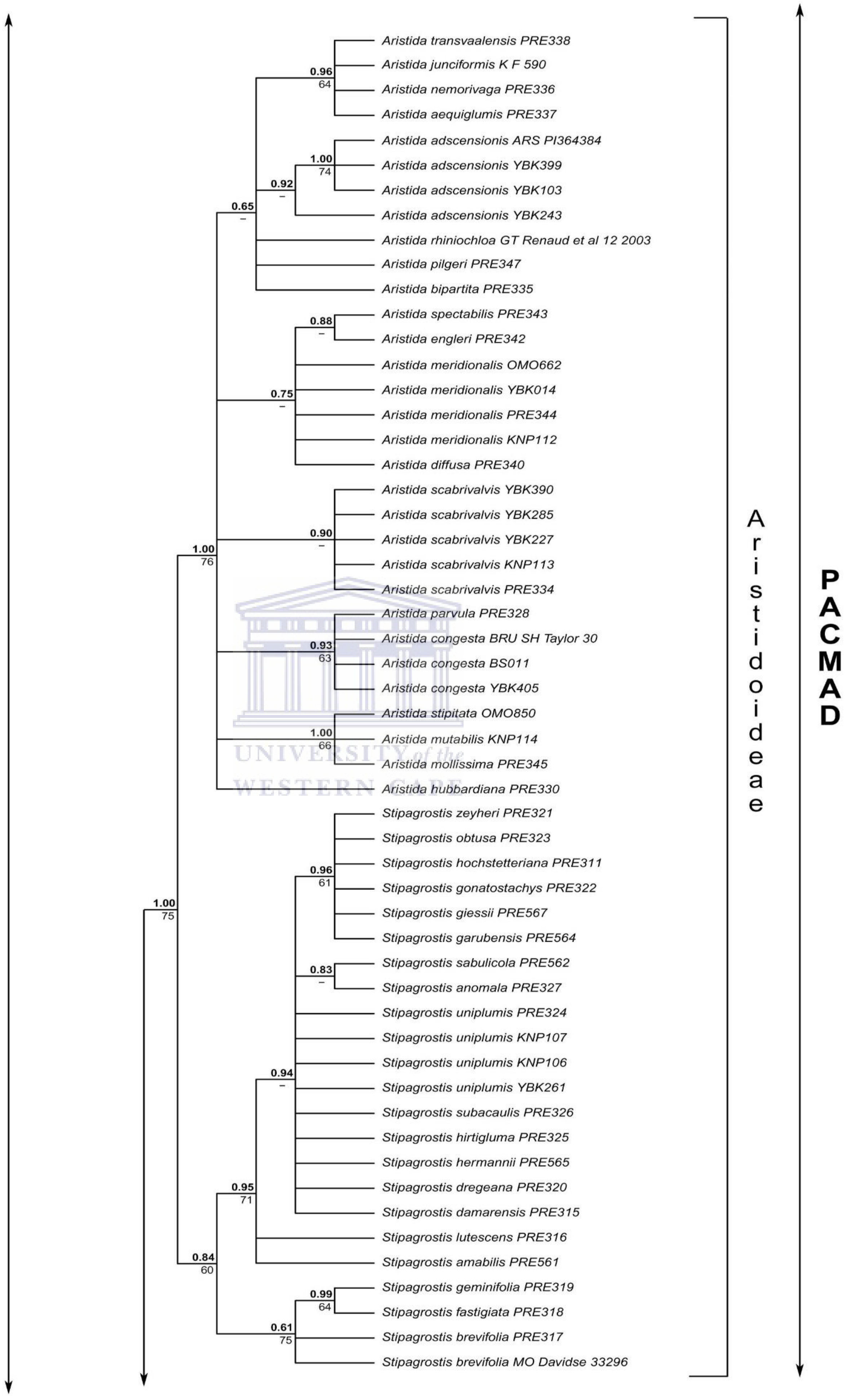
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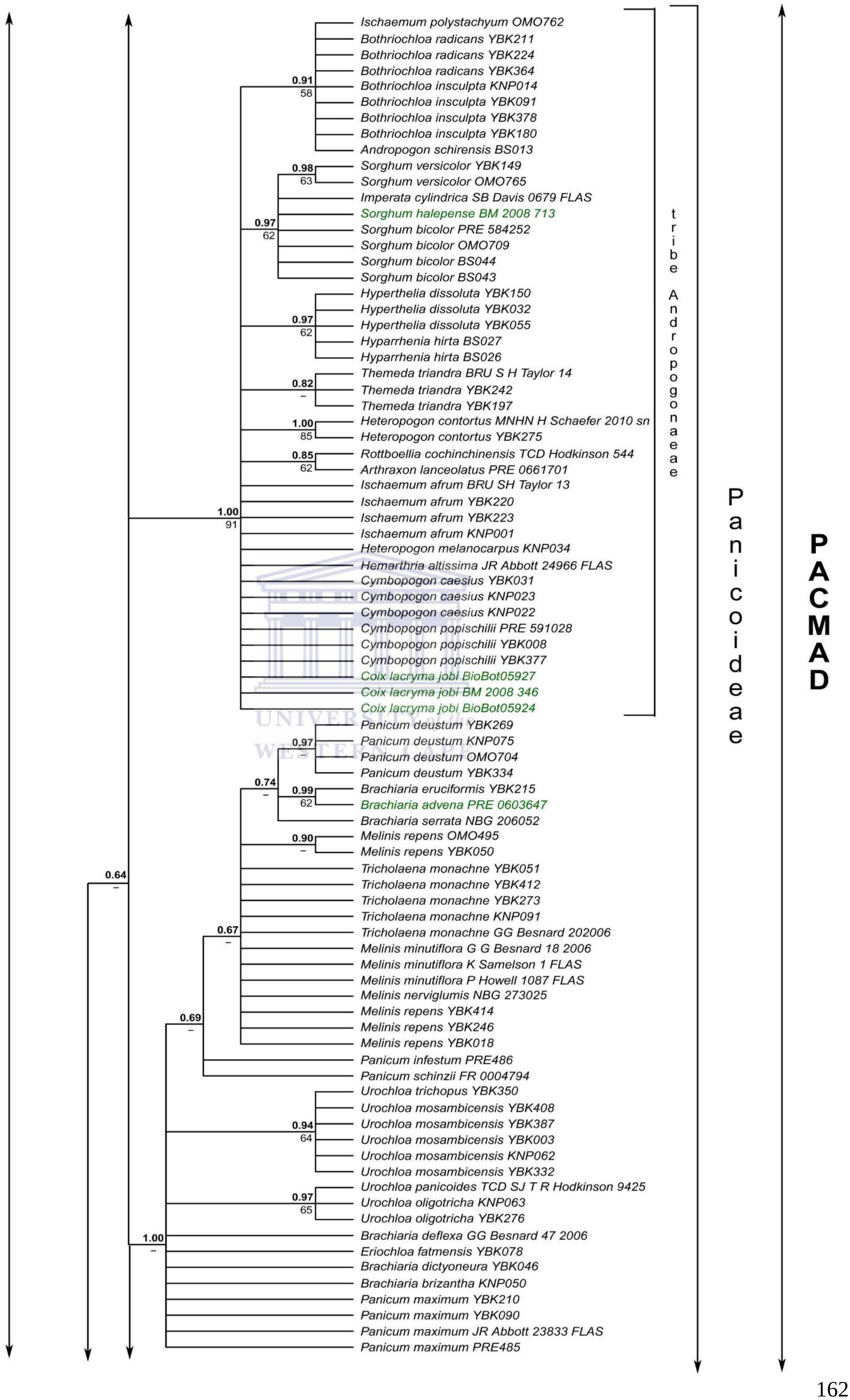
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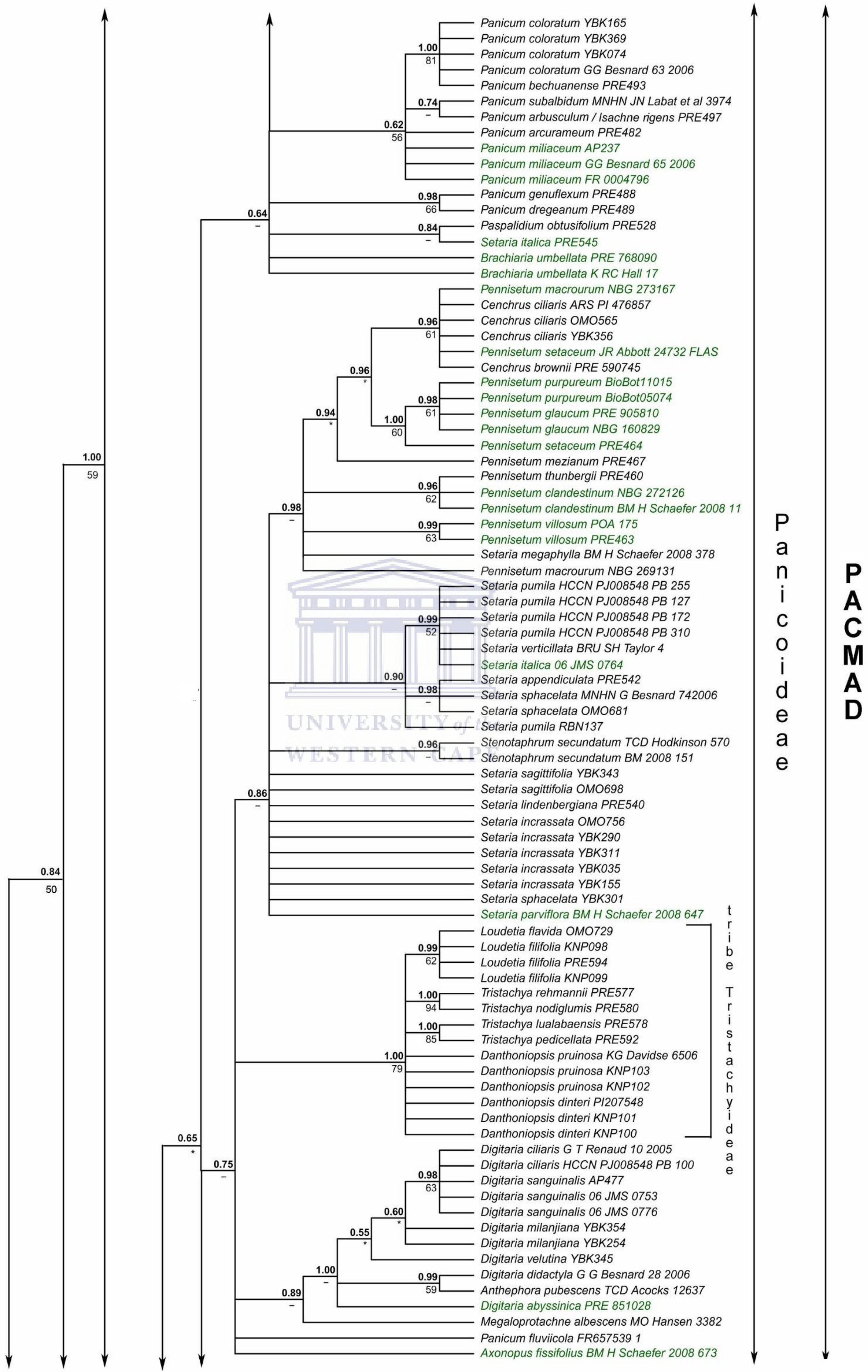
PACMAD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56
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Panicum dregeanum PRE489
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Setaria pumila RBN137
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Setaria sphacelata YBK301
Setaria parviflora BM H Schaefer 2008 647
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Panicoideae
PACMAD

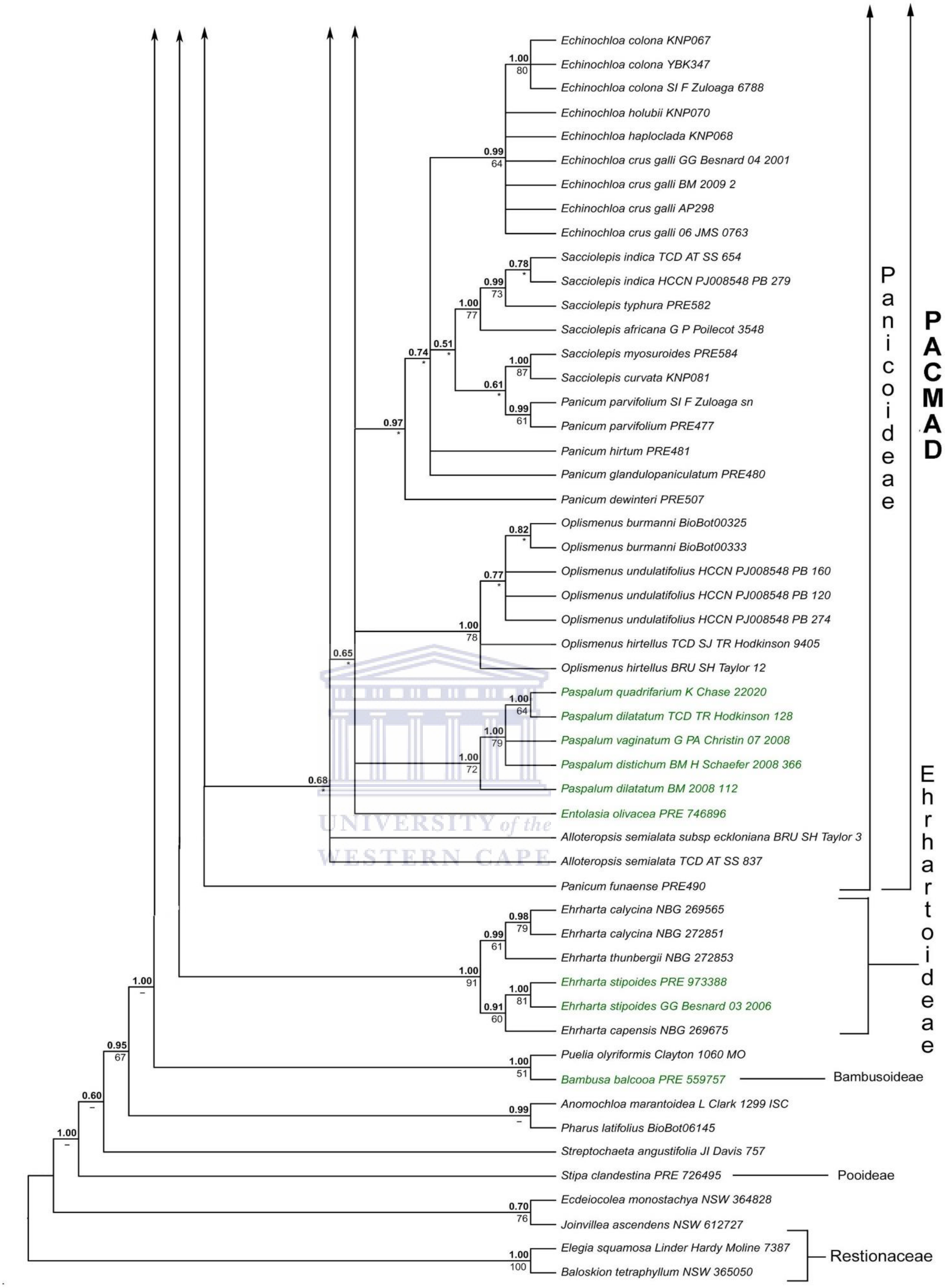


Figure 5.7. 50% majority-rule consensus tree of southern African Poaceae obtained from Bayesian inference (BI) analyses based on *rbcLa* data, showing grass tribes and sub-families, and some sister species. BI posterior probabilities (PP) are above branches in bold type and MP bootstrap percentages (BP) >50% are below branches. Species listed as naturalised in Chapter 2 are highlighted in green, - indicates no MP bootstrap support, * indicates a clade is not present or resolved in the MP analysis.

5.3 Conclusions

While it is generally advised in the literature that DNA-barcoding identification and species delimitation studies should not be integrated (Will & Rubinoff 2004, DeSalle *et al.* 2005, Wheeler 2005, Ebach *et al*. 2008), there is valuable phylogenetic information contained in DNA barcoding markers, which are often the same markers used in systematics studies except that they are usually shortened for ease of use.

As can be seen in the *matK* and *rbcLa + matK* data trees in this study, the two official DNA-barcoding markers can provide good resolution at sub-family and tribe level, with many genera and species also being resolved. However, the overall topologies of the BI trees generated from these two data sets do differ from those of the more recently published trees constructed for family Poaceae, particularly in the arrangements of the BEP and PACMAD clades and their relationships to each other, as discussed above (Bouchenak-Khelladi *et al.* 2008, GPWG II 2012). Both Bouchenak-Khelladi *et al.* (2008) and the GPWG II (2012, Figure 5.1) used three markers to construct their trees, with *matK* and *rbcL* being two of the three markers in both studies. Full gene sequences were used in these studies, rather than the shorter fragments used for DNA-barcoding. The extra marker and longer sequences may have provided the extra resolution seen in the data in these studies in comparison to the data in the present study.

The combined *rbcLa* + *matK* data provided the best species resolution, and *matK* on its own also provided good species resolution, although neither data set were sufficient for resolving all species. *rbcLa* did not perform as well, as was expected, and did not even resolve all grass tribes, although sub-families were generally resolved.

As the phylogeny of the family Poaceae has already been extensively studied with the use of multiple molecular markers combined with morphological characters, this study did not aim to elucidate new undiscovered relationships within the family or improve upon the results already obtained, but it did show that the DNA barcoding markers do contain valuable phylogenetic information, and this data can be combined with other molecular markers and morphological characters to contribute to more in-depth systematics studies, as well as being used for species identification. In fact many of the recently published trees for family Poaceae used the *matK* and *rbcLa* markers in combination with other molecular and/or morphological markers (GPWG 1 2001, Bouchenak-Khelladi *et al.* 2008, GPWG II 2012). As tree-based analyses are currently a popular (although controversial) method of identification in DNA barcoding (Will & Rubinoff 2004, Meier *et al.* 2008), it is useful to examine the performance of the DNA barcoding markers when used in tree reconstruction.

Chapter 6. General Conclusions

Plant invasions have been estimated to cost South Africa about R6.5 billion each year, according to De Lange & Van Wilgen in 2010. That cost may have increased considerably since then. Methods of eradication have and are being researched to deal with existing invasions (Musil *et al.* 2005, Klein 2011, Van Wilgen *et al*. 2012) and legislation regarding usage and control of alien plants has been introduced (NEM:BA 2014). The SANBI Invasive Species Programme (ISP) aims to use early detection methods to rapidly detect new and potential invasions and to prevent them from becoming persistent and thereby reduce the costs of managing and clearing invasions (Wilson *et al.* 2013). In this regard, particularly for plant groups which are challenging to identify using traditional morphological classifications, alternative methods of identification such as DNA barcoding are currently being investigated. Family Poaceae is one such plant family that has proven to be a challenge, as grasses are morphologically relatively homogenous, apart from flower structure. Grass invasions are generally considered of lesser concern in South Africa at present, although there are known grass invasions, particularly in wetland and riparian ecosystems, roadsides and other disturbed areas such as old mines and agricultural land, as well as in the renosterveld of the Western Cape winter-rainfall region (Milton 2004). There are concerns that with global climate change, grass invasions may increase in this region (Milton 2004). There are currently few grass identification experts in South Africa, and it is hoped that DNA barcoding may provide a relatively quick and affordable method of identification of invasive grasses that may allow early detection of emerging grass invasions.

This study aimed to list as many of the naturalised grasses in South Africa as possible so that a DNA barcode library could be assembled for these grasses. In the list compiled as part of this study, 128 naturalised grass species and sub-species were listed (Chapter 3), but it is possible that some naturalised grasses in South Africa have not been sampled or detected. Also many grasses are considered “of unknown origin” and may be naturalised. Alien grasses that may potentially become naturalised, and those used in agriculture, horticulture or for biofuels, are not included in this list. A more comprehensive list of all alien grasses in South

Africa is presently being compiled by the National Working Group on Alien Grasses. Research could be carried out on some of the grasses of unknown origin in order to establish whether they are to be considered naturalised or native.

It could also be worth investigating why some grasses are more successful invaders in South Africa than others. Trait analyses are one type of study that have been used to investigate what makes a plant a successful invader (Baker 1965, Richards *et al.* 2006, Pyšek & Richardson 2007, Gallagher *et al*. 2011, Te Beest *et al.* 2011, Skálová *et al.* 2012), and there is the potential to investigate whether the invasive grasses in South Africa share a common set of traits or if there is another set of factors that determines their success. The DNA barcoding data presented in this study may potentially be used in conjunction with a trait analysis to test for a phylogenetic signal in the results (Lloret *et al*. 2005, Procheş *et al*. 2008, Cadotte *et al.* 2009, Davis *et al*. 2010).

There is now a large database of both native and naturalised grass barcoding sequences for southern Africa available from this study from a combination of sources; samples from South African herbaria extracted in this study and by the ACDB in their grasses of southern Africa project, as well as sequences downloaded from the BOLD and GenBank databases. This database does not include *rbcLa* and *matK* samples for all of the naturalised grasses as listed in Chapter 3, due to lack of available samples or due to PCR issues. Some samples are represented by *matK* only, if no *rbcLa* samples were available or if the *rbcLa* PCR failed. *matK* was considered a more important marker to collect than *rbcLa*, as it is more likely that *matK* can be used as a single marker DNA barcode due to its greater variability.

The database only represents about 43-45% of the grasses found in South Africa, and does not include every native grass sequence available in the BOLD or GenBank databases, so there is much scope for increasing the number of native species represented in the database. Every effort was made to include three replicates of each naturalised and native grass species chosen for the database, but this number of replicates was not always achievable due to lack of samples, lack of online data and issues with PCR. It would be ideal to increase the number of replicates of each grass already in the database to at least three in the future, as having three replicates increases the chances of detecting an incorrectly identified or labelled species in the dataset, as well as allowing for an increased chance of a positive identification of an unknown species, due to variations in available sequence length or potentially missing data in the target sequences, as well as taking natural variation within a species into account. Potentially incorrectly labelled or identified specimens or sequences were occasionally encountered in this study, and it tends to be more of an issue with data taken from GenBank, where sequence data is not electronically linked to an actual herbarium scan of the sequenced specimen, which is how data is presented on the BOLD database. In the past, DNA barcoding has been criticised for attempting to bypass or replace accurate taxonomic identifications and species delimitations, (Will & Rubinoff 2004, DeSalle *et al.* 2005, Wheeler 2005, Ebach *et al*. 2008) but in fact it is presently very difficult to separate the two and have accurately labelled and identified sequence data.

Other problems encountered in the compilation of this DNA barcode database included working with old and degraded DNA samples, inhibition of PCR by polyphenolic compounds, which were not removed by the DNA extraction method used, and issues with *matK* primers that were not specific enough to amplify the grass samples. The inclusion of PVP or another additive into the DNA extraction or PCR protocols may eliminate the inhibition of PCR by the compounds present in some of the grass specimens. This may also improve the amplification of the *matK* DNA barcoding fragment with the widely used universal primers, and may reduce the need for specific *matK* primers, but this was not tested in this study. At present the best *matK* PCR results were obtained by using internal primers including a commonly used universal primer and a primer that is more specific to family Poaceae or order Poales. Due to the extra work and cost involved in these extra steps it is worth testing ways of reducing these steps so that only one PCR and one set of preferably universal primers is required for amplification, as some other studies have had success with the universal *matK* primers (Ragupathy *et al.* 2009, Drumwright *et al.* 2011, Saarela *et al.* 2013).

Once the DNA barcode data was assembled, it was tested for identification efficacy using SPIDER in R. *rbcLa* and *matK* were tested independently and in combination, using three distance based methods of identification (k-NN, BCM and BOLD threshold ID), one tree-based method (species monophyly) with two additional methods that increase the stringency of the tree-based method. The DNA barcode gap was also examined.

The combined *rbcLa* + *matK* data set was generally the best-performing DNA barcode. 81% of individuals in the combined data were calculated to have a barcode gap where the largest intraspecific distance was greater than the smallest interspecific distance (Table 4.2), and 91% of individuals were correctly identified using the Nearest Neighbour criterion (the highest overall species identification success rate) when singletons were excluded from the combined data (Table 4.4). The combined data barcode is currently the official barcode recommended by CBOL for plants (CBOL 2009), so using these two markers for invasive grass identification would be in line with the current protocol for DNA barcoding in plants.

If a single marker was to be chosen to reduce the workload and costs involved in making rapid identifications, then based on this study, *matK* should be chosen over *rbcLa*, although *rbcLa* is usually easier to PCR and sequence using universal primers. In this study, initially *rbcLa* had a higher PCR success rate than *matK*, but once specific *matK* primers were used to amplify two smaller overlapping fragments, then the overall PCR success rate was higher in *matK* than in *rbcLa*. I would not consider this a reliable indication as to the PCR success rates of these two markers though, as compounds inhibiting PCR were present in many of the samples and this made final success rates impossible to measure in this study. In family Poaceae, the *matK* gene has few variable indels and the MUSCLE algorithm was able to align the sequences with few manual adjustments required.

The *matK* data without singletons had a highest identification success rate of 84% with the Nearest Neighbour criterion and 68% of individuals had a barcode gap (Table 4.2), in comparison to the *rbcLa* data, for which 60% of individuals had a barcode gap (Table 4.2), and a highest identification success rate of 76% using the Nearest Neighbour criterion (Table 4.4) for the data without singletons.

While the Nearest Neighbour criterion produced the highest identification success rates in the data without singletons, this method is not currently used to make identifications in practice, but rather as a method of testing DNA barcoding data. The BOLD threshold ID criterion closely resembles the method used by iBOL for making identifications with the *CO1* gene in animals, but in this study (Table 4.4) and in other studies (Gere *et al.* 2012, Mangka *et al.* 2013, Hoveka 2014, Kabongo 2014) it is the criterion with the lowest identification success rates, particularly at the 1% distance threshold chosen by CBOL. As this method is not currently used for species identifications in plants, this low identification success is not of too much concern. Plant identifications are currently made using the BLAST algorithm or using a tree-based method. Further testing needs to be carried out on these data to examine the identification success when using the BLAST algorithm. The tree-based species monophyly criterion had reasonably good identification success rates for these data sets when tested in SPIDER, with a highest identification success rate of 75% in the combined *rbcLa* + *matK* data without singletons (Table 4.5), although this was not the best performing criterion for any of the data sets excluding singletons in this study.

This study did not perform a test of the actual identification success of the data base using known grasses in a simulated identification scenario, or unknown grasses in an attempt to actually make an identification. These tests should be carried out in the future using the current methods of identification for plants to test the actual identification success of the data in comparison with the simulated results in SPIDER.

Data without singletons included were preferentially tested over data with singletons included, as the distance-based criteria match each individual sequence as a query to the rest of the database, and if no other representative of a species is present then a correct identification will not be made. As the purpose of this study was to test the identification efficacy of this database, it was favourable to test how well each species is identified to test the consistency of the sequence data within each species. In reality this database currently contains many single representatives of a species, although the database will be added to over time. The presence of these singletons will be useful once actual grass identifications need to be made, as they increase the representation of species in the data and increase the chances that a match to the unknown sequence will occur.

When identification success was tested at genus level, most grasses could be matched to the correct genus using the four criteria in SPIDER. Once again, the Nearest Neighbour criterion performed best and the BOLD threshold ID criterion at 1% default threshold performed the worst (Tables 4.4 & 4.5). Some naturalised genera in South Africa contain only naturalised species, and in those cases a successful identification to genus level would be sufficient, but other genera contain both native and naturalised species, for example*, Brachiaria, Eleusine, Eragrostis*, *Panicum*, *Pennisetum/Cenchrus* and *Poa*. In these cases it is best to aim for accurate species-level identification. If accurate species identifications cannot be made using the current markers tested in this study, then it is worth investigating other markers that have been shown to give good identification success, such as *trnH-psbA* and ITS, both of which have shown better identification success rates than *matK* in previous studies (Yao *et al.* 2010, China Plant BOL Group 2011, Yan *et al*. 2011, Gere *et al.* 2013, Hoveka 2014).

As tree-based methods of identification are currently commonly used to make actual plant identifications, the efficacy of the DNA barcoding data as phylogenetic loci was tested by constructing phylogenetic trees using the Maximum Parsimony and Bayesian Inference algorithms (Chapter 5). Both *rbcLa* and *matK* have been used in previous systematic studies of family Poaceae (Barker *et al.* 1995, Hilu *et al.* 1999, GPWG I 2001, GPWG II 2012), so these barcoding data can potentially be used in more in-depth studies of the phylogeny of family Poaceae in the future.

Generally, both the *matK* and combined data sets provided good resolution at family and tribe levels, although not all of the relationships within the two major grass clades, the BEP and PACMAD clades, were resolved. While many genera and species were found to be monophyletic, there were numerous exceptions and for a more in-depth analysis of the relationships within these species, it would be better to add these DNA barcoding data (which generally uses shorter sequence fragments than traditional phylogenetic markers) to other phylogenetic and/or morphological data. The *rbcLa* data on its own did not resolve most of grass tribes as monophyletic and was not found to be useful as a phylogenetic marker on its own for investigating relationships at genus and species level.

It is hoped that the large DNA barcoding database of southern African grasses assembled in this study may provide a quick and affordable method of identification in the early detection of grass invasions, and that these data may also be used in applications such as criminal forensics, as well as agricultural, fodder and grazer diet studies, and any other situation in which grasses, both native and naturalised, require identification.

Chapter 7. References

Adams J.B., Grobler A., Rowe C., Riddin T., Bornman T.G., Ayres D.R. (2012) Plant traits and spread of the invasive salt marsh grass, *Spartina alterniflora* Loisel. in the Great Brak Estuary, South Africa. *African Journal of Marine Science* 34:313-322.

AGIS (2007) Agricultural Geo-Referenced Information System, accessed from www.agis.agric.za on [08/11/2013]

Agrawal A.A. & Kotanen P.M. (2003) Herbivores and the success of exotic plants: a phylogenetically controlled experiment. *Ecology Letters* 6:712–715.

Alacs E.A., Georges A., Fitzsimmons N.N., Robertson J. (2010) DNA detective: a review of molecular approaches to wildlife forensics. *Forensic Science, Medicine and Pathology* 6:180–194.

Aliscioni S.S., Giussani L.M., Zuloaga, F.O., Kellogg, E.A., (2003) A molecular phylogeny of *Panicum* (Poaceae: Paniceae): tests of monophyly and phylogenetic placement within the Panicoideae. *American Journal of Botany* 90:796–821.

Baker H.G. (1965) Characteristics and modes of origin of weeds. In: The genetics of colonizing species. Baker H.G., Stebbins G.L., editors. New York: Academic Press:147-168.

Barker N.P., Linder H.P., Harley E. (1995) Phylogeny of Poaceae based on *rbcL* sequences. *Systematic Botan*y 20:423–435.

Barker N.P., Linder H.P., Harley E.H. (1999) Sequences of the grass-specific insert in the chloroplast *rpoC2* gene elucidate generic relationships of the Arundinoideae (Poaceae). *Systematic Botany* 23:327–350.

Baumel B.A., Ainouche M.L., Bayer R.J., Ainouche A.K., and. Misset M.T. (2002) Molecular phylogeny of hybridizing species from the genus *Spartina* Schreb. (Poaceae). *Molecular Phylogenetics and Evolution* 22:303–314.

Benson D.A., Karsch-Mizrachi I., Lipman D.J., Ostell J., Wheeler D.L. (2005) GenBank. *Nucleic Acids Research* 33:D34–D38.

Bezeng B.S., Savolainen V., Yessoufou K., Papadopulos A.S.T., Maurin O., Van der Bank M. (2013) A phylogenetic approach towards understanding the drivers of plant invasiveness on Robben Island, South Africa. *Botanical Journal of the Linnean Society* 172:142-152.

Blackburn T.M., Pyšek P., Bacher S., Carlton J.T., Duncan R.P., Jarošík V., Wilson J.R.U., Richardson D.M. (2011) A proposed unified framework for biological invasions. *Trends in Ecology & Evolution* 26:333-339.

Blossey B., Nötzold R. (1995) Evolution of increased competitive ability in Invasive nonindigenous plants: a hypothesis. *Journal of Ecology* 83:887-889.

Bossdorf D., Prati D., Auge H., Schmid B. (2004) Reduced competitive ability in an invasive plant. *Ecology Letters* 7:346–353.

Bouchenak-Khelladi Y., Salamin N., Savolainen V., Forest F., Van der Bank M., Chase M., Hodkinson T.R. (2008) Large multi-gene phylogenetic trees of the grasses (Poaceae): Progress towards complete tribal and generic level sampling. *Molecular Phylogenetics and Evolution* 47:488–505.

Bouchenak-Khelladi Y., Verboom A., Savolainen V., Hodkinson T.R. (2010) Biogeography of the grasses (Poaceae): a phylogenetic approach to reveal evolutionary history in geographical space and geological time. *Botanical Journal of the Linnean Society* 162:543–557.

Bouchenak-Khelladi Y., Muaysya A.M., Linder H.P. (2014) A revised evolutionary history of Poales: origins and diversification. *Botanical Journal of the Linnean Society* 175:4–16.

Bremer K. (2002) Gondwanan evolution of the grass alliance of families (Poales). *Evolution* 56:1374–1387.

Briggs B.G., Marchant A.D., Gilmore S., Porter C.L. (2000) A molecular phylogeny of Restionaceae and allies. In Monocots: Systematics and evolution. K. L. Wilson and D. A. Morrison [eds.], CSIRO, Melbourne, Australia. Vol. II:661–671.

Bromilow C. (2010) Problem plants and alien weeds of South Africa. *Briza* 3rd ed. South Africa.

Brooks M.L., D'Antonio C.M., Richardson D.M., Di Tomaso J., Grace J.B., Hobbs R.J., Keeley J.E., Pellant M. and Pyke D. (2004) Effects of invasive alien plants on fire regimes. *BioScience* 54:677-688.

Brown B.J., Mitchell R.J., Graham S.A. (2002) Competition for pollination between an invasive species (purple loosestrife) and a native congener. *Ecology* 83:2328-2336.

Brown S.D., Collins R.A., Boyer S., Lefort M.C., Malumbres-Olarte J., Vink C.J., Cruickshank R.H. (2012) Spider: An R package for the analysis of species identity and evolution, with particular reference to DNA barcoding. *Molecular Ecology Resources* 12:562–565.

Briski E., Cristescu M.E., Bailey S.A., MacIsaac H.J. (2011) Use of DNA barcoding to detect invertebrate invasive species from diapausing eggs. *Biological Invasions* 13:1325–1340.

Byrt C.S., Grof C.P., Furbank R.T. (2011) C_4 Plants as biofuel feedstocks: optimising biomass production and feedstock quality from a lignocellulosic perspective. *Journal of Integrative Plant Biology* 53:120–135.

Cadotte M.W., Hamilton M.A., Murray B.R. (2009) Phylogenetic relatedness and plant invader success across two spatial scales. *Diversity and Distributions* 15:481–488.

Callaway R.M., Thelen G.C., Rodriguez A. & Holben W.E. (2004) Soil biota and exotic plant invasion. *Nature* 427:731–733.

CBOL Plant Working Group (2009) A DNA barcode for land plants. *PNAS* 106:12794–12797.

China Plant BOL Group; Li D., Gao L., Li H., Wang H., Ge X., Liu J., Chen Z; Zhou S., Chen S., Yang J., Fu C., Zeng C., Yan H., Zhu Y., Sun Y., Chen S., Zhao L., Wang K., Yang T., Duan G. (2011) Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants.*PNAS* 108: 19641–19646.

Chown S.L., Sinclair B.J. Jansen van Vuuren B. (2008) DNA barcoding and the documentation of alien species establishment on sub-Antarctic Marion Island. Polar Biology 31:651–655.

Chemisquy M.A., Giussani L.M., Scataglini M.A., Kellogg E.A., Morrone O. (2010) Phylogenetic studies favour the unification of *Pennisetum, Cenchrus* and *Odontelytrum* (Poaceae): a combined nuclear, plastid and morphological analysis, and nomenclatural combinations in *Cenchrus*. *Annals of Botany* 106:107-30.

Clark L.G., Zhang W., Wendel J.F. (1995). A phylogeny of the grass family (Poaceae) based on *ndhF* sequence data. *Systematic Botany* 20:436–460.

Clayton W.D., Renvoize S.A. (1986) Genera Graminum, Grasses of the world. Her Majesty's Stationery Office, London.

Clayton W.D., Vorontsova M.S., Harman K.T., Williamson H. (2006 onwards). GrassBase - The Online World Grass Flora. http://www.kew.org/data/grasses-db.html. [accessed 17-09-2014 13:30 GMT+2hr].

Clement W.L., Donoghue M.J. (2012) Barcoding success as a function of phylogenetic relatedness in *Viburnum*, a clade of woody angiosperms. *BMC Evolutionary Biology* 12:73.

Clements D.R., Di Tommaso A., Jordan N , Booth B.D., Cardina J. , Doohan D., Mohler C.L., Murphy S.D., Swanton C.J. (2004) Adaptability of plants invading North American cropland. *Agriculture, Ecosystems & Environment* 104: 379–83.

Coetzee J.A., Hill M.P., Byrne M.J., Bownes A. (2011) A review of the biological control programmes on *Eichhornia crassipes* (C.Mart.) Solms (Pontederiaceae), *Salvinia molesta* D.S.Mitch. (Salviniaceae), *Pistia stratiotes* L. (Araceae), *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae) and *Azolla filiculoides* Lam. (Azollaceae) in South Africa*. African Entomology* 19:451-468.

Colautti R.I., Ricciardi A., Grigorovich I.A., MacIsaac H.J. (2004) Is invasion success explained by the enemy release hypothesis? *Ecology Letters* 7:721–733.

Collins R.A., Cruikshank R.H. (2013) The seven deadly sins of DNA barcoding. *Molecular Ecology Resources* 13:969–97.

Crawley M.J., Harvey P.H., Purvis A. (1996) Comparative Ecology of the Native and Alien Floras of the British Isles. *Philosophical Transactions: Biological Sciences* 351:1251-1259.

Crawley M.J., Keane R.M. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* 17(4):164–17.

Cuénoud P., Savolainen V., Chatrou L.W., Powel M.l, Grayer R.J., and Chase M.W. (2002) Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbcL*, *atpB*, and *matK* DNA sequences. *American Journal of Botany* 89:132-144.

Daehler C.C. & Strong D.R. (1997) Reduced herbivory resistance in introduced smooth cordgrass (*Spartina alterniflora*) after a century of herbivore-free growth. *Oecologia* 110:99–108.

D'Amato M.E., Alechine E., Cloete K.W., Davison S., Corach D. (2013) Where is the game? Wild meat products authentication in South Africa: a case study. *Investigative Genetics* 4:6.

D'Antonio C.M., Vitousek P.M. (1992) Biological invasions by exotic grasses, the grass/fire cycle, and global change. *Annual review of Ecology and Systematics* 23:63-87.

Darriba D., Taboada G.L., Doallo R., Posada D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9:772.

Darwin C. (1859) The origin of the species. J. Murray, London.

Davis J.I., Soreng, R.J. (2007) A preliminary phylogenetic analysis of the grass subfamily Pooideae (Poaceae), with attention to structural features of the plastid and nuclear genomes, including an intron loss in GBSSI. *Aliso* 23:335-348.

Davis C.C., Willis C.G., Primack R.B., Miller-Rushing A.J. (2010) The importance of phylogeny to the study of phenological response to global climate change. *Philosophical Transactions of the Royal Society, B, Biological Sciences* 365:3201-3213.

De Lange W.J., Van Wilgen B.W. (2010) An economic assessment of the contribution of biological control to the management of invasive alien plants and to the protection of ecosystem services in South Africa. *Biological Invasions* 12:4113–4124.

DeSalle R., Egan M.G., Siddall M. (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society, B, Biological Sciences* 360:1905-1916.

DeSalle R. (2006) Species discovery versus species identification in DNA barcoding efforts: response to Rubinoff. *Conservation Biology* 20:1545–1547.

De Vere N., Rich T.C.G., Ford C.R., Trinder S.A., Long C., Moore C.W., Satterthwaite D., Davies H., Allainguillaume J., Ronca S., Tatarinova T., Garbett H., Walker K., Wilkinson M.J. (2012) DNA Barcoding the Native Flowering Plants and Conifers of Wales. *PLoS ONE* 7: e37945. doi: 10.1371/journal.pone.0037945

DeWalt, S.J., Denslow, J.S. & Hamrick J.L. (2004) Biomass allocation, growth, and photosynthesis of genotypes from native and introduced ranges of the tropical shrub *Clidemia hirta*. *Oecologia* 138:521–531.

De Winter B. (1965) The South African Stipeae and Aristidae (Gramineae). *Bothalia* 8:201–404.

Dietz H., Edwards P.J. (2006) Recognition that causal processes change during plant invasion helps explain conflicts in evidence. *Ecology* 87:1359–1367.

Diez J.M., Sullivan J.J., Hulme P.E., Edwards G. & Duncan R.P. (2008) Darwin's naturalization conundrum: dissecting taxonomic patterns of species invasions*. Ecology Letters* 11:674–681.

Doyle J.J., Davis J.I., Soreng R.J., Garvin D., Anderson M.J. (1992) Chloroplast DNA inversions and the origin of the grass family (Poaceae). *PNAS* 89:7722-7726.

Drumwright A.M., Allen B.W., Huff K.A., Ritchey P.A., Cahoon A.B. (2011) Survey and DNA Barcoding of Poaceae in Flat Rock Cedar Glades and Barrens State Natural Area, Murfreesboro, Tennessee. *Castanea,* 76:300-310.

Duncan R.P., Williams P.A. (2002) Darwin's naturalization hypothesis revisited. *Nature* 417:608-609.

Dunning L.T. & Savolainen V. (2010) Broad-scale amplification of *matK* for DNA barcoding plants, a technical note. *Botanical Journal of the Linnean Society* 164:1–9.

Duvall, Melvin R.; Davis, Jerrold I; Clark, Lynn G.; Noll, Jeffrey D.; Goldman, Douglas H.; and Sánchez-Ken, J. Gabriel (2007) Phylogeny of the Grasses (Poaceae) Revisited*. Aliso: A Journal of Systematic and Evolutionary Botany*: Vol. 23:237-247.

Ebach M.C., Williams D.M., Gill A.C. (2008) O Cladistics, Where Art Thou? *Cladistics* 24:851-852.

Edgar R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32:1792-1797.

Edwards E.J., Still C.J. (2008) Climate, phylogeny and the ecological distribution of C_4 grasses. *Ecology Letters* 11:266–276.

Elton C.S. (1958). The ecology of invasions by animals and plants. London: Methuen.

Fazekas A.J., Burgess K.S., Kesanakurti P.R., Graham S.W., Newmaster S.G, Husband B.C., Percy D.M., Hajibabaei M., Barret S.C.H. (2008) Multiple Multilocus DNA Barcodes from the Plastid Genome Discriminate Plant Species Equally Well. *PLoS ONE* 3: e2802. doi:10.1371/journal.pone.0002802

Felsenstein J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791.

Fenner M. & Lee W.G. (2001) Lack of pre-dispersal seed predators in introduced Asteraceae in New Zealand. New Zealand. *Journal of Ecology* 25:95–99.

Fenster C.B. and Barrett S.C.H. (1994) Inheritance of mating-system modifier in *Eichhornia paniculata*. *Heredity* 72:433–445.

Ford C.S., Ayres K.L., Toomey N., Haider N., van Alphen Stahl J., Kelly L.J., Wikstrom N., Hollingsworth P.M., Duff R.J., Hoot S.B., Cowan R.S., Chase M.W., Wilkinson M.J. (2009) Selection of candidate coding DNA barcoding regions for use on land plants. *Botanical Journal of the Linnean Society* 159:1 – 11.

Gallagher R.V., Leishman M.R., Miller J.T., Hui C., Richardson D.M., Suda J., Trávníček P. (2011) Invasiveness in introduced Australian acacias: the role of species' traits and genome size. *Diversity and Distributions* 17:884-897.

Gaut B.S., Tredway L.P., Kubik C., Gaut R.L., Meyer W. (2010) Phylogenetic relationships and genetic diversity among members of the *Festuca-Lolium* complex (Poaceae) based on ITS sequence data*. Plant Systematics and Evolution* 224:33-53.

Gere J., Yessoufou K., Daru B.H., Mankga L.T., Maurin O., van der Bank M. (2013) Incorporating *trnH-psbA* to the core DNA barcodes improves significantly species discrimination within southern African Combretaceae. In: DNA barcoding: a practical tool for fundamental and applied biodiversity research. Nagy Z.T., Backeljau T., De Meyer M., Jordaens K. (eds). *ZooKeys* 365:127–147.

Gibbs Russell G.E., Watson L., Koekemoer, M., Smook L., Barker N.P., Anderson H.M. and Dallwitz M.J. (1991) Grasses of Southern Africa. *Memoirs of the Botanical. Survey of South Africa* 58, National Botanical Institute, Pretoria.

Gielly L., Taberlet P. (1994) The Use of Chloroplast DNA to Resolve Plant Phylogenies: Noncoding versus *rbcL* Sequences. *Molecular Biology and Evolution* 11:769-777.

Givnish T.J., Ames M., McNeal J.R., McKain M.R., Steele P.R., dePamphilis C.W, Graham S.W., Pires J.C., Stevenson D.W., Zomlefer W.B., Briggs B.G., Duvall M.R., Moore M.J., Heaney J.M., Soltis D.E., Soltis P.S., Thiele K., Leebens-Mack. J.H. (2010) Assembling the tree of the monocotyledons: plastome sequence phylogeny and evolution of Poales. *Annals of the Missouri Botanical Gardens* 97:584–616.

Goldblatt P. & Johnson D.E., eds. (1979 - ongoing) Index to plant chromosome numbers. *Missouri Botanical Garden, St. Louis.* http://www.tropicos.org/Project/IPCN, accessed 09-10-2014.

GPWG (2001) Phylogeny and subfamilial classification of the grasses (Poaceae). *Annals of the Missouri Botanical Garden* 88:373-457.

GPWG (2012) New grass phylogeny resolves deep evolutionary relationships and discovers C_4 origins. *New Phytologist* 193:304–312.

Gray A.J., Benham P.E.M., Raybould A.F. (1990) *Spartina anglica* – The evolutionary and ecological background. In: *Spartina anglica – a Research Review*. Gray A.J., Benham P.E.M. (eds). NERC: HMSO, London:5–10.

Gross E.M., Johnson R.L. & Hairston N.G. (2001) Experimental evidence for changes in submersed macrophyte species composition caused by the herbivore *Acentria ephemerella* (Lepidoptera). *Oecologia* 127:105–114.

Guindon S., Gascuel O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52:696-704.

Guo, Y., Mizukami, Y. Yamada, T. (2005) Genetic characterization of androgenic progeny derived from *Lolium perenne* × *Festuca pratensis* cultivars. *New Phytologist* 166:455–464.

Guthrie G. (2007) Impacts of the invasive reed *Arundo donax* on biodiversity at the community-ecosystem level. Unpublished M.Sc thesis, Faculty of Natural Sciences, University of the Western Cape.

Hand M.L., Cogan N.O.I., Stewart A.V., Forster J.W. (2010) Evolutionary history of tall fescue morphotypes inferred from molecular phylogenetics of the *Lolium-Festuca* species complex. *BMC Evolutionary Biology* 10:303.

Handley R.J., Steinger T., Treier U.A., Müller-Schärer H. (2008) Testing the evolution of increased competitive ability (EICA) hypothesis in a novel framework. *Ecology* 89:407-17.

Hao J.H., Qiang S., Chrobock T., van Kleunen M., Liu Q.Q. (2011) A test of Baker’s law: breeding systems of invasive species of Asteraceae in China. *Biological Invasions* 13:571–580.

Harmon-Threatt A.N., Burns J.H., Shemyakina L.A., Knight, T.M. (2009) Breeding system and pollination ecology of introduced plants compared to their native relatives. *American Journal of Botany* 96:1544-1550.

Hebert P.D.N., Cywinska A., Ball S.L, and. De Waard J.R. (2003) Biological identifications through DNA barcodes *Proceedings of the Royal Society B: Biological Sciences* 270:313-321.

Henderson L. (1998) Southern African Plant Invaders Atlas (SAPIA). *Applied Plant Science* 12: 31–32.

Hillis D.M. & Bull J.J. (1993) An Empirical Test of Bootstrapping as a Method for Assessing Confidence in Phylogenetic Analysis. *Systematic Biology* 42:182-192.

Hilu K.W., Alice L.A., Liang H. (1999) A phylogeny of Chloridoideae (Poaceae) based on *matK* sequences. *Annals of the Missouri Botanical Garden* 86:835–851.

Hobbs R.J., Huenneke L.F. (1992) Disturbance, Diversity, and Invasion: Implications for Conservation. *Conservation Biology* 6:324–337.

Hollingsworth P.M. (2011) Refining the DNA barcode for land plants. *PNAS* 8:19451-19452

Hollingsworth P.M., Graham S.W., Little D.P. (2011) Choosing and Using a Plant DNA Barcode. *PLoS ONE* 6: e19254. doi:10.1371/journal.pone.0019254

Holmes P.M., Cowling R.M. (1997) Diversity, composition and guild structure relationships between soil-stored seed banks and mature vegetation in alien plant-invaded South African fynbos shrublands. *Plant Ecology* 133:107–122.

Holmes B.H., Steinke D., Ward R.D. (2009) Identification of shark and ray fins using DNA barcoding. *Fisheries Research* 95:280–288.

Hoveka L. (2014) Invasive alien plants of South Africa’s freshwater systems: accelerating identification of species and climatically suitable areas for species invasion. Unpublished M.Sc. thesis, University of Johannesburg.

Hsiao C., Jacobs S.W.L., Chatterton N.J., Asay K.H. (1999) A molecular phylogeny of the grass family (Poaceae) based on the sequences of nuclear ribosomal DNA (ITS). *Australian Systematic Bot*any 11:667–688.

Huelsenbeck J.P., Ronquist F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754-755.

Hull-Sanders H.M., Clare R., Johnson R.H., Meyer GA. (2007) Evaluation of the evolution of increased competitive ability (EICA) hypothesis: loss of defense against generalist but not specialist herbivores. *Journal of Chemical Ecology* 33:781-799.

The International Plant Names Index (2012) Published on the Internet http://www.ipni.org [accessed 20 July 2013].

Ingram A.L., Doyle J.J. (2004) Is *Eragrostis* (Poaceae) Monophyletic? Insights from nuclear and plastid sequence data. *Systematic Botany* 29:545-552.

Ingram A.L., Doyle J.J. (2007) *Eragrostis* (Poaceae): monophyly and infrageneric classification. *Aliso: A Journal of Systematic and Evolutionary Botany*: Vol. 23:595-604.

Jacobs B.F., Kingston J.D., Jacobs L.L. (1999) The origin of grass-dominated ecosystems. *Annals of the Missouri Botanical Gardens* 86:590–643.

James J.J. (2008) Leaf nitrogen productivity as a mechanism driving the success of invasive annual grasses under low and high nitrogen supply. *Journal of Arid Environments* 72:1775–1784.

Jiang L., Tan J., Pu Z. (2010) An experimental test of Darwin's naturalization hypothesis. *The American Naturalist* 175:415-423.

Kabongo R. (2014) The assessment of DNA barcoding as an identification tool for traded and protected trees in southern Africa: Mozambican commercial timber species as a case study. Unpublished M.Sc. thesis, University of Johannesburg.

Kaufman S.R. & Smouse P.E. (2001) Comparing indigenous and introduced populations of *Melaleuca quinquenervia* (Cav.) Blake: response of seedlings to water and pH levels. *Oecologia*, 127:487–494.

Kellog E.A. (2001) Evolutionary history of the grasses. *Plant Physiology* 125:1198-1205.

Kimura M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111-120.

Klein H. (2011) A catalogue of the insects, mites and pathogens that have been used or rejected, or are under consideration, for the biological control of invasive alien plants in South Africa. *African Entomology* 19:515–549.

Klopper R. R., Chatelain C., Bänninger V., Habashi C., Steyn H. M., De Wet B. C., Arnold T. H., Gautier L., Smith G. F., Spichiger R. (2006) Checklist of the flowering plants of Sub-Saharan Africa. An index of accepted names and synonyms*. Southern African Botanical Diversity Network Report No. 42: 1–894. SABONET,* Pretoria.

Knapp P.A. (1996) Cheatgrass (*Bromus tectorum* L.) dominance in the Great Basin Desert: History, persistence, and influences to human activities. *Global Environmental Change* 6:37-52.

Koonjul P.K., Brandt W.F., Lindsey G.G., Farrant J.M. (1999) Inclusion of polyvinylpyrrolidone in the polymerase chain reaction reverses the inhibitory effects of polyphenolic contamination of RNA. *Nucleic. Acids Research* 27:915-916.

Kress W.J., Wurdack K.J., Zimmer E.A., Weigt L.A., Janzen D.H. (2005) Use of DNA barcodes to identify flowering plants. *PNAS* 102:8369–8374.

Kress W.J., Erickson D.L. (2007) A two-locus global DNA barcode for land plants: the coding *rbcL* gene complements the non-coding *trnH-psbA* spacer region. *PLoS ONE* 2(6): e508. doi:10.1371/journal.pone.0000508

Lahaye, R., Van der Bank, M., Bogarin, D., Warner, J., Pupulin, F., Gigot, G., Maurin, O., Duthoit, S., Barraclough, T.G., & Savolainen, V. (2008) DNA barcoding the floras of biodiversity hotspots. *PNAS* 105:2923-2928.

Lavergne S., Molofsky J. (2007) Increased genetic variation and evolutionary potential drive the success of an invasive grass. *PNAS* 104:3883–3888.

Le Maitre D.C., van Wilgen B.W., Gelderblom C.M., Bailey C., Chapman R.A., Nel J.A. (2002) Invasive alien trees and water resources in South Africa: case studies of the costs and benefits of management. *Forest Ecology and Management* 160:143-159.

Levin R.A., Wagner W.L., Hoch P.C., Nepokroeff M., Pires J.C., Zimmer E.A., Sytsma K.J. (2003) Family-level relationships of Onagraceae based on chloroplast *rbcL* and *ndhF* data. *American Journal of Botany* 90:107-115

Levine J.M. (2000) Species diversity and biological invasions: Relating local process to community pattern. *Science* 288:852–854.

Linder H.P., Ferguson I.K. (1985) On the pollen morphology and phylogeny of the Restionales and Poales. *Grana* 24:65–76.

Linder H.P. (1987) The evolutionary history of the Poales/Restionales: a hypothesis. *Kew Bulletin* 42:297–318.

Linder H.P., Baeza M., Barker N.P., Galley C., Humphreys A.M., Lloyd K.M., Orlovich D.A., Pirie M.D., Simon B.K., Walsh N., Verboom G.A. (2010) A generic classification of the Danthonioideae (Poaceae). *Annals of the Missouri Botanical Garden* 97:306–364.

Lizarazu M.A., Nicola M.V., Salariato D.L. (2014) Taxonomic re-evaluation of *Panicum* sections *Tuerckheimiana* and *Valida* (Poaceae: Panicoideae) using morphological and molecular data (2014). *Taxon* 63: 265–274.

Lloret F., Médail F., Brundu G., Camarda, I., Moragues E., Rita J., Lambdon P., Hulme P. E. (2005) Species attributes and invasion success by alien plants on Mediterranean islands. *Journal of Ecology* 93:512–520.

MacDougall A.S. & Turkington R. (2005) Are invasive species the drivers or passengers of change in degraded ecosystems? *Ecology* 86:42–55.

Mack R.N., Simberloff D., Lonsdale W.M., Evans H., Clout M., & Bazzaz, F.A. (2000) Biotic invasion: causes, epidemiology, global consequences, and control. *Ecological Applications* 10: 689–710.

Mankga L.T., Yessoufou K., Moteetee A.M., Daru B.H., van der Bank M. (2013) Efficacy of the core DNA barcodes in identifying processed and poorly conserved plant materials commonly used in South African traditional medicine. *Zookeys* 365:215–233.

Manning J. & Goldblatt P. (2012) Plants of the Greater Cape Floristic Region 1: the Core Cape flora. *Strelitzia* 29. South African National Biodiversity Institute, Pretoria.

Marlin D., Hill M.P., Byrne M.J. (2013) Interactions within pairs of biological control agents on water hyacinth, *Eichhornia crassipes. Biological Control* 67:483-490.

Marushia R.G, Cadotte M.W., Holt J.S. (2010) Phenology as a basis for management of exotic annual plants in desert invasions. *Journal of Applied Ecology* 47:1290–1299.

Mason-Gamer R.J., Weil C.F., Kellogg E.A. (1998) Granule-bound starch synthase: structure, function, and phylogenetic utility. *Molecular Biology and Evolution* 15:1658–1673.

Mathews S., Tsai R.C., Kellogg E. (2000) Phylogenetic structure in the grass family (Poaceae): evidence from the nuclear gene phytochrome B. *American Journal of Botany* 87:96–107.

McFadyen R.E.C. (1998) Biological control of weeds. *Annual Review of Entomology* 43:369–393.

Meier R., Shiyang K., Vaidya G., Ng P.K.L. (2006) DNA barcoding and taxonomy in *Diptera*: a tale of high intraspecific variability and low identification success. *Systematic Biology* 55:715–728.

Meier R., Zhang G., Ali F. (2008) The use of mean instead of smallest interspecific distances exaggerates the size of the “barcoding gap” and leads to misidentification. *Systematic Biology* 57: 809-813.

Meyer C.P., Paulay G. (2005) DNA Barcoding: Error Rates Based on Comprehensive Sampling. *PLoS Biology* 3: e422. doi:10.1371/journal.pbio.0030422

Michelangeli F.A., Davis J.I. and Stevenson D.W. (2003) Phylogenetic relationships among Poaceae and related families as inferred from morphology, inversions in the plastid genome, and sequence data from the mitochondrial and plastid genomes. *American Journal of Botany* 90:93–106.

Midoko-Iponga D. (2004) Renosterveld Restoration: The role of competition, herbivory and other disturbances. Unpublished M.Sc. thesis, University of Stellenbosch, South Africa.

Milberg P., Lamont B.B., Perez-Fernandez M.A. (1999) Survival and growth of native and exotic composites in response to a nutrient gradient. *Plant Ecology* 145:125–132.

Milton S.J. (2004) Grasses as invasive alien plants in South Africa. *South African Journal of Science* 100:65-79.

Milton S.J., Dean W.R.J., Rahlao S.J. (2008) Evidence for induced pseudo-vivipary in Pennisetum setaceum (Fountain grass) invading a dry river, arid Karoo, South Africa. *South African Journal of Botany* 74:348–349.

Morrone O., Aagesen L., Scataglini M.A., Salariato D.L., Denham S.S., Chemisquy M.A., Sede S. M., Giussani L. M., Kellogg E.A., Zuloaga F.O. (2012) Phylogeny of the Paniceae (Poaceae: Panicoideae): integrating plastid DNA sequences and morphology into a new classification. *Cladistics* 28:333–356.

Muhl S.A. (2008) Alien grass invasion of Renosterveld: Influence of soil variable gradients. Unpublished M.Sc thesis. University of Stellenbosch, Stellenbosch.

Muse S. V. (2000) Examining rates and patterns of nucleotide substitution in plants. *Plant Molecular Biology* 42:25–43.

Musil C.F. (1993) Effect of Invasive Australian Acacias on the Regeneration, Growth and Nutrient Chemistry of South African Lowland Fynbos. *Journal of Applied Ecology* 30:361-372.

Musil C.F., Milton S.J, Davis G.W. (2005) The threat of alien invasive grasses to lowland Cape floral diversity: an empirical appraisal of the effectiveness of practical control strategies. *South African Journal of Science* 101:337-343.

National Environmental Management: Biodiversity Act 2004 (Act no. 10 of 2004) alien and invasive species lists, 2014. *Staatskoerant* 599 no. 37886.

Newmaster S.G., Grguric M., Shanmughanandhan D., Ramalingam S., Ragupathy S. (2013) DNA barcoding detects contamination and substitution in North American herbal products. BMC Medicine 11:222.

Nunes C.F., Ferreira J.L., Fernandes M.C., Breves S., Generoso A.L., Soares B.D., Fontes D., M.S.C., Moacir P., Borem A., Cançado G.M. (2011) An improved method for genomic DNA extraction from strawberry leaves. *Ciência Rural* 41:1383-1389.

Pandit M.K., Pocock M.J.O., Kunin W.E. (2011) Ploidy influences rarity and invasiveness in plants. *Journal of Ecology* 99:1108 –1115.

Pang X., Liu C., Shi L., Liu R., Liang D., et al. (2012) Utility of the *trnH–psbA* intergenic spacer region and its combinations as plant DNA barcodes: a meta-analysis. *PLoS ONE* 7: e48833. doi:10.1371/journal.pone.0048833.

Paradis E., Claude J., Strimmer K. (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20:289-290.

Parker-Allie F., Musil C. F., Thuiller W. (2009) Effects of climate warming on the distributions of invasive Eurasian annual grasses: a South African perspective. *Climatic Change* 94:87–103.

Peng Y.-Y., Baum B.R., Re C.-Z., Jiang Q.-T., Chen G.-Y., Zheng Y.-L., Wei Y.-M. (2010)a The evolution pattern of rDNA ITS in *Avena* and phylogenetic relationship of the *Avena* species (Poaceae: Aveneae). *Hereditas* 147:183–204.

Peng Y.-Y., Wei Y.-M., Baum B.R., Jiang Q.-T, Lan X.-J., Dai S.-F., Zheng Y.-L. (2010)b Phylogenetic investigation of *Avena* diploid species and the maternal genome donor of *Avena* polyploids *Taxon* 59:1472-1482.

Peterson P.M., Romaschenko K., Barker N.P., Linder H.P. (2011) Centropodieae and *Ellisochloa*, a new tribe and genus in Chloridoideae (Poaceae). *Taxon* 60:1113–1122.

Pettengill J.B., Neel M.C. (2010) An evaluation of candidate plant DNA barcodes and assignment methods in diagnosing 29 species in the genus *Agalinis* (Orobanchaceae). *American Journal of Botany* 97:1391-1406.

The Plant List (2013) Version 1.1 Published on the Internet; http://www.theplantlist.org/ (accessed 20 July 2013, 18 July 2014).

Plants of southern Africa – an online checklist. (2009) Version 3.0 Published on the Internet; http://posa.sanbi.org/ (accessed 15 August 2013).

Prentis P.J., Wilson J.R., Dormontt E.E., Richardson D.M., Lowe A.J. (2008) Adaptive evolution in invasive species. *Trends in Plant Science* 13:288-294.

Procheş Ş., Wilson J.R.U., Richardson D.M., Rejmánek, M. (2008) Searching for phylogenetic pattern in biological invasions. *Global Ecology and Biogeography* 17:5–10.

Pyšek P., Richardson D.M. (2007) Traits associated with invasiveness in alien plants:
Where do we stand? In: Biological invasion. Caldwell M.M. (ed.). New York, NY, USA: Springer 97–125.

Pyšek P., Hulme P.E., Meyerson L.A., Smith G.F., Boatwright J.S., Crouch N.R., Figueiredo E., Foxcroft L.C., Jarošík V., Richardson D.M., Suda J., Wilson J.R.U. (2013) Hitting the right target: taxonomic challenges for, and of, plant invasions. *AoB PLANTS* 5: plt042; doi:10.1093/aobpla/

Ragupathy S., Newmaster S.G., Murugesan M., Balasubramaniam V. (2009) DNA barcoding discriminates a new cryptic grass species revealed in an ethnobotany study by the hill tribes of the Western Ghats in southern India. *Molecular Ecology Resources* 9:164–171.

Rambuda T.D., Johnson S.D. (2004) Breeding systems of invasive alien plants in South Africa: does Baker's rule apply? *Diversity and Distributions* 10:409–416.

Ratnasingham S., Hebert P.D. (2007) BOLD: The Barcode of Life Data System. *Molecular Ecology Notes* 7:355–364.

Reinhart K.O., Packer A., Van der Putten W.H., Clay K. (2003) Plant–soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. *Ecology Letters* 6:1046–1050.

Ricciardi A. & Atkinson S.K. (2004) Distinctiveness magnifies the impact of biological invaders in aquatic ecosystems. *Ecology Letters* 7:781–784.

Richards C.L., Bossdorf O., Muth N.Z, Gurevitch J., Pigliucci M. (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecology Letters* 9:981–993.

Richardson D.M., Bond W.J., Dean W.R.J., Higgins S.I., Midgley G.F., Milton S.J., Powrie L.W., Rutherford M.C., Samways M.J., SchulzeR.E.(2000)a Invasive alien organisms and global change: a South African perspective. *In Invasive Species in a Changing World*, eds H.A. Mooney and R.J. Hobbs, p. 303–349. Island Press, Washington, D.C.

Richardson D.M., Pyšek P., Rejmánek M., Barbour M.G., Panetta F.D., West C.J. (2000)b Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions* 6:93–107.

Richardson D.M., van Wilgen B.W. (2004) Invasive alien plants in South Africa: how well do we understand the ecological impacts? *South African Journal of Science* 100:45-52.

Rosenberg N.A. (2007) Statistical tests for taxonomic distinctiveness from observations of monophyly. *Evolution* 61:317-23.

Rouget M., Richardson D.M., Cowling R.M., Lloyd J.W., Lombard A.T. (2003) Current patterns of habitat transformation and future threats to biodiversity in terrestrial ecosystems of the Cape Floristic Region, South Africa. *Biological Conservation* 112:63–85.

Rousseau P. (2012) A molecular systematic study of the African endemic cycads. Unpublished M.Sc thesis, University of Johannesburg, Johannesburg.

Ronquist F., Huelsenbeck J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572-1574.

Saarela J.M., Graham S.W. (2010) Inference of phylogenetic relationships among the subfamilies of grasses (Poaceae: Poales) using meso-scale exemplar-based sampling of the plastid genome. *Botany* 88:65-84.

Saarela J.M., Sokoloff P.C., Gillespie L.J., Consaul L.L., Bull R.D. (2013) DNA barcoding the Canadian Arctic flora: core plastid barcodes (*rbcL* + *matK*) for 490 vascular plant species. *PLoS ONE* 8: e77982. doi:10.1371/journal.pone.0077982.

Sánchez-Ken, J.G., Clark, L.G. (2007) Phylogenetic relationships within the Centothecoideae + Panicoideae clade (Poaceae) based on *ndhF* and *rpl16* intron sequences and structural data. *Aliso: A Journal of Systematic and Evolutionary Botany*, 23:487-502.

Sánchez-Ken, J.G., Clark, L.G., Kellogg, E.A., Kay, E.E. (2007) Reinstatement and emendation of subfamily Micrairoideae (Poaceae). *Systematic.Botany* 32:71–80.

Sánchez-Ken J.G., Clark L.G. (2010) Phylogeny and a new tribal classification of the Panicoideae s.l. (Poaceae) based on plastid and nuclear sequence data and structural data. *American Journal of Botany* 97:1732-1748.

SAPIA news (2008) No. 7. Accessed on the Internet: http://www.invasives.org.za/resources/downloadable-resources/finish/41-sapia-newsletters/520-sapia-newsletter-no-7-april-2008-reed-sweet-grass-tussock-paspalum-lindenleaf-sage-creeping-knotweed-polka-dot-plant.html (24 July 2014).

Schaefer H., Hardy O.J., Silva L., Barraclough T.G., Savolainen V. (2011) Testing Darwin's naturalization hypothesis in the Azores. *Ecology Letters* 14:389–396.

Schmidt J.P., Drake J.M. (2011) Why Are Some Plant Genera More Invasive Than Others? *PLoS ONE* 6: e18654. doi:10.1371/journal.pone.0018654

Sede S.M., Morrone O., Giussani L.M., Zuloaga F.O., (2008) Phylogenetic studies in the Paniceae (Poaceae): a realignment of section Lorea of *Panicum*. *Systematic Botany* 33:284–300.

Shantz H.L. (1954) The place of grasslands in the earth's cover of vegetation. *Ecology* 35:143–145.

Sharma G.P., Muhl S.A., Esler K.J., Milton S.J. (2010) Competitive interactions between the alien invasive annual grass *Avena fatua* and indigenous herbaceous plants in South African Renosterveld: the role of nitrogen enrichment. *Biological Invasions* 12:3371-3378.

Shiponeni N.N., Milton S.J. (2006) Seed dispersal in the dung of large herbivores: implications for restoration of Renosterveld shrubland old fields*. Biodiversity and Conservation* 15:3161–3175.

Siemann R. & Rogers W.E. (2001) Genetic differences in growth of an invasive tree species. *Ecology Letters* 4:514–518.

Skálová H., Havlíčková V., and Pyšek P. (2012) Seedling traits, plasticity and local differentiation as strategies of invasive species of Impatiens in central Europe. *Annals of Botany* 110:1429-1438.

Snijman, D.A. (ed.). (2013) Plants of the Greater Cape Floristic Region, Vol.2: the Extra Cape flora. *Strelitzia* 30. South African National Biodiversity Institute, Pretoria.

Soreng R.J., Davis J.I. (1998) Phylogenetics and character evolution in the grass family (Poaceae): simultaneous analysis of morphological and chloroplast DNA restriction site character sets. *The Botanical Review* 64:1–85.

Soreng R.J., Davis J.I. (2000) Phylogenetic structure in Poaceae subfamily Pooideae as inferred from molecular and morphological characters: misclassification versus reticulation. In: *Grasses: systematics and evolution*, Jacobs, S. W. L. & Everett, J.(eds.). CSIRO Publishing, Collingwood, Victoria, Australia:61-74.

Srivathsan A. & Meier R. (2012) On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. *Cladistics* 28:190-194.

Stachowicz, J.J. & Tilman, D. (2005) Species invasions and the relationships between species diversity, community saturation, and ecosystem functioning. In: Species Invasions: insights into ecology, evolution, and biogeography, Sax D.F, Stachowicz, J.J. &. Gaines S.D.), Sinauer Sunderland, MA. 41–64.

Stewart Jr. C.N. (2009) Weedy and Invasive Plant Genomics. *Wiley-Blackwell.*

Strauss S.Y., Webb C.O., Salamin N. (2006) Exotic taxa less related to native species are more invasive. *PNAS* 103:5841–5845.

Stuessy T.F. (2009) Plant taxonomy: the systematic evaluation of comparative data. 2nd edition**.** *Columbia University Press.*

Swofford D.L. (2002) Phylogenetic analysis using parsimony (* and other methods). Version 4. *Sunderland, MA: Sinauer Associates.*

Tamura K., Stecher G., Peterson D., Filipski A., and Kumar S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30:2725-2729.

Tautz D., Arctander P., Minelli A., Thomas R.H., Vogler A.P. (2003) A plea for DNA taxonomy. *Trends in Ecology & Evolution* 18:70-74.

Te Beest M., Le Roux J.J., Richardson D.M., Brysting A.K., Suda J., Kubešová M., Pyšek P. (2011) The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany* 109:19-45.

Teerawatananon A., Jacobs S.W.L., Hodkinson T.R. (2011) Phylogenetics of Panicoideae (Poaceae) based on chloroplast and nuclear DNA sequences. *Telopea* 13:115–142.

Thuiller W., Gallien L., Boulangeat I., de Bello F., Mükemüller T., Roquet C., Lavergne S. (2010) Resolving Darwin's naturalization conundrum: a quest for evidence. *Diversity and Distributions* 16:461–475.

Todd S. (2008) The abundance and impact of alien annual grasses on Hantam-Roggeveld Dolerite renosterveld vegetation at Nieuwoudtville, Northern Cape, South Africa. *Prepared for Indigo Development and Change.*

Van Kleunen M., Schmid B. (2003) No evidence for evolutionary increased competitive ability (EICA) in the invasive plant *Solidago canadensis*. *Ecology* 84:2824–2831.

Van Oudtshoorn F. (2006) Guide to grasses of southern Africa. Second edition, Fourth impression. *Briza* Pretoria.

Van Wilgen B.W., Richardson D.M., Le Maitre D.C., Marais C., Magadlela D. (2001) The economic consequences of alien plant invasions: examples of impacts and approaches to sustainable management in South Africa. *Environment, Development and Sustainability* 3:145–168.

Van Wilgen B.W., Forsyth G.G., Le Maitre D.C., Wannenburgh A., Kotzé J.D.F., Van den Berg E., Henderson L. (2012) An assessment of the effectiveness of a large, national-scale invasive alien plant control strategy in South Africa. *Biological Conservation* 148:28-38.

Van Wyk E., Van Wilgen B.W. (2002) The cost of water hyacinth control in South Africa: a case study of three options. *African Journal of Aquatic* Science 27:141-149.

Verloove F. (2010) Invaders in disguise. Conservation risks derived from misidentifications of invasive plants. *Management of Biological Invasions* 1:1-5.

Verloove F. (2012) New combinations in *Cenchrus* (Paniceae, Poaceae) in Europe and the Mediterranean. *Willdenowia* 42:77-78.

Versfeld, D.B., Le Maitre, D.C., Chapman, R.A. (1998) Alien Invading Plants and Water Resources in South Africa: A preliminary assessment, Report TT99/98, Water Research Commission, Pretoria.

Vila M., Gomez A. & Maron J.L. (2003) Are alien plants more competitive than their native conspecifics? A test using *Hypericum perforatum* L. *Oecologia* 137:211–215.

Vitousek P.M., D'Antonio C.M., Loope L.L., Westbrooks R. (1996). Biological invasions as global environmental change. *American Scientist*, *84*(5), 468-478.

Wagner, W. L., Herbst, D. R., Sohmer. S. H. (1990) Manual of the flowering plants of Hawaii. *University of Hawaii Press*, Honolulu, Hawaii, USA.

Wang Y., Cerling T.E., MacFadden B.J. (1994) Fossil horses and carbon isotopes: new evidence for Cenozoic dietary, habitat, and ecosystem changes in North America. *Palaeogeography, Palaeoclimatology, Palaeoecology* 107:269-279.

Ward J., Gilmore S. R., Robertson J., Peakall R. (2009) A grass molecular identification system for forensic botany: A critical evaluation of the strengths and limitations. *Journal of Forensic Sciences* 54:1254-1260.

Watson, L., Dallwitz, M.J. (1992) The Grass Genera of the World. CAB International, Wallingford, UK.

Wheeler Q.D. (2005) Losing the plot: DNA "barcodes" and taxonomy. *Cladistics* 21:405-407.

Whisenant S.G. (1990) Changing fire frequencies on Idaho's Snake River plains: ecological and management implications. In: P*roceedings of the Symposium on Cheatgrass Invasion, Shrub Die-off and Other Aspects of Shrub Biology and Management. Intermountain Research Station General Technical Report* INT-276 McAurthur, D., Romney M., Smith S.D. & Tueller P.T. (eds.). Ogden, Utah:4–10.

Will K.W., Rubinoff D. (2004) Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics* 20:47-55.

Williams J.L., Auge H., Maron J.L. (2008) Different gardens, different results: native and introduced populations exhibit contrasting phenotypes across common gardens. *Oecologia* 157:239–248.

Williamson M., Fitter A. (1996) The varying success of invaders. *Ecology* 77:1661–1666.

Willis A.J., Thomas M.B. & Lawton J.H. (1999) Is the increased vigour of invasive weeds explained by a trade-off between growth and herbivore resistance? *Oecologia* 120:632–640.

Wilson J.R.U., Ivey P., Manyama P., Nänni I. (2013) A new national unit for invasive species detection, assessment and eradication planning. *South African Journal of Science* 109:5-6.

Wolfe, K. H., Li, W.-H. & Sharp, P. M. (1987) Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. *PNAS* 84:9054–9058.

Wolkovich E.M., Cleland E.E. (2010) The phenology of plant invasions: a community ecology perspective. *Frontiers in Ecology and the Environment* 9:287–294.

Yan H., Hao G., Hu C., Ge X. (2011) DNA barcoding in closely related species: A case study of *Primula* L. sect.Proliferae Pax (Primulaceae) in China*. Journal of Systematics and Evolution* 49:225–236.

Yao H., Song J., Liu C., Luo K., Han J. Li Y., Pang X., Xu H., Zhu Y., Xiao P., Chen S., (2010) Use of ITS2 Region as the Universal DNA Barcode for Plants and Animals*. PLoS ONE* 5(10): e13102. doi:10.1371/journal.pone.0013102

Young J.A., Allen F.L. (1997) Cheat grass and range science 1930–1950*. Journal of Rangeland Management* 50:530–535.

Yu J., Xue J.H., Zhou S.L. (2011) New universal *mat*K primers for DNA barcoding angiosperms. *Journal of Systematics and Evolution* 49:176–181.

Zedler J.B., Kercher S. (2004) Causes and Consequences of Invasive Plants in Wetlands: Opportunities, Opportunists, and Outcomes. *Critical Reviews in Plant Sciences* 23:431–452.

Zuloaga F.O., Morrone O., Davids G., Pennington S.J. (2007) Classification and Biogeography of Panicoideae (Poaceae) in the New World. *Aliso: A Journal of Systematic and Evolutionary Botany* 23:503-529.

APPENDICES

Appendix 1A. Alphabetical list of all species represented in this study, including species from sister clades and early diverging grass lineages not found in southern Africa.

Family	Scientific name	Common synonyms	Origin	Grass sub-family	*matK* replicates	*rbcLa* replicates
Poaceae	*Acrachne racemosa* (B.Heyne ex Roth) Ohwi		native	Chloridoideae	5	4
Poaceae	*Agrostis avenacea* J.F.Gmel.		naturalised	Pooideae	1	1
Poaceae	*Agrostis gigantea* Roth		naturalised	Pooideae	3	3
Poaceae	*Agrostis montevidensis* Spreng. ex Nees		naturalised	Pooideae	2	2
Poaceae	*Aira cupaniana* Guss.		naturalised	Pooideae	0	2
Poaceae	*Aira praecox* L.		naturalised	Pooideae	3	3
Poaceae	*Alloteropsis semialata* subsp. *eckloniana* (Nees) Gibbs Russ.		native	Panicoideae	1	1
Poaceae	*Alloteropsis semialata* (R.Br.) Hitchc.	*Alloteropsis semialata* subsp. *semialata*	native	Panicoideae	1	1
Poaceae	*Alopecurus arundinaceus* Poir.		naturalised	Pooideae	0	1
Poaceae	*Ammophila arenaria* (L.) Link		naturalised	Pooideae	4	4

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Andropogon schirensis* Hochst.		native	Panicoideae	1	1
Poaceae	*Anomochloa marantoidea* Brongn.		Early diverging grass lineage	Anomochlooideae	1	1
Poaceae	*Anthephora pubescens* Nees		native	Panicoideae	1	1
Poaceae	*Anthoxanthum odoratum* L.		naturalised	Pooideae	5	5
Poaceae	*Aristida adscensionis* L.		native	Aristidoideae	4	4
Poaceae	*Aristida aequiglumis* Hack.		native	Aristidoideae	1	1
Poaceae	*Aristida bipartita* (Nees) Trin. & Rupr.		native	Aristidoideae	1	1
Poaceae	*Aristida congesta* Roem. & Schult.		native	Aristidoideae	3	3
Poaceae	*Aristida diffusa* Trin.		native	Aristidoideae	1	1
Poaceae	*Aristida engleri* Mez		native	Aristidoideae	1	1
Poaceae	*Aristida hubbardiana* Schweick.		native	Aristidoideae	1	1

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Aristida junciformis* Trin. & Rupr.		native	Aristidoideae	1	1
Poaceae	*Aristida meridionalis* Henrard		native	Aristidoideae	4	4
Poaceae	*Aristida mollissima* Pilg.		native	Aristidoideae	1	1
Poaceae	*Aristida mutabilis* Trin. & Rupr.	*Aristida spicata*	native	Aristidoideae	1	1
Poaceae	*Aristida nemorivaga* Henrard	*Aristida canescens*	native	Aristidoideae	1	1
Poaceae	*Aristida parvula* (Nees) De Winter		native	Aristidoideae	1	1
Poaceae	*Aristida pilgeri* Henrard		native	Aristidoideae	1	1
Poaceae	*Aristida rhiniochloa* Hochst.		native	Aristidoideae	1	1
Poaceae	*Aristida scabrivalvis* Hack.		native	Aristidoideae	5	5
Poaceae	*Aristida spectabilis* Hack.		native	Aristidoideae	1	1
Poaceae	*Aristida stipitata* Hack.		native	Aristidoideae	1	1
Poaceae	*Aristida transvaalensis* Henrard		native	Aristidoideae	1	1

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Arrhenatherum elatius* (L.) P.Beauv. ex J.Presl & C.Presl		naturalised	Pooideae	3	3
Poaceae	*Arthraxon lanceolatus* Hochst.		uncertain origin	Panicoideae	1	3
Poaceae	*Arundo donax* L.		naturalised	Arundinoideae	2	3
Poaceae	*Avena barbata* Pott ex Link		native	Pooideae	1	3
Poaceae	*Avena fatua* L.		naturalised	Pooideae	4	4
Poaceae	*Avena sativa* L.		naturalised	Pooideae	2	5
Poaceae	*Avena sterilis* L.		naturalised	Pooideae	1	4
Poaceae	*Axonopus fissifolius* (Raddi) Kuhlm.	*Axonopus affinis*	naturalised	Panicoideae	1	4
Restionaceae	*Baloskion tetraphyllum* (Labill.) B.G.Briggs & L.A.S.Johnson		Sister clade		1	1
Poaceae	*Bambusa balcooa* Roxb. Ex Roxb.		naturalised	Bambusoideae	1	3
Poaceae	*Bothriochloa insculpta* (A.Rich.) A.Camus		native	Panicoideae	4	4

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Bothriochloa radicans* (Lehm.) A.Camus		native	Panicoideae	3	3
Poaceae	*Brachiaria advena* Vickery		naturalised	Panicoideae	1	1
Poaceae	*Brachiaria brizantha* (A.Rich.) Stapf		native	Panicoideae	1	1
Poaceae	*Brachiaria deflexa* (Schumach.) C.E.Hubb. ex Robyns	*Pseudobrachiaria deflexa*	native	Panicoideae	1	1
Poaceae	*Brachiaria dictyoneura* (Fig. & De Not.) Stapf		native	Panicoideae	1	1
Poaceae	*Brachiaria eruciformis* (Sm.) Griseb.		uncertain origin	Panicoideae	1	1
Poaceae	*Brachiaria umbellata* (Trin.) Clayton		naturalised	Panicoideae	2	2
Poaceae	*Brachypodium distachyon* (L.) P.Beauv.		naturalised	Pooideae	1	3
Poaceae	*Briza maxima* L.		naturalised	Pooideae	3	3
Poaceae	*Briza minor* L.		naturalised	Pooideae	2	4
Poaceae	*Briza subaristata* Lam.		naturalised	Pooideae	0	2

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK*** **replicates**	***rbcLa*** **replicates**
Poaceae	*Bromus alopecuros* Poir.		naturalised	Pooideae	0	1
Poaceae	*Bromus catharticus* Vahl	*Bromus wildenowii*	naturalised	Pooideae	2	2
Poaceae	*Bromus commutatus* Schrad		naturalised	Pooideae	2	3
Poaceae	*Bromus diandrus* Roth		naturalised	Pooideae	3	3
Poaceae	*Bromus hordeaceus* L.		naturalised	Pooideae	3	3
Poaceae	*Bromus inermis* Leyss.		naturalised	Pooideae	3	3
Poaceae	*Bromus leptoclados* Nees		native	Pooideae	1	1
Poaceae	*Bromus madritensis* L.		naturalised	Pooideae	1	1
Poaceae	*Bromus pectinatus* Thunb.	*Bromus japonicus*	uncertain origin	Pooideae	1	1
Poaceae	*Bromus rigidus* Roth	*Bromus diandrus var. rigidus*	naturalised	Pooideae	5	5
Poaceae	*Bromus rubens* L.		naturalised	Pooideae	0	2
Poaceae	*Bromus tectorum* L.		naturalised	Pooideae	3	3
Poaceae	*Calamagrostis epigejos* (L.) Roth		uncertain origin	Pooideae	3	3

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Capeochloa arundinacea* (P.J.Bergius) N.P.Barker & H.P.Linder	*Merxmuellera arundinacea, Andropogon arundinaceus*	Native	Danthonioideae	2	2
Poaceae	*Capeochloa cincta* subsp. *sericea*		native	Danthonioideae	1	1
Poaceae	*Capeochloa cincta* (Nees) N.P.Barker & H.P.Linder	*Merxmuellera cincta*	native	Danthonioideae	1	1
Poaceae	*Catapodium rigidum* (L.) C.E.Hubb.		naturalised	Pooideae	3	3
Poaceae	*Cenchrus biflorus* Roxb.		naturalised	Panicoideae	0	3
Poaceae	*Cenchrus brownii* Roem. & Schult.		naturalised	Panicoideae	1	1
Poaceae	*Cenchrus ciliaris* L.		native	Panicoideae	3	3
Poaceae	*Cenchrus incertus* M.A.Curtis		naturalised	Panicoideae	0	1
Poaceae	*Chaetobromus involucratus* subsp. *dregeanus* (Nees) Verboom	*Chaetobromus involucratus*	native	Danthonioideae	0	2
Poaceae	*Chloris gayana* Kunth		uncertain origin	Chloridoideae	0	2
Poaceae	*Chloris pycnothrix* Trin.		uncertain origin	Chloridoideae	0	1

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Chloris roxburghiana* Schult.		native	Chloridoideae	2	2
Poaceae	*Chloris truncata* R.Br.		naturalised	Chloridoideae	0	2
Poaceae	*Chloris virgata* Sw.		uncertain origin	Chloridoideae	7	7
Poaceae	*Coix lacryma-jobi* L.		naturalised	Panicoideae	3	3
Poaceae	*Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn.		naturalised	Danthonioideae	2	4
Poaceae	*Corynephorus fasciculatus* Boiss. & Reut.		naturalised	Pooideae	0	1
Poaceae	*Ctenium concinnum* Nees		native	Chloridoideae	1	1
Poaceae	*Cymbopogon pospischilii* (K.Schum.) C.E.Hubb	*Cymbopogon plurinodis*	native	Panicoideae	3	3
Poaceae	*Cymbopogon caesius* (Hook. & Arn.) Stapf	*Cymbopogon excavatus*	native	Panicoideae	3	3
Poaceae	*Cymbopogon marginatus* (Steud.) Stapf ex Burtt-Davy		native	Panicoideae	0	1
Poaceae	*Cynodon nlemfuensis* Vanderyst		naturalised	Chloridoideae	3	4

Family	Scientific name	Common synonyms	Origin	Grass sub-family	*matK* replicates	*rbcLa* replicates
Poaceae	*Cynodon aethiopicus* Clayton & Harlan		native	Chloridoideae	2	0
Poaceae	*Cynodon dactylon* (L.) Pers.		native	Chloridoideae	3	3
Poaceae	*Cynodon transvaalensis* Burtt Davy		uncertain origin	Chloridoideae	1	2
Poaceae	*Cynosurus echinatus* L.		naturalised	Pooideae	3	3
Poaceae	*Dactylis glomerata* L.		naturalised	Pooideae	3	3
Poaceae	*Dactyloctenium aegyptium* (L.) Willd.	*Dactyloctenium aegyptiacum*	native	Chloridoideae	2	3
Poaceae	*Dactyloctenium giganteum* B.S.Fisher & Schweick.		native	Chloridoideae	2	2
Poaceae	*Danthoniopsis dinteri* (Pilg.) C.E.Hubb.		native	Panicoideae	3	3
Poaceae	*Danthoniopsis pruinosa* C.E.Hubb.		native	Panicoideae	3	3
Poaceae	*Deschampsia cespitosa* (L.) P.Beauv.		naturalised	Pooideae	3	3

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Dichanthium aristatum* (Poir.) C.E.Hubb.		naturalised	Panicoideae	0	1
Poaceae	*Digitaria abyssinica* (A.Rich.) Stapf.	*Digitaria scalarum*	naturalised	Panicoideae	1	2
Poaceae	*Digitaria ciliaris* (Retz.) Koeler		uncertain origin	Panicoideae	2	2
Poaceae	*Digitaria didactyla* Willd		uncertain origin	Panicoideae	1	1
Poaceae	*Digitaria eriantha* Steud.		native	Panicoideae	0	1
Poaceae	*Digitaria milanjiana* (Rendle) Stapf		native	Panicoideae	2	2
Poaceae	*Digitaria sanguinalis* (L.) Scop.		uncertain origin	Panicoideae	3	5
Poaceae	*Digitaria velutina* (Forssk.) P.Beauv.		native	Panicoideae	1	1
Poaceae	*Digitaria violascens* Link		naturalised	Panicoideae	0	1
Poaceae	*Dinebra retroflexa* (Vahl) Panz.		native	Chloridoideae	0	2

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Dinebra retroflexa* (Vahl) Panz*. var. condensata* S.M.Phillips		native	Chloridoideae	1	1
Ecdeiocoleaceae	*Ecdeiocolea monostachya* F. Muell.		sister clade		1	1
Poaceae	*Echinochloa colona* (L.) Link		uncertain origin	Panicoideae	3	4
Poaceae	*Echinochloa crus-galli* (L.) P.Beauv.		uncertain origin	Panicoideae	3	3
Poaceae	*Echinochloa haploclada* (Stapf) Stapf		native	Panicoideae	1	1
Poaceae	*Echinochloa holubii* (Stapf) Stapf		native	Panicoideae	1	1
Poaceae	*Ehrharta stipoides* Labill.	*Microlaena stipoides*	naturalised	Ehrhartoideae	3	3
Poaceae	*Ehrharta calycina* Sm.		native	Ehrhartoideae	2	3
Poaceae	*Ehrharta capensis* Thunb.		native	Ehrhartoideae	1	1
Poaceae	*Ehrharta erecta* Lam.		native	Ehrhartoideae	0	1
Poaceae	*Ehrharta longifolia* Schrad.		native	Ehrhartoideae	0	1
Poaceae	*Ehrharta thunbergii* Gibbs Russ.		native	Ehrhartoideae	1	1

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Ehrharta villosa* Schult.f.	*Ehrharta villosa var. villosa*	native	Ehrhartoideae	0	1
Restionaceae	*Elegia squamosa* Mast.		sister clade		1	1
Poaceae	*Eleusine coracana* (L.) Gaertn.	*Eleusine coracana* subsp*. africana*	uncertain origin	Chloridoideae	1	1
Poaceae	*Eleusine indica* (L.) Gaertn.	*Eleusine indica* subsp. *indica*	naturalised	Chloridoideae	2	3
Poaceae	*Eleusine multiflora* Hochst. ex A.Rich.		naturalised	Chloridoideae	0	2
Poaceae	*Eleusine tristachya* (Lam.) Lam.		naturalised	Chloridoideae	1	3
Poaceae	*Ellisochloa papposa* (Nees) P.M.Peterson & N.P.Barker	*Merxmuellera papposa, Danthonia papposa*	native	Chloridoideae	2	2
Poaceae	*Ellisochloa rangei* (Pilg.) P.M.Peterson & N.P.Barker	*Merxmuellera rangei, Danthonia rangei*	native	Chloridoideae	2	2
Poaceae	*Elymus repens* (L.) Gould	*Elytrigia repens*	naturalised	Pooideae	3	3
Poaceae	*Enneapogon cenchroides* (Licht. ex Roem. & Schult.) C.E.Hubb.		native	Chloridoideae	4	5

Family	Scientific name	Common synonyms	Origin	Grass sub-family	*matK* replicates	*rbcLa* replicates
Poaceae	*Enteropogon macrostachyus* (A.Rich.) Munro ex Benth.		native	Chloridoideae	1	2
Poaceae	*Entolasia olivacea* Stapf		naturalised	Panicoideae	1	1
Poaceae	*Entoplocamia aristulata* (Hack. ex Rendle) Stapf		native	Chloridoideae	2	3
Poaceae	*Eragrostis virescens* J.Presl	*Eragrostis mexicana*	naturalised	Chloridoideae	2	3
Poaceae	*Eragrostis aspera* (Jacq.) Nees		native	Chloridoideae	1	1
Poaceae	*Eragrostis barrelieri* Daveau		naturalised	Chloridoideae	2	2
Poaceae	*Eragrostis bergiana* (Kunth) Trin.		native	Chloridoideae	1	1
Poaceae	*Eragrostis brizantha* Nees		native	Chloridoideae	1	1
Poaceae	*Eragrostis capensis* (Thunb.) Trin.		native	Chloridoideae	1	1
Poaceae	*Eragrostis cilianensis* (All.) Vignolo ex Janch.		native	Chloridoideae	0	1
Poaceae	*Eragrostis crassinervis* Hack.		native	Chloridoideae	1	1

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Eragrostis curvula* (Schrad.) Nees	*Eragrostis jeffreysii*	native	Chloridoideae	4	5
Poaceae	*Eragrostis cylindriflora* Hochst.		native	Chloridoideae	4	4
Poaceae	*Eragrostis glandulosipedata* De Winter		native	Chloridoideae	1	1
Poaceae	*Eragrostis gummiflua* Nees		native	Chloridoideae	2	2
Poaceae	*Eragrostis homomalla* Nees		native	Chloridoideae	1	1
Poaceae	*Eragrostis inamoena* K.Schum.		native	Chloridoideae	2	2
Poaceae	*Eragrostis japonica* (Thunb.) Trin.	*Diandrochloa namaquensis*	native	Chloridoideae	1	1
Poaceae	*Eragrostis lappula* Nees		native	Chloridoideae	1	1
Poaceae	*Eragrostis lehmanniana* Nees		native	Chloridoideae	3	3
Poaceae	*Eragrostis macrochlamys* Pilg.		native	Chloridoideae	1	1

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Eragrostis minor* Host		naturalised	Chloridoideae	2	4
Poaceae	*Eragrostis obtusa* Munro ex Ficalho & Hiern		native	Chloridoideae	1	1
Poaceae	*Eragrostis omahekensis* De Winter		native	Chloridoideae	1	1
Poaceae	*Eragrostis pallens* Hack.		native	Chloridoideae	1	1
Poaceae	*Eragrostis patens* Oliv.		native	Chloridoideae	1	1
Poaceae	*Eragrostis pilosa* (L.) P.Beauv.	*Eragrostis pilosa* subsp. *pilosa*	uncertain origin	Chloridoideae	0	2
Poaceae	*Eragrostis porosa* Nees		native	Chloridoideae	1	1
Poaceae	*Eragrostis procumbens* Nees		native	Chloridoideae	1	1
Poaceae	*Eragrostis pusilla* Hack.	*Diandrochloa pusilla*	native	Chloridoideae	1	1
Poaceae	*Eragrostis rotifer* Rendle		native	Chloridoideae	3	3
Poaceae	*Eragrostis superba* Peyr.		native	Chloridoideae	6	6
Poaceae	*Eragrostis tef* (Zucc.) Trotter		naturalised	Chloridoideae	1	1
Poaceae	*Eragrostis truncata* Hack.		native	Chloridoideae	1	1

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Eragrostis viscosa* (Retz.) Trin.		native	Chloridoideae	1	1
Poaceae	*Eragrostis volkensii* Pilg.		native	Chloridoideae	1	1
Poaceae	*Eriochloa fatmensis* (Hochst. & Steud.) Clayton		native	Panicoideae	1	1
Poaceae	*Eustachys paspaloides* (Vahl) Lanza & Mattei		native	Chloridoideae	3	3
Poaceae	*Festuca arundinacea* Schreb.		naturalised	Pooideae	3	3
Poaceae	*Festuca scabra* Vahl		native	Pooideae	0	1
Poaceae	*Fingerhuthia africana* Lehm.		native	Chloridoideae	4	5
Poaceae	*Gastridium phleoides* (Nees & Meyen) C.E.Hubb.	*Gastridium ventricosum*	naturalised	Pooideae	3	4
Poaceae	*Geochloa decora* (Nees) N.P.Barker & H.P.Linde	*Merxmuellera decora, Danthonia decora*	native	Danthonioideae	2	2
Poaceae	*Geochloa lupulina* (L.f.) N.P.Barker & H.P.Linder	*Merxmuellera lupulina, Danthonia lupulina*	native	Danthonioideae	2	2

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Geochloa rufa* (Nees) N.P.Barker & H.P.Linder	*Merxmuellera rufa, Danthonia rufa*	native	Danthonioideae	1	1
Poaceae	*Glyceria maxima* (Hartm.) Holmb.	*Poa aquatica*	naturalised	Pooideae	3	3
Poaceae	*Hainardia cylindrica* (Willd.) Greuter		naturalised	Pooideae		
Poaceae	*Harpochloa falx* (L.f.) Kuntze		native	Chloridoideae	1	1
Poaceae	*Helictotrichon imberbe* (Nees) Veldkamp	*Helictotrichon turgidulum*	native	Pooideae	2	2
Poaceae	*Helictotrichon longum* (Stapf) Schweick.		native	Pooideae	1	1
Poaceae	*Hemarthria altissima* (Poir.) Stapf & C.E.Hubb.		native	Panicoideae	1	1
Poaceae	*Heteropogon contortus* (L.) Roem. & Schult.		native	Panicoideae	2	3
Poaceae	*Heteropogon melanocarpus* (Elliott) Benth.		native	Panicoideae	1	1
Poaceae	*Holcus lanatus* L.		naturalised	Pooideae	3	4

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Hordeum marinum* subsp. *gussoneanum* (Parl.) Thell.	*Hordeum geniculatum*	naturalised	Pooideae	3	4
Poaceae	*Hordeum murinum* L.	*Hordeum murinum* subsp*. murinum*	naturalised	Pooideae	4	8
Poaceae	*Hordeum murinum* L. subsp*. glaucum* (Steud.) Tzvelev		naturalised	Pooideae	1	1
Poaceae	*Hordeum murinum* L. subsp*. leporinum* (Link) Arcang.		naturalised	Pooideae	2	6
Poaceae	*Hordeum stenostachys* Godr.		naturalised	Pooideae	1	2
Poaceae	*Hyparrhenia hirta* (L.) Stapf		native	Panicoideae	2	3
Poaceae	*Hyperthelia dissoluta* (Nees ex Steud.) Clayton		native	Panicoideae	3	3
Poaceae	*Imperata cylindrica* (L.) Raeusch.		uncertain origin	Panicoideae	1	2
Poaceae	*Ischaemum afrum* (J.F.Gmel.) Dandy		native	Panicoideae	4	4
Poaceae	*Ischaemum polystachyum* J.Presl	*Ischaemum fasciculatum*	native	Panicoideae	1	1

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Joinvilleaceae	*Joinvillea ascendens* Gaudich. ex Brongn. & Gris		sister clade		1	1
Poaceae	*Koeleria capensis* Nees		native	Pooideae	1	1
Poaceae	*Lagurus ovatus* L.		naturalised	Pooideae	2	3
Poaceae	*Lamarckia aurea* (L.) Moench		naturalised	Pooideae	1	2
Poaceae	*Leersia hexandra* Sw.		native	Ehrhartoideae	0	2
Poaceae	*Leptochloa eleusine* (Nees) Cope & N.Snow	*Diplachne eleusine*	native	Chloridoideae	1	1
Poaceae	*Leptochloa fusca* (L.) Kunth	*Diplachne fusca*	native	Chloridoideae	3	2
Poaceae	*Lepturus repens* (G.Forst.) R.Br.		naturalised	Chloridoideae	1	3
Poaceae	*Lintonia nutans* Stapf		naturalised	Chloridoideae	0	2
Poaceae	*Lolium multiflorum* Lam.		naturalised	Pooideae	3	3
Poaceae	*Lolium multiflorum* Lam. x *Lolium perenne* L.		naturalised	Pooideae	1	1

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Lolium perenne* L.		naturalised	Pooideae	3	3
Poaceae	*Lolium rigidum* Gaudin		naturalised	Pooideae	1	2
Poaceae	*Lolium temulentum* L.		naturalised	Pooideae	2	2
Poaceae	*Loudetia filifolia* Schweick.		native	Panicoideae	3	3
Poaceae	*Loudetia flavida* (Stapf) C.E.Hubb.		native	Panicoideae	1	1
Poaceae	*Megaloprotachne albescens* C.E.Hubb.		native	Panicoideae	1	1
Poaceae	*Melica racemosa* Thunb.		native	Pooideae	1	1
Poaceae	*Melinis minutiflora* P.Beauv.		native	Panicoideae	3	3
Poaceae	*Melinis nerviglumis* (Franch.) Zizka		native	Panicoideae	1	1
Poaceae	*Melinis repens* (Willd.) Zizka		uncertain origin	Panicoideae	5	7
Poaceae	*Merxmuellera davyi* (C.E.Hubb.) Conert	*Danthonia davyi*	native	Danthonioideae	1	1
Poaceae	*Merxmuellera drakensbergensis* (Schweick.) Conert	*Danthonia drakensbergensis*	native	Danthonioideae	1	1

Family	Scientific name	Common synonyms	Origin	Grass sub-family	*matK* replicates	*rbcLa* replicates
Poaceae	*Merxmuellera macowanii* (Stapf) Conert	*Danthonia macowanii*	native	Danthonioideae	0	1
Poaceae	*Merxmuellera stereophylla* (J.G.Anderson) Conert	*Danthonia stereophylla*	native	Danthonioideae	1	1
Poaceae	*Monelytrum luederitzianum* Hack.		native	Chloridoideae	1	2
Poaceae	*Nassella neesiana* (Trin. & Rupr.) Barkworth		naturalised	Pooideae	2	3
Poaceae	*Nassella tenuissima* (Trin.) Barkworth		naturalised	Pooideae	0	1
Poaceae	*Nassella trichotoma* (Nees) Hack. ex Arechav.		naturalised	Pooideae	1	1
Poaceae	*Oplismenus burmanni* (Retz.) P.Beauv.		native	Panicoideae	2	3
Poaceae	*Oplismenus hirtellus* (L.) P.Beauv.	*Oplismenus hirtellus* subsp*. Imbecillus*	native	Panicoideae	2	2
Poaceae	*Oplismenus undulatifolius* (Ard.) Roem. & Schult.		native	Panicoideae	3	4

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Oropetium capense* Stapf		native	Chloridoideae	3	3
Poaceae	*Isachne rigens* (Sw.) Trin. (unresolved)	*Panicum arbusculum* Mez	native	Panicoideae	1	1
Poaceae	*Panicum schinzii* Hack.		native	Panicoideae	1	1
Poaceae	*Panicum arcurameum* Stapf		native	Panicoideae	1	1
Poaceae	*Panicum bechuanense* Bremek. & Oberm.		native	Panicoideae	1	1
Poaceae	*Panicum coloratum* L.		native	Panicoideae	4	4
Poaceae	*Panicum deustum* Thunb.		native	Panicoideae	4	4
Poaceae	*Panicum dewinteri* J.G.Anderson		native	Panicoideae	1	1
Poaceae	*Panicum dregeanum* Nees		native	Panicoideae	1	1
Poaceae	*Panicum fluviicola* Steud.		native	Panicoideae	1	1
Poaceae	*Panicum funaense* Vanderyst		native	Panicoideae	1	1
Poaceae	*Panicum genuflexum* Stapf		native	Panicoideae	1	1

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Panicum glandulopaniculatum* Renvoize		native	Panicoideae	1	1
Poaceae	*Panicum hirtum* Lam.	*Panicum heterostachyum*	native	Panicoideae	1	1
Poaceae	*Panicum infestum* Andersson		native	Panicoideae	1	1
Poaceae	*Panicum maximum* Jacq.	*Megathyrsus maximus*	native	Panicoideae	4	4
Poaceae	*Panicum miliaceum* L.		naturalised	Panicoideae	3	3
Poaceae	*Panicum parvifolium* Lam.		native	Panicoideae	2	2
Poaceae	*Panicum subalbidum* Kunth		native	Panicoideae	1	1
Poaceae	*Parapholis incurva* (L.) C.E.Hubb.		naturalised	Pooideae	4	2
Poaceae	*Paspalidium obtusifolium* (Delile) Simpson		uncertain origin	Panicoideae	1	1
Poaceae	*Paspalum dilatatum* Poir.		naturalised	Panicoideae	2	2
Poaceae	*Paspalum distichum* L.	*Paspaum paspalodes*	naturalised	Panicoideae	1	2
Poaceae	*Paspalum notatum* Flüggé		naturalised	Panicoideae	0	2

Family	Scientific name	Common synonyms	Origin	Grass sub-family	*matK* replicates	*rbcLa* replicates
Poaceae	*Paspalum quadrifarium* Lam.		naturalised	Panicoideae	1	1
Poaceae	*Paspalum urvillei* Steud.		naturalised	Panicoideae	0	2
Poaceae	*Paspalum vaginatum* Sw.		uncertain origin	Panicoideae	1	2
Poaceae	*Pennisetum clandestinum* Hochst. ex Chiov	*Cenchrus clandestinus*	naturalised	Panicoideae	2	2
Poaceae	*Pennisetum glaucum* (L.) R.Br.	*Cenchrus spicatus*	naturalised	Panicoideae	2	3
Poaceae	*Pennisetum macrourum* Trin.	*Cenchrus caudatus*	uncertain origin	Panicoideae	2	2
Poaceae	*Pennisetum mezianum* Leeke	*Cenchrus mezianus*	native	Panicoideae	1	1
Poaceae	*Pennisetum purpureum* Schumach.	*Cenchrus purpureus*	naturalised	Panicoideae	1	1
Poaceae	*Pennisetum setaceum* (Forssk.) Chiov.	*Cenchrus setaceus*	naturalised	Panicoideae	2	3
Poaceae	*Pennisetum thunbergii* Kunth	*Cenchrus geniculatus*	native	Panicoideae	1	1
Poaceae	*Pennisetum villosum* R.Br. ex Fresen.	*Cenchrus longisetus*	naturalised	Panicoideae	2	3

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Pentameris glacialis* N.P.Barker		native	Danthonioideae	0	1
Poaceae	*Pentameris longiglumis* (Nees) Steud.		native	Danthonioideae	1	1
Poaceae	*Pentameris obtusifolia* (Hochst.) Schweick.	*Pseudopentameris obtusifolia*	native	Danthonioideae	1	1
Poaceae	*Pentameris oreophila* N.P.Barker		native	Danthonioideae	0	1
Poaceae	*Pentaschistis airoides* (Nees) Stapf	*Pentameris airoides*	native	Danthonioideae	1	1
Poaceae	*Pentaschistis aurea* (Steud.) McClean	*Pentameris aurea*	native	Danthonioideae	1	1
Poaceae	*Pentaschistis capensis* (Nees) Stapf	*Pentameris capensis*	native	Danthonioideae	1	1
Poaceae	*Pentaschistis colorata* (Steud.) Stapf	*Pentameris colorata*	native	Danthonioideae	1	1
Poaceae	*Pentaschistis ecklonii* (Nees) McClean	*Pentameris bachmanni i*	native	Danthonioideae	1	1

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Pentaschistis eriostoma* (Nees) Stapf	*Pentameris eriostoma*	native	Danthonioideae	1	1
Poaceae	*Pentaschistis glandulosa* (Schrad.) H.P.Linder	*Pentameris glandulosa*	native	Danthonioideae	1	1
Poaceae	*Pentaschistis heptamera* (Nees) Stapf	*Pentameris heptamera*	native	Danthonioideae	1	1
Poaceae	*Pentaschistis longipes* Stapf		native	Danthonioideae	1	1
Poaceae	*Pentaschistis natalensis* Stapf	*Pentameris natalensis*	native	Danthonioideae	1	1
Poaceae	*Pentaschistis patula* (Nees) Stapf	*Pentameris patula*	native	Danthonioideae	1	1
Poaceae	*Pentaschistis pyrophila* H.P.Linder	*Pentameris pyrophila*	native	Danthonioideae	1	1
Poaceae	*Pentaschistis reflexa* H.P.Linder	*Pentameris reflexa*	native	Danthonioideae	1	1
Poaceae	*Pentaschistis tomentella* Stap	*Pentameris tomentella*	native	Danthonioideae	1	1
Poaceae	*Pentaschistis tysonii* Stapf	*Pentameris tysonii*	native	Danthonioideae	0	1

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Pentaschistis veneta* H.P.Linder	*Pentameris veneta*	native	Danthonioideae	1	1
Poaceae	*Pentaschistis viscidula* (Nees) Stapf	*Pentameris viscidula*	native	Danthonioideae	1	1
Poaceae	*Perotis patens* Gand.		native	Chloridoideae	4	4
Poaceae	*Phalaris arundinacea* L.		naturalised	Pooideae	2	3
Poaceae	*Phalaris canariensis* L.		naturalised	Pooideae	0	1
Poaceae	*Pharus latifolius* L.		Early diverging grass lineage	Pharoideae	1	1
Poaceae	*Phragmites australis* (Cav.) Steud.		uncertain origin	Arundinoideae.	1	1
Poaceae	*Phragmites mauritianus* Kunth		native	Arundinoideae.	4	5
Poaceae	*Poa annua* L.		naturalised	Pooideae	3	3
Poaceae	*Poa binata* Nees		native	Pooideae	1	1
Poaceae	*Poa bulbosa* L.		uncertain origin	Pooideae	5	5
Poaceae	*Poa pratensis* L.		naturalised	Pooideae	3	4
Poaceae	*Poa trivialis* L.		naturalised	Pooideae		3

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Pogonarthria squarrosa* (Roem. & Schult.) Pilg.		native	Chloridoideae	9	9
Poaceae	*Polypogon monspeliensis* (L.) Desf.		naturalised	Pooideae	2	3
Poaceae	*Polypogon viridis* (Gouan) Breistr.		naturalised	Pooideae	1	2
Poaceae	*Pseudopentameris macrantha* (Schrad.) Conert	*Pentameris macrantha*	native	Danthonioideae	1	1
Poaceae	*Puccinellia acroxantha* C.A.Sm. & C.E.Hubb.		native	Pooideae	1	1
Poaceae	*Puccinellia angustata* (R.Br.) E.L.Rand & Redfield		native	Pooideae	5	5
Poaceae	*Puccinellia distans* L. Parl.		naturalised	Pooideae	3	3
Poaceae	*Puccinellia fasciculata* (Torr.) E.P.Bicknell		naturalised	Pooideae	0	3
Poaceae	*Puelia olyriformis* (Franch.) Clayton		Early diverging grass lineage	Puelioideae	1	1
Poaceae	*Rostraria cristata* (L.) Tzvelev		naturalised	Pooideae	1	3

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Rostraria pumila* (Desf.) Tzvelev		naturalised	Pooideae	1	1
Poaceae	*Rottboellia cochinchinensis* (Lour.) Clayton		uncertain origin	Panicoideae	1	**1**
Poaceae	*Sacciolepis africana* C.E.Hubb. & Snowden		native	Panicoideae	1	1
Poaceae	*Sacciolepis curvata* (L.) Chase	*Panicum curvatum*	native	Panicoideae	1	1
Poaceae	*Sacciolepis indica* (L.) Chase		native	Panicoideae	2	2
Poaceae	*Sacciolepis myosuroides* (R.Br.) A.Camus	*Sacciolepis spiciformis*	native	Panicoideae	1	1
Poaceae	*Sacciolepis typhura* (Stapf) Stapf		native	Panicoideae	1	1
Poaceae	*Schismus barbatus* (L.) Thell.		native	Danthonioideae	2	3
Poaceae	*Schismus scaberrimus* Nees		native	Danthonioideae	1	1
Poaceae	*Schmidtia pappophoroides* Steud. ex J.A.Schmidt		native	Chloridoideae	5	5

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Schoenefeldia transiens* (Pilg.) Chiov.		native	Chloridoideae	1	1
Poaceae	*Setaria appendiculata* (Hack.) Stapf		native	Panicoideae	1	1
Poaceae	*Setaria incrassata* (Hochst.) Hack.		native	Panicoideae	5	5
Poaceae	*Setaria italica* (L.) P.Beauv.		naturalised	Panicoideae	2	3
Poaceae	*Setaria lindenbergiana* (Nees) Stapf		native	Panicoideae	1	1
Poaceae	*Setaria megaphylla* (Steud.) T.Durand & Schinz		native	Panicoideae	1	1
Poaceae	*Setaria parviflora* (Poir.) M.Kerguelen	*Setaria geniculata*	naturalised	Panicoideae	1	1
Poaceae	*Setaria pumila* (Poir.) Roem. & Schult		uncertain origin	Panicoideae	5	5
Poaceae	*Setaria sagittifolia* (A.Rich.) Walp.		native	Panicoideae	2	2
Poaceae	*Setaria sphacelata* (Schumach.) Stapf & C.E.Hubb. ex Moss		native	Panicoideae	3	3

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Setaria verticillata* (L.) P.Beauv.		uncertain origin	Panicoideae	1	1
Poaceae	*Sorghum × drummondii* (Nees ex Steud.) Millsp. & Chase	*Sorghum bicolor* subsp*. drummondii*	naturalised	Panicoideae	0	1
Poaceae	*Sorghum bicolor* (L.) Moench		native	Panicoideae	4	4
Poaceae	*Sorghum halepense* (L.) Pers.		naturalised	Panicoideae	3	3
Poaceae	*Sorghum versicolor* Andersson		native	Panicoideae	2	2
Poaceae	*Sphenopus divaricatus* (Gouan) Rchb.		naturalised	Pooideae	0	2
Poaceae	*Sporobolus acinifolius* Stapf		native	Chloridoideae	1	1
Poaceae	*Sporobolus africanus* (Poir.) Robyns & Tournay		native	Chloridoideae	3	3
Poaceae	*Sporobolus albicans* (Nees ex Trin.) Nees		native	Chloridoideae	1	1
Poaceae	*Sporobolus centrifugus* (Trin.) Nees		native	Chloridoideae	1	1

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Sporobolus coromandelianus* (Retz.) Kunth		native	Chloridoideae	1	1
Poaceae	*Sporobolus festivus* Hochst. ex A.Rich.		native	Chloridoideae	2	2
Poaceae	*Sporobolus fimbriatus* (Trin.) Nees		native	Chloridoideae	3	3
Poaceae	*Sporobolus ludwigii* Hochst.		native	Chloridoideae	1	1
Poaceae	*Sporobolus natalensis* (Steud.) T.Durand & Schinz		native	Chloridoideae	1	1
Poaceae	*Sporobolus nebulosus* Hack.		native	Chloridoideae	1	1
Poaceae	*Sporobolus nitens* Stent		native	Chloridoideae	5	5
Poaceae	*Sporobolus panicoides* A.Rich.		native	Chloridoideae	1	1
Poaceae	*Sporobolus pectinatus* Hack.		native	Chloridoideae	1	1
Poaceae	*Sporobolus pellucidus* Hochst.		native	Chloridoideae	1	1

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Sporobolus pyramidalis* P.Beauv.		native	Chloridoideae	3	3
Poaceae	*Sporobolus salsus* Mez		native	Chloridoideae	1	1
Poaceae	*Sporobolus spicatus* (Vahl) Kunth		native	Chloridoideae	1	1
Poaceae	*Sporobolus stapfianus* Gand.		native	Chloridoideae	2	2
Poaceae	*Sporobolus subulatus* Hack. ex Scott-Elliot		native	Chloridoideae	1	1
Poaceae	*Sporobolus virginicus* (L.) Kunth		uncertain origin	Chloridoideae	1	1
Poaceae	*Steinchisma hians* (Elliott) Nash & Small	*Panicum hians*	naturalised	Panicoideae	0	1
Poaceae	*Stenotaphrum secundatum* (Walter) Kuntze		uncertain origin	Panicoideae	2	2
Poaceae	*Stipa capensis* Thunb.	*Achnatherum clandestinum*	native	Pooideae	1	1
Poaceae	*Stipa clandestina* Hack.		naturalised	Pooideae	1	1
Poaceae	*Stipa dregeana* Steud.	*Jarava plumosa*	native	Pooideae	1	1
Poaceae	*Stipa papposa* Nees	*Austrostipa variabilis*	naturalised	Pooideae	0	1

Family	Scientific name	Common synonyms	Origin	Grass sub-family	*matK* replicates	*rbcLa* replicates
Poaceae	*Stipa variabilis* Hughes		naturalised	Pooideae	1	1
Poaceae	*Stipagrostis amabilis* (Schweick.) De Winter		native	Aristidoideae	1	1
Poaceae	*Stipagrostis anomala* De Winter		native	Aristidoideae	1	1
Poaceae	*Stipagrostis brevifolia* (Nees) De Winter		native	Aristidoideae	1	1
Poaceae	*Stipagrostis damarensis* (Mez) De Winter		native	Aristidoideae	1	1
Poaceae	*Stipagrostis dregeana* Nees		native	Aristidoideae	1	1
Poaceae	*Stipagrostis fastigiata* (Hack.) De Winter		native	Aristidoideae	1	1
Poaceae	*Stipagrostis garubensis* (Pilg.) De Winter		native	Aristidoideae	1	1
Poaceae	*Stipagrostis geminifolia* Nees		native	Aristidoideae	1	1
Poaceae	*Stipagrostis giessii* Kers		native	Aristidoideae	1	1

Family	Scientific name	Common synonyms	Origin	Grass sub-family	*matK* replicates	*rbcLa* replicates
Poaceae	*Stipagrostis gonatostachys* (Pilg.) De Winter		native	Aristidoideae	1	1
Poaceae	*Stipagrostis hermannii* (Mez) De Winter		native	Aristidoideae	1	1
Poaceae	*Stipagrostis hirtigluma* (Steud. ex Trin. & Rupr.) De Winter		native	Aristidoideae	1	1
Poaceae	*Stipagrostis hochstetteriana* (Beck ex Hack.) De Winter		native	Aristidoideae	1	1
Poaceae	*Stipagrostis lutescens* (Nees) De Winter		native	Aristidoideae	1	1
Poaceae	*Stipagrostis obtusa* (Delile) Nees		native	Aristidoideae	1	1
Poaceae	*Stipagrostis sabulicola* (Pilg.) De Winter		native	Aristidoideae	1	1
Poaceae	*Stipagrostis subacaulis* (Nees) De Winter		native	Aristidoideae	1	1
Poaceae	*Stipagrostis uniplumis* (Licht.) De Winter		native	Aristidoideae	4	4

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Stipagrostis zeyheri* (Nees) De Winter		native	Aristidoideae	1	2
Poaceae	*Streptochaeta angustifolia* Soderstr.		Early diverging grass lineage	Anomochlooideae	2	2
Poaceae	*Tenaxia aureocephala* (J.G.Anderson) N.P.Barker & H.P.Linder	*Merxmuellera aureocephala, Danthonia aureocephala*	native	Danthonioideae	1	1
Poaceae	*Tenaxia disticha* (Nees) N. P. Barker & H. P. Linder	*Merxmuellera disticha*	native	Danthonioideae	2	2
Poaceae	*Tenaxia stricta* (Schrad.) N.P.Barker & H.P.Linder	*Merxmuellera stricta*	native	Danthonioideae	2	2
Poaceae	*Tenaxia dura* (Stapf) N.P.Barker & H.P.Linder	*Merxmuellera dura*	native	Danthonioideae	1	1
Poaceae	*Tetrachne dregei* Nees		native	Chloridoideae	0	1
Poaceae	*Tetrapogon tenellus* (Roxb.) Chiov.		native	Chloridoideae	1	2
Poaceae	*Themeda triandra* Forssk.		native	Panicoideae	3	3

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Trachypogon spicatus* (L.f.) Kuntze		native	Panicoideae	0	1
Poaceae	*Tragus berteronianus* Schult.		uncertain origin	Chloridoideae	4	5
Poaceae	*Tragus racemosus* (L.) All.		native	Chloridoideae	1	1
Poaceae	*Tribolium curvum* (Nees) Verboom & H.P. Linder	*Karroochloa curva, Danthonia curva*	native	Danthonioideae	1	1
Poaceae	*Tribolium brachystachyum* (Nees) Renvoize		native	Danthonioideae	1	1
Poaceae	*Tribolium echinatum* (Thunb.) Renvoize		native	Danthonioideae	1	2
Poaceae	*Tribolium hispidum* (Thunb.) Desv		native	Danthonioideae	2	2
Poaceae	*Tribolium obtusifolium* (Nees) Renvoize	*Dactylis ovata*	native	Danthonioideae	1	1
Poaceae	*Tribolium pleuropogon* (Stapf) Verboom & H.P.Linder	*Schismus pleuropogon*	native	Danthonioideae	1	1

Family	**Scientific name**	**Common synonyms**	**Origin**	**Grass sub-family**	***matK* replicates**	***rbcLa* replicates**
Poaceae	*Tribolium purpureum* (L.f.) Verboom & H.P.Linder	*Karroochloa purpurea*	native	Danthonioideae	1	2
Poaceae	*Tribolium utriculosum* (Nees) Renvoize		native	Danthonioideae	1	1
Poaceae	*Tricholaena monachne* (Trin.) Stapf & C.E.Hubb.		native	Panicoideae	5	5
Poaceae	*Trichoneura grandiglumis* (Nees) Ekman		native	Chloridoideae	2	3
Poaceae	*Tripogon minimus* (A.Rich.) Hochst. ex Steud.		native	Chloridoideae	1	1
Poaceae	*Triraphis purpurea* Hack.		native	Chloridoideae	1	1
Poaceae	*Triraphis ramosissima* Hack.		native	Chloridoideae	1	2
Poaceae	*Triraphis schinzii* Hack.		native	Chloridoideae	1	1
Poaceae	*Tristachya lualabaensis* (De Wild.) J.B.Phipps		native	Panicoideae	1	1
Poaceae	*Tristachya nodiglumis* K.Schum.		native	Panicoideae	1	1
Poaceae	*Tristachya pedicellata* Stent	*Loudetia pedicellata*	native	Panicoideae	1	1

Family	Scientific name	Common synonyms	Origin	Grass sub-family	*matK* replicates	*rbcLa* replicates
Poaceae	*Tristachya rehmannii* Hack.		native	Panicoideae	1	1
Poaceae	*Urochloa mosambicensis* (Hack.) Dandy		native	Panicoideae	5	5
Poaceae	*Urochloa oligotricha* (Fig. & De Not.) Henrard		native	Panicoideae	2	2
Poaceae	*Urochloa panicoides* P.Beauv.		native	Panicoideae	1	1
Poaceae	*Urochloa trichopus* (Hochst.) Stapf		native	Panicoideae	1	1
Poaceae	*Vulpia bromoides* (L.) Gray		naturalised	Pooideae	3	3
Poaceae	*Vulpia fasciculata* (Forssk.) Samp.		naturalised	Pooideae	2	2
Poaceae	*Vulpia muralis* (Kunth) Nees		naturalised	Pooideae	1	1
Poaceae	*Vulpia myuros* (L.) C.C.Gmel.		naturalised	Pooideae	3	3

Appendix 1B List of taxa included in this study from the Grasses of southern Africa project carried out at ACDB, including both naturalised and native grass species.

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Acrachne racemosa (B.Heyne ex Roth) Ohwi	native	PRE275	KNPB085-10	✓	✓
Acrachne racemosa (B.Heyne ex Roth) Ohwi	native	KNP165	KNPB640-10	✓	✓
Acrachne racemosa (B.Heyne ex Roth) Ohwi	native	YBK340	KNPA293-09	✓	✓
Andropogon schirensis Hochst.	native	BS013	KNPB678-10	✓	✓
Aristida adscensionis L.	native	YBK243	KNPA438-09	✓	✓
Aristida adscensionis L.	native	YBK103	KNPA387-09	✓	✓
Aristida adscensionis L.	native	YBK399	KNPA322-09	✓	✓
Aristida aequiglumis Hack.	native	PRE337	KNPB147-10	✓	✓
Aristida bipartita (Nees) Trin. & Rupr.	native	PRE335	KNPB145-10	✓	✓
Aristida nemorivaga Henrard	native	PRE336	KNPB146-10	✓	✓
Aristida congesta Roem. & Schult.	native	YBK405	KNPA327-09	✓	✓
Aristida congesta Roem. & Schult.	native	BS011	KNPB676-10	✓	✓
Aristida diffusa Trin.	native	PRE340	KNPB150-10	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Aristida engleri Mez	native	PRE342	KNPB152-10	✓	✓
Aristida hubbardiana Schweick.	native	PRE330	KNPB140-10	✓	✓
Aristida meridionalis Henrard	native	KNP112	KNPB587-10	✓	✓
Aristida meridionalis Henrard	native	PRE344	KNPB154-10	✓	✓
Aristida meridionalis Henrard	native	YBK014	KNPA255-09	✓	✓
Aristida meridionalis Henrard	native	OM0662	KNPA217-09	✓	✓
Aristida mollissima Pilg.	native	PRE345	KNPB155-10	✓	✓
Aristida parvula (Nees) De Winter	native	PRE328	KNPB138-10	✓	✓
Aristida pilgeri Henrard	native	PRE347	KNPB157-10	✓	✓
Aristida scabrivalvis Hack.	native	PRE334	KNPB144-10	✓	✓
Aristida scabrivalvis Hack.	native	KNP113	KNPB588-10	✓	✓
Aristida scabrivalvis Hack.	native	YBK227	KNPA433-09	✓	✓
Aristida scabrivalvis Hack.	native	YBK285	KNPA457-09	✓	✓
Aristida scabrivalvis Hack.	native	YBK390	KNPA320-09	✓	✓
Aristida spectabilis Hack.	native	PRE343	KNPB153-10	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Aristida mutabilis Trin. & Rupr.	native	KNP114	KNPB589-10	✓	✓
Aristida stipitata Hack.	native	OM0850	KNPA236-09	✓	✓
Aristida transvaalensis Henrard	native	PRE338	KNPB148-10	✓	✓
Bothriochloa insculpta (A.Rich.) A.Camus	native	YBK180	KNPA414-09	✓	✓
Bothriochloa insculpta (A.Rich.) A.Camus	native	YBK378	KNPA315-09	✓	✓
Bothriochloa insculpta (A.Rich.) A.Camus	native	YBK091	KNPA382-09	✓	✓
Bothriochloa insculpta (A.Rich.) A.Camus	native	KNP014	KNPB489-10	✓	✓
Bothriochloa radicans (Lehm.) A.Camus	native	YBK364	KNPA307-09	✓	✓
Bothriochloa radicans (Lehm.) A.Camus	native	YBK224	KNPA431-09	✓	✓
Bothriochloa radicans (Lehm.) A.Camus	native	YBK211	KNPA426-09	✓	✓
Brachiaria brizantha (A.Rich.) Stapf	native	KNP050	KNPB525-10	✓	✓
Brachiaria dictyoneura (Fig. & De Not.) Stapf	native	YBK046	KNPA269-09	✓	✓
Brachiaria eruciformis (Sm.) Griseb.	uncertain origin	YBK215	KNPA428-09	✓	✓
Bromus catharticus Vahl	naturalised	PRE469	KNPB279-10	✓	✓
Bromus hordeaceus L.	naturalised	PRE473	KNPB283-10	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Bromus leptoclados Nees	native	PRE472	KNPB282-10	✓	✓
Calamagrostis epigejos (L.) Roth	uncertain origin	PRE309	KNPB119-10	✓	✓
Capeochloa arundinacea (P.J.Bergius) N.P.Barker & H.P.Linder	native	PRE520	KNPB330-10	✓	✓
Capeochloa cincta (Nees) N.P.Barker & H.P.Linder	native	PRE521	KNPB331-10	✓	✓
Cenchrus ciliaris L.	native	YBK356	KNPA306-09	✓	✓
Cenchrus ciliaris L.	native	OM0565	KNPA205-09	✓	✓
Chloris roxburghiana Schult.	native	OM0389	KNPA196-09	✓	✓
Chloris roxburghiana Schult.	native	KNP155	KNPB630-10	✓	✓
Chloris virgata Sw.	naturalised	YBK147	KNPA402-09	✓	✓
Chloris virgata Sw.	naturalised	YBK190	KNPA417-09	✓	✓
Chloris virgata Sw.	naturalised	YBK013	KNPA254-09	✓	✓
Chloris virgata Sw.	naturalised	YBK393	KNPA321-09	✓	✓
Chloris virgata Sw.	naturalised	YBK355	KNPA305-09	✓	✓
Chloris virgata Sw.	naturalised	KNP156	KNPB631-10	✓	✓
Cymbopogon caesius (Hook. & Arn.) Stapf	native	KNP022	KNPB497-10	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Cymbopogon caesius (Hook. & Arn.) Stapf	native	KNP023	KNPB498-10	✓	✓
Cymbopogon caesius (Hook. & Arn.) Stapf	native	YBK031	KNPA262-09	✓	✓
Cymbopogon pospischilii (K.Schum.) C.E.Hubb	native	YBK008	KNPA251-09	✓	✓
Cymbopogon pospischilii (K.Schum.) C.E.Hubb	native	YBK377	KNPA314-09	✓	✓
Dactyloctenium aegyptium (L.) Willd.	native	YBK335	KNPA288-09	✓	✓
Dactyloctenium giganteum B.S.Fisher & Schweick.	native	YBK336	KNPA289-09	✓	✓
Dactyloctenium giganteum B.S.Fisher & Schweick.	native	PRE279	KNPB089-10	✓	✓
Danthoniopsis dinteri (Pilg.) C.E.Hubb.	native	KNP100	KNPB575-10	✓	✓
Danthoniopsis dinteri (Pilg.) C.E.Hubb.	native	KNP101	KNPB576-10	✓	✓
Danthoniopsis pruinosa C.E.Hubb.	native	KNP102	KNPB577-10	✓	✓
Danthoniopsis pruinosa C.E.Hubb.	native	KNP103	KNPB578-10	✓	✓
Eragrostis japonica (Thunb.) Trin.	native	PRE379	KNPB189-10	✓	✓
Eragrostis pusilla Hack.	native	PRE380	KNPB190-10	✓	✓
Digitaria milanjiana (Rendle) Stapf	native	YBK254	KNPA441-09	✓	✓
Digitaria milanjiana (Rendle) Stapf	native	YBK354	KNPA304-09	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Digitaria velutina (Forssk.) P.Beauv.	native	YBK345	KNPA297-09	✓	✓
Dinebra retroflexa (Vahl) Panz. var. condensata S.M.Phillips	native	KNP161	KNPB636-10	✓	✓
Leptochloa fusca (L.) Kunth	native	PRE281	KNPB091-10	✓	✓
Echinochloa colona (L.) Link	uncertain origin	YBK347	KNPA299-09	✓	✓
Echinochloa colona (L.) Link	uncertain origin	KNP067	KNPB542-10	✓	✓
Echinochloa haploclada (Stapf) Stapf	native	KNP068	KNPB543-10	✓	✓
Echinochloa holubii (Stapf) Stapf	native	KNP070	KNPB545-10	✓	✓
Eleusine indica (L.) Gaertn.	naturalised	YBK349	KNPA300-09	✓	✓
Ellisochloa papposa (Nees) P.M.Peterson & N.P.Barker	native	PRE525	KNPB335-10	✓	✓
Ellisochloa rangei (Pilg.) P.M.Peterson & N.P.Barker	native	PRE526	KNPB336-10	✓	✓
Enneapogon cenchroides (Licht. ex Roem. & Schult.) C.E.Hubb.	native	YBK232	KNPA435-09	✓	✓
Enneapogon cenchroides (Licht. ex Roem. & Schult.) C.E.Hubb.	native	YBK195	KNPA420-09	✓	✓
Enneapogon cenchroides (Licht. ex Roem. & Schult.) C.E.Hubb.	native	KNP176	KNPB651-10	✓	✓
Enneapogon cenchroides (Licht. ex Roem. & Schult.) C.E.Hubb.	native	YBK351	KNPA302-09	✓	✓
Enneapogon cenchroides (Licht. ex Roem. & Schult.) C.E.Hubb.	native	RBN279	KNPA242-09	✓	-

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Enteropogon macrostachyus (A.Rich.) Munro ex Benth.	native	YBK330	KNPA473-09	✓	✓
Entoplocamia aristulata (Hack. ex Rendle) Stapf	native	PRE270	KNPB080-10	✓	✓
Eragrostis aspera (Jacq.) Nees	native	BS009	KNPB674-10	✓	✓
Eragrostis bergiana (Kunth) Trin.	native	PRE231	KNPB041-10	✓	✓
Eragrostis brizantha Nees	native	PRE227	KNPB037-10	✓	✓
Eragrostis crassinervis Hack.	native	KNP134	KNPB609-10	✓	✓
Eragrostis cylindriflora Hochst.	native	YBK376	KNPA313-09	✓	✓
Eragrostis cylindriflora Hochst.	native	YBK118	KNPA391-09	✓	✓
Eragrostis cylindriflora Hochst.	native	KNP135	KNPB610-10	✓	✓
Eragrostis cylindriflora Hochst.	native	YBK402	KNPA324-09	✓	✓
Eragrostis glandulosipedata De Winter	native	PRE238	KNPB048-10	✓	✓
Eragrostis gummiflua Nees	native	BS019	KNPB684-10	✓	✓
Eragrostis gummiflua Nees	native	YBK047	KNPA270-09	✓	✓
Eragrostis homomalla Nees	native	PRE199	KNPB009-10	✓	✓
Eragrostis inamoena K.Schum.	native	KNP137	KNPB612-10	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Eragrostis inamoena K.Schum.	native	KNP138	KNPB613-10	✓	✓
Eragrostis curvula (Schrad.) Nees	native	PRE239	KNPB049-10	✓	✓
Eragrostis lappula Nees	native	PRE213	KNPB023-10	✓	✓
Eragrostis lehmanniana Nees	native	YBK225	KNPA432-09	✓	✓
Eragrostis lehmanniana Nees	native	YBK072	KNPA281-09	✓	✓
Eragrostis lehmanniana Nees	native	YBK346	KNPA298-09	✓	✓
Eragrostis macrochlamys Pilg.	native	PRE228	KNPB038-10	✓	✓
Eragrostis obtusa Munro ex Ficalho & Hiern	native	PRE225	KNPB035-10	✓	✓
Eragrostis omahekensis De Winter	native	PRE240	KNPB050-10	✓	✓
Eragrostis pallens Hack.	native	PRE219	KNPB029-10	✓	✓
Eragrostis patens Oliv.	native	RL1143	KNPA244-09	✓	✓
Eragrostis porosa Nees	native	YBK337	KNPA290-09	✓	✓
Eragrostis procumbens Nees	native	PRE207	KNPB017-10	✓	✓
Eragrostis rotifer Rendle	native	PRE204	KNPB014-10	✓	✓
Eragrostis rotifer Rendle	native	YBK077	KNPA283-09	✓	✓

Scientific name	Origin	Voucher number	BOLD accession number	*matK* sequence	*rbcLa* sequence
Eragrostis rotifer Rendle	native	KNP141	KNPB616-10	✓	✓
Eragrostis superba Peyr.	native	KNP142	KNPB617-10	✓	✓
Eragrostis superba Peyr.	native	YBK374	KNPA312-09	✓	✓
Eragrostis superba Peyr.	native	YBK006	KNPA249-09	✓	✓
Eragrostis superba Peyr.	native	OM0422	KNPA198-09	✓	✓
Eragrostis superba Peyr.	native	YBK101	KNPA386-09	✓	✓
Eragrostis superba Peyr.	native	YBK191	KNPA418-09	✓	✓
Eragrostis truncata Hack.	native	PRE230	KNPB040-10	✓	✓
Eragrostis virescens J.Presl	naturalised	PRE202	KNPB012-10	✓	✓
Eragrostis viscosa (Retz.) Trin.	native	KNP143	KNPB618-10	✓	✓
Eragrostis volkensii Pilg.	native	PRE217	KNPB027-10	✓	✓
Eriochloa fatmensis (Hochst. & Steud.) Clayton	native	YBK078	KNPA284-09	✓	✓
Eustachys paspaloides (Vahl) Lanza & Mattei	native	OM0575	KNPA206-09	✓	✓
Eustachys paspaloides (Vahl) Lanza & Mattei	native	OM0720	KNPA228-09	✓	✓
Eustachys paspaloides (Vahl) Lanza & Mattei	native	PRE264	KNPB074-10	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Fingerhuthia africana Lehm.	native	BS033	KNPB698-10	✓	✓
Fingerhuthia africana Lehm.	native	YBK256	KNPA442-09	✓	✓
Fingerhuthia africana Lehm.	native	BS034	KNPB699-10	✓	✓
Fingerhuthia africana Lehm.	native	KNP181	KNPB656-10	✓	✓
Geochloa decora (Nees) N.P.Barker & H.P.Linde	native	PRE513	KNPB323-10	✓	✓
Geochloa lupulina (L.f.) N.P.Barker & H.P.Linder	native	PRE512	KNPB322-10	✓	✓
Geochloa rufa (Nees) N.P.Barker & H.P.Linder	native	PRE511	KNPB321-10	✓	✓
Helictotrichon longum (Stapf) Schweick.	native	PRE622	KNPB432-10	✓	✓
Helictotrichon imberbe (Nees) Veldkamp	native	PRE617	KNPB427-10	✓	✓
Heteropogon contortus (L.) Roem. & Schult.	native	YBK275	KNPA451-09	✓	✓
Heteropogon melanocarpus (Elliott) Benth.	native	KNP034	KNPB509-10	✓	✓
Hyparrhenia anamesa Clayton	naturalised	BS035	KNPB700-10	✓	✓
Hyparrhenia hirta (L.) Stapf	native	BSO27	KNPB692-10	✓	✓
Hyparrhenia hirta (L.) Stapf	native	BS026	KNPB691-10	✓	✓
Hyperthelia dissoluta (Nees ex Steud.) Clayton	native	YBK055	KNPA274-09	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Hyperthelia dissoluta (Nees ex Steud.) Clayton	native	YBK032	KNPA263-09	✓	✓
Hyperthelia dissoluta (Nees ex Steud.) Clayton	native	YBK150	KNPA405-09	✓	✓
Ischaemum afrum (J.F.Gmel.) Dandy	native	KNP001	KNPB476-10	✓	✓
Ischaemum afrum (J.F.Gmel.) Dandy	native	YBK223	KNPA430-09	✓	✓
Ischaemum afrum (J.F.Gmel.) Dandy	native	YBK220	KNPA429-09	✓	✓
Ischaemum polystachyum J.Presl	native	OM0762	KNPB460-10	✓	✓
Leptochloa eleusine (Nees) Cope & N.Snow	native	PRE282	KNPB092-10	✓	✓
Loudetia filifolia Schweick.	native	KNP099	KNPB574-10	✓	✓
Loudetia filifolia Schweick.	native	PRE594	KNPB404-10	✓	✓
Loudetia filifolia Schweick.	native	KNP098	KNPB573-10	✓	✓
Loudetia flavida (Stapf) C.E.Hubb.	native	OM0729	KNPA231-09	✓	✓
Tristachya pedicellata Stent	native	PRE592	KNPB402-10	✓	✓
Melinis repens (Willd.) Zizka	naturalised	YBK018	KNPA258-09	✓	✓
Melinis repens (Willd.) Zizka	naturalised	YBK050	KNPA272-09	✓	✓
Melinis repens (Willd.) Zizka	naturalised	OM0495	KNPA202-09	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Melinis repens (Willd.) Zizka	naturalised	YBK246	KNPA440-09	✓	✓
Melinis repens (Willd.) Zizka	naturalised	YBK414	KNPA335-09	✓	✓
Merxmuellera davyi (C.E.Hubb.) Conert	native	PRE523	KNPB333-10	✓	✓
Merxmuellera stereophylla (J.G.Anderson) Conert	native	PRE517	KNPB327-10	✓	✓
Monelytrum luederitzianum Hack.	native	PRE352	KNPB162-10	✓	✓
Oropetium capense Stapf	native	KNP159	KNPB634-10	✓	✓
Oropetium capense Stapf	native	PRE267	KNPB077-10	✓	✓
Panicum arbusculum Sieber ex Griseb /*Isachne rigens* (Sw.) Trin	native	PRE497	KNPB307-10	✓	✓
Panicum arcurameum Stapf	native	PRE482	KNPB292-10	✓	✓
Panicum bechuanense Bremek. & Oberm.	native	PRE493	KNPB303-10	✓	✓
Panicum coloratum L.	native	YBK074	KNPA282-09	✓	✓
Panicum coloratum L.	native	YBK369	KNPA310-09	✓	✓
Panicum coloratum L.	native	YBK165	KNPA409-09	✓	✓
Panicum deustum Thunb.	native	YBK334	KNPA287-09	✓	✓
Panicum deustum Thunb.	native	OM0704	KNPA223-09	✓	✓

Scientific name	Origin	Voucher number	BOLD accession number	*matK* sequence	*rbcLa* sequence
Panicum deustum Thunb.	native	KNP075	KNPB550-10	✓	✓
Panicum deustum Thunb.	native	YBK269	KNPA448-09	✓	✓
Panicum dewinteri J.G.Anderson	native	PRE507	KNPB317-10	✓	✓
Panicum dregeanum Nees	native	PRE489	KNPB299-10	✓	✓
Panicum funaense Vanderyst	native	PRE490	KNPB300-10	✓	✓
Panicum genuflexum Stapf	native	PRE488	KNPB298-10	✓	✓
Panicum glandulopaniculatum Renvoize	native	PRE480	KNPB290-10	✓	✓
Panicum hirtum Lam.	native	PRE481	KNPB291-10	✓	✓
Panicum infestum Andersson	native	PRE486	KNPB296-10	✓	✓
Panicum maximum Jacq.	native	PRE485	KNPB295-10	✓	✓
Panicum maximum Jacq.	native	YBK090	KNPA381-09	✓	✓
Panicum maximum Jacq.	native	YBK210	KNPA425-09	✓	✓
Panicum parvifolium Lam.	native	PRE477	KNPB287-10	✓	✓
Paspalidium obtusifolium (Delile) Simpson	uncertain origin	PRE528	KNPB338-10	✓	✓
Pennisetum mezianum Leeke	native	PRE467	KNPB277-10	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Pennisetum setaceum (Forssk.) Chiov.	naturalised	PRE464	KNPB274-10	✓	✓
Pennisetum thunbergii Kunth	native	PRE460	KNPB270-10	✓	✓
Pennisetum villosum R.Br. ex Fresen.	naturalised	PRE463	KNPB273-10	✓	✓
Pentameris longiglumis (Nees) Steud.	native	PRE289	KNPB099-10	✓	✓
Pentaschistis capensis	native	PRE441	KNPB251-10	✓	✓
Pentaschistis colorata (Steud.) Stapf	native	PRE423	KNPB233-10	✓	✓
Pentaschistis ecklonii (Nees) McClean	native	PRE407	KNPB217-10	✓	✓
Pentaschistis heptamera (Nees) Stapf	native	PRE443	KNPB253-10	✓	✓
Pentaschistis longipes Stapf	native	PRE395	KNPB205-10	✓	✓
Pentaschistis natalensis Stapf	native	PRE409	KNPB219-10	✓	✓
Pentaschistis patula (Nees) Stapf	native	PRE401	KNPB211-10	✓	✓
Pentaschistis pyrophila H.P.Linder	native	PRE425	KNPB235-10	✓	✓
Pentaschistis reflexa H.P.Linder	native	PRE408	KNPB218-10	✓	✓
Pentaschistis tomentella Stap	native	PRE399	KNPB209-10	✓	✓
Pentaschistis veneta H.P.Linder	native	PRE390	KNPB200-10	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Pentaschistis viscidula (Nees) Stapf	native	PRE416	KNPB226-10	✓	✓
Perotis patens Gand.	native	OM0637	KNPA211-09	✓	✓
Perotis patens Gand.	native	YBK009	KNPA252-09	✓	✓
Perotis patens Gand.	native	YBK403	KNPA325-09	✓	✓
Phragmites australis (Cav.) Steud.	native	PRE296	KNPB106-10	✓	✓
Phragmites mauritianus Kunth	native	KNP105	KNPB580-10	✓	✓
Phragmites mauritianus Kunth	native	BS038	KNPB703-10	✓	✓
Phragmites mauritianus Kunth	native	BS039	KNPB704-10	✓	✓
Phragmites mauritianus Kunth	native	PRE297	KNPB107-10	✓	✓
Poa binata Nees	native	PRE532	KNPB342-10	✓	✓
Poa bulbosa L.	naturalised	PRE530	KNPB340-10	✓	✓
Pogonarthria squarrosa (Roem. & Schult.) Pilg.	native	YBK272	KNPA449-09	✓	✓
Pogonarthria squarrosa (Roem. & Schult.) Pilg.	native	YBK132	KNPA397-09	✓	✓
Pogonarthria squarrosa (Roem. & Schult.) Pilg.	native	YBK407	KNPA329-09	✓	✓
Pogonarthria squarrosa (Roem. & Schult.) Pilg.	native	KNP171	KNPB646-10	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Pogonarthria squarrosa (Roem. & Schult.) Pilg.	native	PRE283	KNPB093-10	✓	✓
Pogonarthria squarrosa (Roem. & Schult.) Pilg.	native	OM0646	KNPA215-09	✓	✓
Pogonarthria squarrosa (Roem. & Schult.) Pilg.	native	YBK010	KNPA253-09	✓	✓
Pogonarthria squarrosa (Roem. & Schult.) Pilg.	native	YBK380	KNPA317-09	✓	✓
Pentameris obtusifolia (Hochst.) Schweick.	native	PRE385	KNPB195-10	✓	✓
Puccinellia acroxantha C.A.Sm. & C.E.Hubb.	native	PRE535	KNPB345-10	✓	✓
Sacciolepis curvata (L.) Chase	native	KNP081	KNPB556-10	✓	✓
Sacciolepis speciformis	native	PRE584	KNPB394-10	✓	✓
Sacciolepis typhura (Stapf) Stapf	native	PRE582	KNPB392-10	✓	✓
Schismus scaberrimus Nees	native	PRE589	KNPB399-10	✓	✓
Schmidtia pappophoroides Steud. ex J.A.Schmidt	native	YBK049	KNPA271-09	✓	✓
Schmidtia pappophoroides Steud. ex J.A.Schmidt	native	YBK288	KNPA458-09	✓	✓
Schmidtia pappophoroides Steud. ex J.A.Schmidt	native	YBK116	KNPA390-09	✓	✓
Schmidtia pappophoroides Steud. ex J.A.Schmidt	native	YBK105	KNPA388-09	✓	✓
Schmidtia pappophoroides Steud. ex J.A.Schmidt	native	KNP180	KNPB655-10	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Schoenefeldia transiens (Pilg.) Chiov.	native	KNP147	KNPB622-10	✓	✓
Setaria appendiculata (Hack.) Stapf	native	PRE542	KNPB352-10	✓	✓
Setaria incrassata (Hochst.) Hack.	native	YBK155	KNPA406-09	✓	✓
Setaria incrassata (Hochst.) Hack.	native	YBK035	KNPA265-09	✓	✓
Setaria incrassata (Hochst.) Hack.	native	YBK311	KNPA468-09	✓	✓
Setaria incrassata (Hochst.) Hack.	native	YBK290	KNPA459-09	✓	✓
Setaria incrassata (Hochst.) Hack.	native	OM0756	KNPA233-09	✓	✓
Setaria lindenbergiana (Nees) Stapf	native	PRE540	KNPB350-10	✓	✓
Setaria pumila (Poir.) Roem. & Schult	uncertain origin	RBN137	KNPA240-09	✓	✓
Setaria sagittifolia (A.Rich.) Walp.	native	OM0698	KNPA222-09	✓	✓
Setaria sagittifolia (A.Rich.) Walp.	native	YBK343	KNPA296-09	✓	✓
Setaria italica (L.) P.Beauv.	naturalised	PRE545	KNPB355-10	✓	✓
Setaria sphacelata (Schumach.) Stapf & C.E.Hubb. ex Moss	native	YBK301	KNPA463-09	✓	✓
Setaria sphacelata (Schumach.) Stapf & C.E.Hubb. ex Moss	native	OM0681	KNPA219-09	✓	✓
Sorghum bicolor (L.) Moench	naturalised	BS043	KNPB708-10	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Sorghum bicolor (L.) Moench	naturalised	BS044	KNPB709-10	✓	✓
Sorghum bicolor (L.) Moench	naturalised	OM0709	KNPA224-09	✓	✓
Sorghum versicolor Andersson	native	OM0765	KNPA234-09	✓	✓
Sorghum versicolor Andersson	native	YBK149	KNPA404-09	✓	✓
Sporobolus acinifolius Stapf	native	PRE358	KNPB168-10	✓	✓
Sporobolus africanus (Poir.) Robyns & Tournay	native	PRE372	KNPB182-10	✓	✓
Sporobolus albicans (Nees ex Trin.) Nees	native	PRE359	KNPB169-10	✓	✓
Sporobolus centrifugus (Trin.) Nees	native	PRE366	KNPB176-10	✓	✓
Sporobolus coromandelianus (Retz.) Kunth	native	PRE553	KNPB363-10	✓	✓
Sporobolus festivus Hochst. ex A.Rich.	native	KNP118	KNPB593-10	✓	✓
Sporobolus ludwigii Hochst.	native	PRE552	KNPB362-10	✓	✓
Sporobolus natalensis (Steud.) T.Durand & Schinz	native	PRE376	KNPB186-10	✓	✓
Sporobolus nebulosus Hack.	native	PRE357	KNPB167-10	✓	✓
Sporobolus nitens Stent	native	KNP122	KNPB597-10	✓	✓
Sporobolus nitens Stent	native	OM0501	KNPA203-09	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Sporobolus nitens Stent	native	OM0643	KNPA213-09	✓	✓
Sporobolus nitens Stent	native	YBK121	KNPA392-09	✓	✓
Sporobolus nitens Stent	native	YBK148	KNPA403-09	✓	✓
Sporobolus panicoides A.Rich.	native	PRE369	KNPB179-10	✓	✓
Sporobolus pectinatus Hack.	native	PRE368	KNPB178-10	✓	✓
Sporobolus pellucidus Hochst.	native	PRE557	KNPB367-10	✓	✓
Sporobolus pyramidalis P.Beauv.	native	KNP124	KNPB599-10	✓	✓
Sporobolus pyramidalis P.Beauv.	native	BS029	KNPB694-10	✓	✓
Sporobolus pyramidalis P.Beauv.	native	YBK088	KNPA285-09	✓	✓
Sporobolus salsus Mez	native	PRE558	KNPB368-10	✓	✓
Sporobolus spicatus (Vahl) Kunth	native	PRE374	KNPB184-10	✓	✓
Sporobolus stapfianus Gand.	native	OM0551	KNPA204-09	✓	✓
Sporobolus stapfianus Gand.	native	PRE356	KNPB166-10	✓	✓
Sporobolus subulatus Hack. ex Scott-Elliot	native	PRE367	KNPB177-10	✓	✓
Sporobolus fimbriatus (Trin.) Nees	native	YBK415	KNPA336-09	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***matK* sequence**	***rbcLa* sequence**
Sporobolus fimbriatus (Trin.) Nees	native	YBK004	KNPA248-09	✓	✓
Sporobolus fimbriatus (Trin.) Nees	native	YBK125	KNPA395-09	✓	✓
Stipa capensis Thunb.	native	PRE591	KNPB401-10	✓	✓
Stipa dregeana Steud.	native	PRE590	KNPB400-10	✓	✓
Stipagrostis amabilis (Schweick.) De Winter	native	PRE561	KNPB371-10	✓	✓
Stipagrostis anomala De Winter	native	PRE327	KNPB137-10	✓	✓
Stipagrostis brevifolia (Nees) De Winter	native	PRE317	KNPB127-10	✓	✓
Stipagrostis damarensis (Mez) De Winter	native	PRE315	KNPB125-10	✓	✓
Stipagrostis dregeana Nees	native	PRE320	KNPB130-10	✓	✓
Stipagrostis fastigiata (Hack.) De Winter	native	PRE318	KNPB128-10	✓	✓
Stipagrostis garubensis (Pilg.) De Winter	native	PRE564	KNPB374-10	✓	✓
Stipagrostis geminifolia Nees	native	PRE319	KNPB129-10	✓	✓
Stipagrostis giessii Kers	native	PRE567	KNPB377-10	✓	✓
Stipagrostis gonatostachys (Pilg.) De Winter	native	PRE322	KNPB132-10	✓	✓
Stipagrostis hermannii (Mez) De Winter	native	PRE565	KNPB375-10	✓	✓

Scientific name	**Origin**	**Voucher number**	**BOLD accession number**	***mat*K sequence**	***rbc*La sequence**
Stipagrostis hirtigluma (Steud. ex Trin. & Rupr.) De Winter	native	PRE325	KNPB135-10	✓	✓
Stipagrostis hochstetteriana (Beck ex Hack.) De Winter	native	PRE311	KNPB121-10	✓	✓
Stipagrostis lutescens (Nees) De Winter	native	PRE316	KNPB126-10	✓	✓
Stipagrostis obtusa (Delile) Nees	native	PRE323	KNPB133-10	✓	✓
Stipagrostis sabulicola (Pilg.) De Winter	native	PRE562	KNPB372-10	✓	✓
Stipagrostis subacaulis (Nees) De Winter	native	PRE326	KNPB136-10	✓	✓
Stipagrostis uniplumis (Licht.) De Winter	native	YBK261	KNPA445-09	✓	✓
Stipagrostis uniplumis (Licht.) De Winter	native	KNP106	KNPB581-10	✓	✓
Stipagrostis uniplumis (Licht.) De Winter	native	KNP107	KNPB582-10	✓	✓
Stipagrostis uniplumis (Licht.) De Winter	native	PRE324	KNPB134-10	✓	✓
Stipagrostis zeyheri (Nees) De Winter	native	PRE321	KNPB131-10	✓	✓
Tenaxia aureocephala (J.G.Anderson) N.P.Barker & H.P.Linder	native	PRE522	KNPB332-10	✓	✓
Tetrapogon tenellus (Roxb.) Chiov.	native	KNP160	KNPB635-10	✓	✓
Themeda triandra Forssk.	native	YBK197	KNPA421-09	✓	✓
Themeda triandra Forssk.	native	YBK242	KNPA437-09	✓	✓

Scientific name	Origin	Voucher number	BOLD accession number	*mat*K sequence	*rbc*La sequence
Tragus berteronianus Schult.	uncertain origin	OM0576	KNPA207-09	✓	✓
Tragus berteronianus Schult.	uncertain origin	YBK157	KNPA407-09	✓	✓
Tragus berteronianus Schult.	uncertain origin	YBK342	KNPA295-09	✓	✓
Tragus berteronianus Schult.	uncertain origin	YBK183	KNPA415-09	✓	✓
Tribolium echinatum (Thunb.) Renvoize	native	PRE453	KNPB263-10	✓	✓
Tribolium obtusifolium (Nees) Renvoize	native	PRE451	KNPB261-10	✓	✓
Tribolium utriculosum (Nees) Renvoize	native	PRE455	KNPB265-10	✓	✓
Tricholaena monachne (Trin.) Stapf & C.E.Hubb.	native	KNP091	KNPB566-10	✓	✓
Tricholaena monachne (Trin.) Stapf & C.E.Hubb.	native	YBK273	KNPA450-09	✓	✓
Tricholaena monachne (Trin.) Stapf & C.E.Hubb.	native	YBK412	KNPA334-09	✓	✓
Tricholaena monachne (Trin.) Stapf & C.E.Hubb	native	YBK051	KNPA273-09	✓	✓
Trichoneura grandiglumis (Nees) Ekman	native	YBK135	KNPA398-09	✓	✓
Triraphis purpurea Hack.	native	PRE574	KNPB384-10	✓	✓
Triraphis ramosissima Hack.	native	PRE571	KNPB381-10	✓	✓
Triraphis schinzii Hack.	native	PRE572	KNPB382-10	✓	✓

Scientific name	Origin	Voucher number	BOLD accession number	*mat*K sequence	*rbc*L*a* sequence
Tristachya lualabaensis (De Wild.) J.B.Phipps	native	PRE578	KNPB388-10	✓	✓
Tristachya nodiglumis K.Schum.	native	PRE580	KNPB390-10	✓	✓
Tristachya rehmannii Hack.	native	PRE577	KNPB387-10	✓	✓
Urochloa mosambicensis (Hack.) Dandy	native	YBK332	KNPA474-09	✓	✓
Urochloa mosambicensis (Hack.) Dandy	native	KNP062	KNPB537-10	✓	✓
Urochloa mosambicensis (Hack.) Dandy	native	YBK003	KNPA247-09	✓	✓
Urochloa mosambicensis (Hack.) Dandy	native	YBK387	KNPA319-09	✓	✓
Urochloa mosambicensis (Hack.) Dandy	native	YBK408	KNPA330-09	✓	✓
Urochloa oligotricha (Fig. & De Not.) Henrard	native	YBK276	KNPA452-09	✓	✓
Urochloa oligotricha (Fig. & De Not.) Henrard	native	KNP063	KNPB538-10	✓	✓
Urochloa trichopus (Hochst.) Stapf	native	YBK350	KNPA301-09	✓	✓
Vulpia myuros (L.) C.C.Gmel.	naturalised	PRE570	KNPB380-10	✓	✓

Appendix 1C. List of all taxa sampled from BOLD or Genbank databases that are native to or naturalised in South Africa, as well as sister clades and early diverging grass lineages not found in South Africa.

Family	**Scientific name**	**Country sampled**	**Origin**	**Vouher number**	**BOLD accession**	**Genbank accession *rbc*La**	**Genbank accession *mat*K**
Poaceae	*Acrachne racemosa* (B.Heyne ex Roth) Ohwi	-	native	Snow & Chatukuta 6833 (MO)	GBVT3782-13	JN681648.1	JN681616.1
Poaceae	*Acrachne racemosa* (B.Heyne ex Roth) Ohwi	South Africa	native	Smook 9899 (US	-	-	JF729080.1
Poaceae	*Agrostis avenacea* J.F.Gmel.	-	naturalised	TCD:S.J. & T.R. Hodkinson 9374	-	HE573287.1	HE574415.1
Poaceae	*Agrostis gigantea* Roth	Canada	naturalised	KSRB 022	KSRB 022	HQ589940.1	HQ593158.1
Poaceae	*Agrostis gigantea* Roth	-	naturalised	-	NMW3165	JN893046.1	JN895346.1
Poaceae	*Agrostis gigantea* Roth	Wales	naturalised	-	NMW013	-	-
Poaceae	*Aira cupaniana* Guss.	-	naturalised	-	-	-	DQ786879.1
Poaceae	*Aira praecox* L.	Canada	naturalised	-	07-JMS-0807	-	-
Poaceae	*Aira praecox* L.	Wales	naturalised	NMW3171	NMW3171	JN893320	JN895889.1
Poaceae	*Aira praecox* L.	Portugal	naturalised	BM 2008/730	-	HM849752	HM850558.1
Poaceae	*Alloteropsis semialata* subsp. *eckloniana* (Nees) Gibbs Russ.	-	native	BRU: S.H. Taylor 3	-	FR821343.1	FR821327.1

Family	**Scientific name**	**Country sampled**	**Origin**	**Vouher number**	**BOLD accession**	**Genbank accession *rbc*L*a***	**Genbank accession *mat*K**
Poaceae	*Alloteropsis semialata* (R.Br.) Hitchc.	Thailand	native	A.T. & S.S. 837 (TCD)	-	AM849345.1	HE574026.1
Poaceae	*Alopecurus arundinaceus* Poir.	-	naturalised	UZ 53.07	-	-	JX438080.1
Poaceae	*Ammophila arenaria* (L.) Link	Canada	naturalised	-	07-JMS-1207	-	-
Poaceae	*Ammophila arenaria* (L.) Link	Wales	naturalised	-	NMW6411	JN893253	JN895834.1
Poaceae	*Ammophila arenaria* (L.) Link	Wales	naturalised	-	NMW5116	JN891031	JN894221.1
Poaceae	*Ammophila arenaria* (L.) Link	-	naturalised	NMW3184	NMW3184	JN890707	JN893970.1
Poaceae	*Anthephora pubescens* Nees	-	native	TCD:Acocks 12637	-	HE575846.1	HE575878.1
Poaceae	*Anthoxanthum odoratum* L.	Wales	naturalised	-	-	JN890708	-
Poaceae	*Anthoxanthum odoratum* L.	Wales	naturalised	-	NMW3188	JN890711	JN893974.1
Poaceae	*Anthoxanthum odoratum* L.	-	naturalised	-	07-JMS-1293	-	-

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*L*a*	Genbank accession *ma*tK
Poaceae	*Anthoxanthum odoratum* L.	-	naturalised	-	07-JMS-0815	-	-
Poaceae	*Anthoxanthum odoratum* L.	-	naturalised	-	07-JMS-1278	-	-
Poaceae	*Aristida adscensionis* L.	-	native	ARS PI364384(seeds)	-	HE573453.1	HE573938.1
Poaceae	*Aristida congesta* Roem. & Schult.	-	native	BRU: S.H. Taylor 30	-	FR821334.1	FR821317.1
Poaceae	*Aristida junciformis* Trin. & Rupr.	-	native	K:F 590	-	HE575817.1	HE586078.1
Poaceae	*Aristida rhiniochloa* Hochst.	-	native	T. Renaud et al. 12-2003 (G)	-	AM849346	HE573948
Poaceae	*Arrhenatherum elatius* (L.) P.Beauv. ex J.Presl & C.Presl	Canada	naturalised	-	07-JMS-0971	-	-
Poaceae	*Arrhenatherum elatius* (L.) P.Beauv. ex J.Presl & C.Presl	Wales	naturalised	-	NMW3189	-	JN893973.1
Poaceae	*Arrhenatherum elatius* (L.) P.Beauv. ex J.Presl & C.Presl	Azores	naturalised	BM 2008/392	-	HM849793	HM850561.1

Family	**Scientific name**	**Country sampled**	**Origin**	**Voucher number**	**BOLD accession**	**Genbank accession *rbc*La**	**Genbank accession *mat*K**
Poaceae	*Arthraxon lanceolatus* Hochst.	-	uncertain origin	G:G. Besnard 67-2006	-	-	HE574004.1
Poaceae	*Arundo donax* L.	-	naturalised	BM 2008/12	-	HM849795	HM850568.1
Poaceae	*Arundo donax* L.	-	naturalised	BS0142	SAFH2529-11	JQ412319	JQ412201.1
Poaceae	*Arundo donax* L.	-	naturalised	K. Hilu 5546 (VPI)	-	-	AF164408.1
Poaceae	*Avena barbata* Pott ex Link	-	naturalised	BM 2008/585	-	HM849803	HM850559.1
Poaceae	*Avena barbata* Pott ex Link	-	naturalised	PI 337802	-	-	EU833841.1
Poaceae	*Avena barbata* Pott ex Link	-	naturalised	PI 282723	-	-	GU367265.1
Poaceae	*Avena fatua* L.	Canada	naturalised	Saarela_779	GRASS1403-10	-	-
Poaceae	*Avena fatua* L.	Canada	naturalised	06-JMS-0741	GRASS788-07	-	-
Poaceae	*Avena fatua* L.	Wales	naturalised	NMW080	POWNA065-10	JN893067	JN895698.1
Poaceae	*Avena fatua* L.	-	naturalised	07-JMS-0999	GRII015-08	-	-
Poaceae	*Avena sativa* L.	-	naturalised	06-JMS-0775	GRASS818-07	-	-

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*La	Genbank accession *mat*K
Poaceae	*Avena sativa* L.	-	naturalised	CN 53095	-	-	GU367304.1
Poaceae	*Avena sativa* L.	-	naturalised	-	-	-	DQ786886.1
Poaceae	*Avena sterilis* L.	-	naturalised	PI 378763	-	-	GU367305.1
Poaceae	*Avena sterilis* L.	-	naturalised	PI 411503	-	-	EU833858.1
Poaceae	*Avena sterilis* L.	-	naturalised	PI 411656	-	-	GU367306.1
Poaceae	*Avena sterilis* L.	-	naturalised	MIB:ZPL:03241	-	HE963347.1	HE966879.1
Poaceae	*Axonopus fissifolius* (Raddi) Kuhlm.	Azores	naturalised	BM<GBR-LONDON>:H. Schaefer 2008/673	-	FN870383.1	FN908047.1
Poaceae	*Bambusa balcooa* Roxb. Ex Roxb.	India	naturalised	AUS-TB2	-	-	JX966236.1
Poaceae	*Brachiaria deflexa* (Schumach.) C.E.Hubb. ex Robyns	-	native	G. Besnard 47-2006 (G)	-	AM849408.1	HE574037.1
Poaceae	*Brachiaria umbellata* (Trin.) Clayton		naturalised	K:RC Hall 17	-	HF558490.1	HF558523.1
Poaceae	*Brachypodium distachyon* (L.) P.Beauv.	Portugal (Azores)	naturalised	BM 2008/544	-	HM849819.1	HM850578.1
Poaceae	*Brachypodium distachyon* (L.) P.Beauv.	-	naturalised	-	-	-	AM234568.1

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*L*a*	Genbank accession *mat*K
Poaceae	*Briza maxima* L.	Portugal (Azores)	naturalised	BM<GBR-LONDON>:H. Schaefer 2008/15	-	FN870384.1	FN908048.1
Poaceae	*Briza minor* L.	Portugal (Azores)	naturalised	BM 2008/292	-	HM849825	HM850580.1
Poaceae	*Briza minor* L.	-	naturalised	-	-	-	DQ786892.1
Poaceae	*Briza subaristata* Lam.	-	naturalised	-	-	-	DQ786899.1
Poaceae	*Briza subaristata* Lam.	-	naturalised	-	-	-	AM234608.1
Poaceae	*Bromus catharticus* Vahl	Portugal (Azores)	naturalised	BM<GBRLONDON>:H. Schaefer 2008/16	-	FN870385.1	FN908049.1
Poaceae	*Bromus commutatus* Schrad	Canada	naturalised	06-JMS-0644	GRASS714-07	-	-
Poaceae	*Bromus commutatus* Schrad	Wales	naturalised	NMW102	POWNA084-10	JN890976.1	JN895848.1
Poaceae	*Bromus commutatus* Schrad	UK	naturalised	BM000954745	-	-	FJ395418.1
Poaceae	*Bromus diandrus* Roth	-	naturalised	07-JMS-0847	GRASS983-07	-	-
Poaceae	*Bromus diandrus* Roth	-	naturalised	06-JMS-0544	GRASS653-07	-	-

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*La	Genbank accession *mat*K
Poaceae	*Bromus diandrus* Roth	-	naturalised	06-JMS-0509	GRASS634-07	-	-
Poaceae	*Bromus hordeaceus* L.	Canada	naturalised	07-JMS-0934a	GRASS1046-07	-	-
Poaceae	*Bromus hordeaceus* L.	Wales	naturalised	NMW3209	POWNA1503-12	JN892852.1	JN895538.1
Poaceae	*Bromus inermis* Leyss.	Canada	naturalised	04-PMP-18610	GRASS374-07	-	-
Poaceae	*Bromus inermis* Leyss.	Canada	naturalised	06-JMS-0378	GRASS547-07	-	-
Poaceae	*Bromus inermis* Leyss.	Canada	naturalised	AP142	KSR106-07	HQ589977	HQ593194.1
Poaceae	*Bromus japonicus* Thunb.	-	naturalised	06-JMS-0673	GRASS736-07	-	-
Poaceae	*Bromus madritensis* L.	-	naturalised	BM 2008/439	-	HM849827	HM850583.1
Poaceae	*Bromus rigidus* Roth	Portugal	naturalised	BM 2008/18	-	HM849828.1	HM850584.1
Poaceae	*Bromus rigidus* Roth	South Korea	naturalised	HCCN-PJ008548-PB-383	-	KF712966.1	KF713103.1
Poaceae	*Bromus rigidus* Roth	South Korea	naturalised	HCCN-PJ008548-PB-15	-	KF712964.1	KF713102.1
Poaceae	*Bromus rigidus* Roth	South Korea	naturalised	NIBRVP0000361195	-	KF712967.1	KF713101.1
Poaceae	*Bromus tectorum* L.	-	naturalised	06-JMS-0450	GRASS597-07	-	-

Family	**Scientific name**	**Country sampled**	**Origin**	**Voucher number**	**BOLD accession**	**Genbank accession *rbc*L*a***	**Genbank accession *ma*tK**
Poaceae	*Bromus tectorum* L.	-	naturalised	06-JMS-0608	GRASS686-07	-	-
Poaceae	*Bromus tectorum* L.	-	naturalised	06-JMS-0761	GRASS804-07	-	-
Poaceae	*Calamagrostis epigejos* (L.) Roth	-	uncertain origin	NMW111	POWNA089-10	JN890760.1	JN894015.1
Poaceae	*Calamagrostis epigejos* (L.) Roth	-	uncertain origin	NMW3218	POWNA2839-12	JN892647.1	JN894974.1
Poaceae	*Capeochloa arundinacea* (P.J.Bergius) N.P.Barker & H.P.Linder	South Africa	native	Barker, N.P. 1017 (BOL)	-	DQ887106.1	EU400738.1
Poaceae	*Capeochloa cincta* (Nees) N.P.Barker & H.P.Linder subsp. sericea	South Africa	native	Barker, N.P. 1545 (GRA)	-	DQ887107.1	EU400739.1
Poaceae	*Catapodium rigidum* (L.) C.E.Hubb.	Wales	naturalised	NMW3223	POWNA2153-12	JN892363.1	JN895185.1
Poaceae	*Catapodium rigidum* (L.) C.E.Hubb.	Wales	naturalised	NMW181	POWNA152-10	JN892799.1	JN895497.1
Poaceae	*Catapodium rigidum* (L.) C.E.Hubb.	Azores	naturalised	BM 2008/678	-	HM849870.1	HM850566.1

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession ***rbc*L*a***	Genbank accession ***mat*K**
Poaceae	*Cenchrus ciliaris* L.	-	native	ARS PI 476857	-	FR821344.1	FR821328.1
Poaceae	*Chaetobromus involucratus* subsp. *dregeanus* (Nees) Verboom	-	native	Barker, N.P. 1715 (GRA)	-	-	EU400717.1
Poaceae	*Chloris gayana* Kunth	Uganda	uncertain origin	USDA-NPGS PI 205251	-	-	AF144582.1
Poaceae	*Chloris gayana* Kunth	South Africa	uncertain origin	USDA-NPGS PI 207542/K. Hilu 5726 (VPI)	-	-	AF164424.1
Poaceae	*Chloris pycnothrix* Trin.	-	uncertain origin	PI 204172/K. Hilu 5711 (VPI)	-	-	AF312329.1
Poaceae	*Chloris truncata* R.Br.	-	naturalised	PI 279931/K. Hilu 5673 (VPI)	-	-	AF312330.1
Poaceae	*Chloris virgata* Sw.	-	uncertain origin	K:Chase 21994	-	HE575821.1	HE586081.1
Poaceae	*Coix lacryma-jobi* L.	Costa Rica	naturalised	BioBot05924	MHPAD3275-10	JQ593308	JQ588705.1
Poaceae	*Coix lacryma-jobi* L.	Costa Rica	naturalised	BioBot05927	MHPAD3278-10	JQ593310	JQ588708.1
Poaceae	*Coix lacryma-jobi* L.	Azores	naturalised	BM 2008/346	-	HM849905.1	HM850570.1

Family	**Scientific name**	**Country sampled**	**Origin**	**Voucher number**	**BOLD accession**	**Genbank accession *rbc*L*a***	**Genbank accession *mat*K**
Poaceae	*Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn.	-	naturalised	BM 2008/557	-	HM849917.1	HM850581.1
Poaceae	*Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn.	-	naturalised	B&T seeds 73	-	-	HF558497.1
Poaceae	*Ctenium concinnum* Nees	South Africa	native	K:GR 67	-	HE575822.1	HE586074.1
Poaceae	*Cynodon dactylon* (L.) Pers.	USA	native	06-PMP-19701	GRII035-08	-	-
Poaceae	*Cynodon dactylon* (L.) Pers.	-	native	BS0132	SAFH2527-11	JQ412349	JQ412229
Poaceae	*Cynodon dactylon* (L.) Pers.	Azores	native	BM 2008/40	-	HM849930	HM850567.1
Poaceae	*Cynodon nlemfuensis* Vanderyst	Costa Rica	naturalised	BioBot10220	MHPAD991-09	JQ593322.1	JQ588717.1
Poaceae	*Cynodon nlemfuensis* Vanderyst	Costa Rica	naturalised	BioBot10219	MHPAD990-09	JQ593321.1	JQ588716.1
Poaceae	*Cynodon nlemfuensis* Vanderys	Costa Rica	naturalised	BioBot10218	MHPAD989-09	JQ593320.1	JQ588715.1

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbcLa*	Genbank accession *mat*K
Poaceae	*Cynodon transvaalensis* Burtt Davy	-	uncertain origin	TCD:T.R. Hodkinson 116	-	HE575823.1	HE591380.1
Poaceae	*Cynodon transvaalensis* Burtt Davy	-	uncertain origin	PI 290812/K. Hilu 5773 (VPI)	-	-	AF312331.1
Poaceae	*Cynosurus echinatus* L.	Canada	naturalised	07-JMS-0852	GRASS988-07	-	-
Poaceae	*Cynosurus echinatus* L.	Canada	naturalised	07-JMS-0809	GRASS945-07	-	-
Poaceae	*Cynosurus echinatus* L.	Azores	naturalised	BM 2008/303		HM849933.1	HM850530.1
Poaceae	*Dactylis glomerata* L.	Canada	naturalised	AP080	KSR057-07	HQ590058	HQ593262.1
Poaceae	*Dactylis glomerata* L.	Wales	naturalised	NMW3228	POWNA2149-12	JN892359	JN895180.1
Poaceae	*Dactylis glomerata* L.	Azores	naturalised	BM 2008/42	-	HM849945	HM850569.1
Poaceae	*Dactyloctenium aegyptium* (L.) Willd.	-	native	G:T. Renaud 05-2005	-	AM887877.1	HE573967.1
Poaceae	*Dactyloctenium aegyptium* (L.) Willd.	Mexico	native	Peterson 22283 & Saarela (US)	-	-	JF729102.1
Poaceae	*Danthoniopsis dinteri* (Pilg.) C.E.Hubb.	-	native	PI207548	-	HE573373.1	HE573998.1

Family	**Scientific name**	**Country sampled**	**Origin**	**Voucher number**	**BOLD accession**	**Genbank accession *rbc*L*a***	**Genbank accession *mat*K**
Poaceae	*Danthoniopsis pruinosa* C.E.Hubb.	-	native	K:G. Davidse 6506	-	HE575839.1	HE575874.1
Poaceae	*Deschampsia cespitosa* (L.) P.Beauv.	Canada	Naturalised	06-JMS-0789	GRASS832-07	-	-
Poaceae	*Deschampsia cespitosa* (L.) P.Beauv.	-	naturalised	07-JMS-1200	GRASS1205-07	-	-
Poaceae	*Deschampsia cespitosa* (L.) P.Beauv.	Wales	naturalised	NBGW1009	POWNA218-10	JN892311	JN894900.1
Poaceae	*Deschampsia flexuosa* (L.) Trin.	Wales	naturalised	-	NMW262	JN892037.1	JN894954.1
Poaceae	*Deschampsia flexuosa* (L.) Trin.	Wales	naturalised	-	NMW3234	JN892161.1	JN895040.1
Poaceae	*Deschampsia flexuosa* (L.) Trin.	USA	naturalised	07-PMP-20793	GRII066-08		
Poaceae	*Digitaria ciliaris* (Retz.) Koeler	South Korea	uncertain origin	HCCN-PJ008548-PB100a	-	KC164299.1	KF163715.1
Poaceae	*Digitaria ciliaris* (Retz.) Koeler	-	uncertain origin	T. Renaud 10-2005 (G)	-	AM849336.1	HE574067.1
Poaceae	*Digitaria didactyla* Willd	-	uncertain origin	G. Besnard 28-2006 (G)	-	AM849404.1	HE574051.1

Family	**Scientific name**	**Country sampled**	**Origin**	**Voucher number**	**BOLD accession**	**Genbank accession *rbc*La**	**Genbank accession *mat*K**
Poaceae	*Digitaria eriantha* Steud.	-	native	-	-	-	HE574068.1
Poaceae	*Digitaria sanguinalis* (L.) Scop.	Canada	naturalised	06-JMS-0776	GRASS819-07	-	-
Poaceae	*Digitaria sanguinalis* (L.) Scop.	Canada	naturalised	06-JMS-0753	GRASS796-07	-	-
Poaceae	*Digitaria sanguinalis* (L.) Scop.	-	naturalised	BM 2008/756	-	-	HM850571.1
Poaceae	*Digitaria sanguinalis* (L.) Scop.	-	naturalised	K. Hilu 6 (VPI)	-	-	AF164421.1
Poaceae	*Digitaria sanguinalis* (L.) Scop.	Canada	naturalised	AP477	KSR341-07	HQ590067	HQ593271.1
Poaceae	*Dinebra retroflexa* (Vahl) Panz. var. condensata S.M.Phillips	-	native	USDA-NPGS PI 275326	-	-	AF144594.1
Poaceae	*Dinebra retroflexa* (Vahl) Panz. var. condensata S.M.Phillips	-	native	Ndegwa 610 (US)	-	-	JF729105.1
Poaceae	*Echinochloa colona* (L.) Link	-	uncertain origin	HCCN-PJ007898-E91; NUC 2011-1	-	-	KC164263.1

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*La	Genbank accession *mat*K
Poaceae	*Echinochloa colona* (L.) Link	-	uncertain origin	SI:F. Zuloaga 6788	-	HE573377.1	HE574053.1
Poaceae	*Echinochloa crus-galli* (L.) P.Beauv.	Canada	uncertain origin	06-JMS-0763	GRASS806-07	-	-
Poaceae	*Echinochloa crus-galli* (L.) P.Beauv.	Canada	uncertain origin	AP298	KSR219-07	HQ590071	HQ593274.1
Poaceae	*Echinochloa crus-galli* (L.) P.Beauv.	-	uncertain origin	BM 2009/2	-	HM849963	HM850573
Poaceae	*Echinochloa crus-galli* (L.) P.Beauv.	Cananda	uncertain origin	AP298	-	HQ590071.1	HQ593274.1
Poaceae	*Echinochloa crus-galli* (L.) P.Beauv.	Portugal	uncertain origin	BM 2009/2	-	HM849963.1	HM850573.1
Poaceae	*Echinochloa crus-galli* (L.) P.Beauv.	-	uncertain origin	G:G. Besnard 04-2001	-	AM887871.1	HE574054.1
Poaceae	*Ehrharta erecta* Lam.	-	native	4698 RSA	-	-	AY792568.1
Poaceae	*Ehrharta longifolia* Schrad.	-	native	USDA-NPGS PI 270493/K. Hilu 5728 (VPI)	-	-	AF164392.1
Poaceae	*Eleusine coracana* (L.) Gaertn.	-	uncertain origin	J.H. Leebens-Mack 1003-2010	-	HQ182427.1	HQ180864.1

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbcLa*	Genbank accession *mat*K
Poaceae	*Eleusine indica* (L.) Gaertn.	-	naturalised	PS0419MT01	-	GQ436380 (species)	GQ434091.1
Poaceae	*Eleusine indica* (L.) Gaertn.	-	naturalised	Peterson 21362, Saarela & Flores Villegas (US)	-	-	JF729107.1
Poaceae	*Eleusine multiflora* Hochst. ex A.Rich.	-	naturalised	DU/Bot/cyto/57	-	-	KF357743.1
Poaceae	*Eleusine tristachya* (Lam.) Lam.	-	naturalised	BM<GBR-LONDON>:H. Schaefer 2008/602	-	FN870389.1	FN908053.1
Poaceae	*Ellisochloa papposa* (Nees) P.M.Peterson & N.P.Barker	South Africa	native	N.P. 1759 (GRA)	-	DQ887114.1	EU400746.1
Poaceae	*Ellisochloa rangei* (Pilg.) P.M.Peterson & N.P.Barker	South Africa	native	Barker, N.P. 960 (BOL)	-	DQ887115.1	EU400747.1
Poaceae	*Elymus repens* (L.) Gould	Canada	naturalised	07-JMS-0985	GRII005-08		
Poaceae	*Elymus repens* (L.) Gould	Canada	naturalised	AP481	KSR344-07	HQ590076	HQ593279.1
Poaceae	*Elymus repens* (L.) Gould	-	naturalised	BM 2008/229	-	HM849973	HM850576.1

Family	**Scientific name**	**Country sampled**	**Origin**	**Voucher number**	**BOLD accession**	**Genbank accession *rbc*La**	**Genbank accession *mat*K**
Poaceae	*Enteropogon macrostachyus* (A.Rich.) Munro ex Benth .	-	native	Snow & Chatukuta 6873 (MO)	-	-	JN681630.1
Poaceae	*Entoplocamia aristulata* (Hack. ex Rendle) Stapf	-	native	Snow & Burgoyne 7197 (MO)	-	-	JN681646.1
Poaceae	*Entoplocamia aristulata* (Hack. ex Rendle) Stapf	-	native	BRU:S.H. Taylor 21	-	FR821338.1	FR821321.1
Poaceae	*Eragrostis capensis* (Thunb.) Trin.	-	native	G:G. Besnard 59-2006	-	-	HE573970.1
Poaceae	*Eragrostis cilianensis* (All.) Vignolo ex Janch .	Azores	native	-	-	-	FN908054.1
Poaceae	*Eragrostis curvula* (Schrad.) Nees	-	native	K:M. Chase 9268.B	-	HE577857.1	HE586097.1
Poaceae	*Eragrostis minor* Host	Canada	naturalised	06-JMS-0783	GRASS826-07	-	-
Poaceae	*Eragrostis minor* Host	Canada	naturalised	06-JMS-0636	GRASS707-07	-	-
Poaceae	*Eragrostis minor* Host	Portugal	naturalised	BM 2008/761	-	HM849978	HM850577.1
Poaceae	*Eragrostis pilosa* (L.) P.Beauv.	USA	uncertain origin	07-PMP-20912	GRII068-08		

Family	**Scientific name**	**Country sampled**	**Origin**	**Voucher number**	**BOLD accession**	**Genbank accession *rbc*L*a***	**Genbank accession *mat*K**
Poaceae	*Eragrostis pilosa* (L.) P.Beauv.	Trieste, Italy	uncertain origin	MIB:ZPL:03980	-		HE966914.1
Poaceae	*Eragrostis racemosa* (Thunb.) Steud.	-	uncertain origin	-	-	-	-
Poaceae	*Eragrostis tef* (Zucc.) Trotter	-	Naturalised	K:M. Chase 36829	-	HE577859.1	HE586095.1
Poaceae	*Eragrostis virescens* J.Presl	-	naturalised	K:Chase 9270	-	HE575824.1	HE586093.1
Poaceae	*Festuca arundinacea* Schreb.	Wales	naturalised	NMW3253	POWNA1676-12	JN891699	JN894714.1
Poaceae	*Festuca arundinacea* Schreb.	Canada	naturalised	AP450	KSR314-07	HQ590100	HQ593296.1
Poaceae	*Festuca arundinacea* Schreb.	Portugal	naturalised	BM 2008/409	-	HM850001	HM850531.1
Poaceae	*Festuca scabra* Vahl	-	native	PRE 522214	-	-	JX438108.1
Poaceae	*Fingerhuthia africana* Lehm.	-	native	TCD:Smook 6942	-	-	HE575866.1
Poaceae	*Gastridium phleoides* (Nees & Meyen) C.E.Hubb	Wales	naturalised	NMW3267	POWNA1548-12	JN891468	-

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*La	Genbank accession *mat*K
Poaceae	*Gastridium phleoides* (Nees & Meyen) C.E.Hubb.	Wales	naturalised	NMW3266	POWNA1547-12	JN891467	JN894879.1
Poaceae	*Gastridium phleoides* (Nees & Meyen) C.E.Hubb.	Wales	naturalised	NMW3265	POWNA1546-12	JN891466	JN894538.1
Poaceae	*Gastridium phleoides* (Nees & Meyen) C.E.Hubb.	-	naturalised	-	-	-	FN908056.1
Poaceae	*Geochloa decora* (Nees) N.P.Barker & H.P.Linder	South Africa	native	Barker 1168 (BOL)	-	DQ887108.1	EU400740.1
Poaceae	*Geochloa lupulina* (L.f.) N.P.Barker & H.P.Linder	South Africa	native	Linder, H.P. 7004 (BOL)	-	DQ887113.1	EU400744.1
Poaceae	*Glyceria maxima* (Hartm.) Holmb.	Wales	naturalised	NMW3275	POWNA1379-12	JN891215	JN894364
Poaceae	*Glyceria maxima* (Hartm.) Holmb.	-	naturalised	NMW400	POWNA343-10	JN892962	JN895612.1
Poaceae	*Glyceria maxima* (Hartm.) Holmb .	Canada	naturalised	06-JMS-0780	GRASS823-07	-	-
Poaceae	*Hainardia cylindrica* (Willd.) Greuter	-	naturalised	BM<GBR-LONDON>:H. Schaefer 2008/613	-	FN870394.1	FN908058.1

Family	**Scientific name**	**Country sampled**	**Origin**	**Voucher number**	**BOLD accession**	**Genbank accession *rbcLa***	**Genbank accession *mat*K**
Poaceae	*Hainardia cylindrica* (Willd.) Greuter	-	naturalised	-	-	-	HE646577.1
Poaceae	*Harpochloa falx* (L.f.) Kuntze	-	native	Snow, Burgoyne, McKibbin, & Roux 7020 (MO)	-	JN681673.1	JN681620.1
Poaceae	*Hemarthria altissima* (Poir.) Stapf & C.E.Hubb.	-	native	J.R. Abbott 24966 (FLAS)	-	GU135209.1	GU135047.1
Poaceae	*Heteropogon contortus* (L.) Roem. & Schult.	-	native	MNHN:H. Schaefer 2010/sn	-	HE575844.1	HE575877.1
Poaceae	*Heteropogon contortus* (L.) Roem. & Schult.	-	native	BRU:S.H. Taylor 20	-	-	FR821324.1
Poaceae	*Holcus lanatus* L.	Canada	naturalised	07-JMS-0839	GRASS975-07	-	-
Poaceae	*Holcus lanatus* L.	Wales	naturalised	NBGW1001	POWNA356-10	JN892327	JN894905.1
Poaceae	*Holcus lanatus* L.	Portugal	naturalised	BM 2008/71	-	HM850053	HM850540.1
Poaceae	*Holcus lanatus* L.	-	naturalised	BM000954772	-	-	FJ395394.1

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*L*a*	Genbank accession *mat*K
Poaceae	*Hordeum marinum* subsp. *gussoneanum* (Parl.) Thell.	-	naturalised	H299	-	AY137434.1	EU118397.1
Poaceae	*Hordeum marinum* subsp. *gussoneanum* (Parl.) Thell.	-	naturalised	H28	-	AY137433.1	AB078115.1
Poaceae	*Hordeum marinum* subsp. *gussoneanum* (Parl.) Thell.	-	naturalised	H826	-	AY137428.1	AB078118.1
Poaceae	*Hordeum marinum* subsp. *gussoneanum* (Parl.) Thell.	-	naturalised	H834	-	-	EU118398.1
Poaceae	*Hordeum murinum* L.	Canada	naturalised	07-JMS-0936	GRASS1049-07	-	-
Poaceae	*Hordeum murinum* L.	Wales	naturalised	NMW6168	POWNA1185-12	JN890940	JN894152.1
Poaceae	*Hordeum murinum* L.	Wales	naturalised	NMW6167	POWNA1194-12	JN890949	JN894159.1
Poaceae	*Hordeum murinum* L.	-	naturalised	BRU:Christin 156	-	-	HF558528.1
Poaceae	*Hordeum murinum* L.	Azores	naturalised	BM 2008/72	-	-	HM850543.1

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*L*a*	Genbank accession *ma*tK
Poaceae	*Hordeum murinum* L. subsp. glaucum (Steud.) Tzvelev	-	naturalised	H801	-	AY836172.1	EU118379.1
Poaceae	*Hordeum murinum* L. subsp. leporinum (Link) Arcang .	-	naturalised	H0812	-	-	AB078122.1
Poaceae	*Hordeum murinum* L. subsp. leporinum (Link) Arcang.	-	naturalised	H0561	-	-	AB078121.1
Poaceae	*Hordeum murinum* L. subsp. leporinum (Link) Arcang.	-	naturalised	H591	-	-	EU118380.1
Poaceae	*Hordeum murinum* L. subsp. leporinum (Link) Arcang.	-	naturalised	H796	-	-	EU118378.1
Poaceae	*Hordeum murinum* L.	-	naturalised	MIB:ZPL:03078	-	HE963513.1	HE966940.1
Poaceae	*Hordeum murinum* L.	-	naturalised	H0721	-	-	AB078123.1
Poaceae	*Hordeum murinum* L.	-	naturalised	H721	-	-	EU118377.1
Poaceae	*Hordeum stenostachys* Godr.	-	naturalised	H1783	-	AY137448.1	AB078136.1

Family	**Scientific name**	**Country sampled**	**Origin**	**Voucher number**	**BOLD accession**	**Genbank accession *rbc*L*a***	**Genbank accession *mat*K**
Poaceae	*Hordeum stenostachys* Godr.	-	naturalised	-	-	-	EU118388.1
Poaceae	*Hyparrhenia hirta* (L.) Stapf	South Africa	native	Kirstenbosch Gardens/N. Barker 1134	-	-	AF164417.1
Poaceae	*Imperata cylindrica* (L.) Raeusch.	USA	uncertain origin	S.B. Davis 0679 (FLAS)	-	GU135272.1	GU135105.1
Poaceae	*Imperata cylindrica* (L.) Raeusch.	-	uncertain origin	K:J. Marsden 3	-		HE574013.1
Poaceae	*Ischaemum afrum* (J.F.Gmel.) Dandy	South Africa	native	BRU:S.H. Taylor 13	-	FR821341.1	FR821325.1
Poaceae	*Tribolium curvum* (Nees) Verboom & H.P. Linder	South Africa	native	Verboom, G.A. 604 (BOL)	-	EU400666.1	EU400735.1
Poaceae	*Tribolium purpureum* (L.f.) Verboom & H.P.Linder	-	native	GH:H. P. Linder 5449	-	-	HE573984.1
Poaceae	*Lagurus ovatus* L.	-	naturalised	BM<GBR-LONDON>:H. Schaefer 2008/80	-	FN870395.1	FN908059.1
Poaceae	*Lagurus ovatus* L.	-	naturalised	-	-	-	AM234563.1

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*La	Genbank accession *mat*K
Poaceae	*Lamarckia aurea* (L.) Moench	-	naturalised	UZ 36.07	-	-	JX438085.1
Poaceae	*Leersia hexandra* Sw.	-	native	-	-	-	AF489909.1
Poaceae	*Leersia hexandra* Sw.	-	native	Hodkinson 636/TCD	-	-	EU434285.1
Poaceae	*Leptochloa fusca* (L.) Kunth	South Africa	native	PI 203357/K. Hilu 5761 (VPI)	-	-	AF312345.1
Poaceae	*Leptochloa fusca* (L.) Kunth	-	native	TCD:Smook 6677	-	HE575827.1	HE575867.1
Poaceae	*Lepturus repens* (G.Forst.) R.Br.	-	naturalised	Latz 10843, MO	-	-	AF144598.1
Poaceae	*Lepturus repens* (G.Forst.) R.Br.	-	naturalised		-	-	HE573971.1
Poaceae	*Lintonia nutans* Stapf	Tanzania	native	M. Wasumbi 14374 (MO)	-	-	AF312337.1
Poaceae	*Lintonia nutans* Stapf	Tanzania	native	Mwasumbi 14374 (US)	-	-	JF729121.1
Poaceae	*Lolium multiflorum* Lam.	-	naturalised		-	JX871942.1	JX871942.1
Poaceae	*Lolium multiflorum* Lam.	-	naturalised		-	FN870397.1	FN908061.1

Family	**Scientific name**	**Country sampled**	**Origin**	**Voucher number**	**BOLD accession**	**Genbank accession *rbcLa***	**Genbank accession *mat*K**
Poaceae	*Lolium multiflorum* Lam.	Canada	naturalised	07-JMS-0841	GRASS977-07	-	-
Poaceae	*Lolium perenne* L.	Canada	naturalised	07-JMS-0850	GRASS986-07	-	-
Poaceae	*Lolium perenne* L.	Wales	naturalised	NBGW1013	POWNA434-10	JN891739	JN894742.1
Poaceae	*Lolium perenne* L.	Azores	naturalised	BM 2008/692	-	HM850132	HM850533.1
Poaceae	*Lolium rigidum* Gaudin	Azores	naturalised	BM 2008/356	-	HM850133	HM850534
Poaceae	*Lolium rigidum* Gaudin	-	naturalised	-	-	-	DQ786926.1
Poaceae	*Lolium temulentum* L.	Wales	naturalised	NMW3305	POWNA2649-12	JN893065	JN895696.1
Poaceae	*Lolium temulentum* L.	Canada	naturalised	07-JMS-1189	GRASS1195-07	-	-
Poaceae	*Megaloprotachne albescens* C.E.Hubb.	-	native	MO:Hansen 3382	-	HE573396.1	HE574078.1
Poaceae	*Melinis minutiflora* P.Beauv.	-	native	P. Howell 1087 (FLAS)	-	GU135253.1	GU135090.1
Poaceae	*Melinis minutiflora* P.Beauv.	-	native	K. Samelson 1 (FLAS)	-	GU135248.1	GU135085.1

Family	**Scientific name**	**Country sampled**	**Origin**	**Voucher number**	**BOLD accession**	**Genbank accession *rbc*La**	**Genbank accession *mat*K**
Poaceae	*Melinis minutiflora* P.Beauv.	-	native	G:G. Besnard 18-2006	-	AM849412.1	HE574079.1
Poaceae	*Melinis repens* (Willd.) Zizka	-	naturalised	J.R. Abbott 22668 (FLAS)	-	-	GU134976.1
Poaceae	*Melinis repens* (Willd.) Zizka	-	naturalised	G:P.Rondeau 06-2005	-	-	HE574080.1
Poaceae	*Merxmuellera drakensbergensis* (Schweick.) Conert	South Africa	native	Mafa, P. 4 (BOL; GRA)	-	DQ887110.1	EU400742.1
Poaceae	*Tenaxia dura* (Stapf) N.P.Barker & H.P.Linder	South Africa	native	Barker, N.P. 983 (BOL)	-	DQ887111.1	EU400743.1
Poaceae	*Merxmuellera macowanii* (Stapf) Conert	South Africa	native	Barker, N.P. 1008 (BOL)	-	-	EU400745.1
Poaceae	*Ehrharta stipoides* Labill.	-	naturalised	Besnard 03-2006 (G)	-	AM849377.1	HE573926.1
Poaceae	*Monelytrum luederitzianum* Hack.	South Africa	native	Smook 10031 (US)	-	-	JF729127.1
Poaceae	*Nassella neesiana* (Trin. & Rupr.) Barkworth	-	naturalised	Nnees764	-	KC129629.1	KC129531.1

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*L*a*	Genbank accession *mat*K
Poaceae	*Nassella neesiana* (Trin. & Rupr.) Barkworth	-	naturalised	RA22	-	-	KC129530.1
Poaceae	*Nassella tenuissima* (Trin.) Barkworth	-	naturalised	BRU:Christin 129	-	-	HF558510.1
Poaceae	*Oplismenus burmanni* (Retz.) P.Beauv.	Costa Rica	native	BioBot00333	-	JQ593372.1	JQ588760.1
Poaceae	*Oplismenus burmanni* (Retz.) P.Beauv.	Costa Rica	native	BioBot00325	-	JQ593370.1	JQ588759.1
Poaceae	*Oplismenus burmanni* (Retz.) P.Beauv.	-	native	K:RC Hall 41	-		HF558512.1
Poaceae	*Oplismenus hirtellus* (L.) P.Beauv.	-	native	BRU:S.H. Taylor 12	-	FR821345.1	FR821329.1
Poaceae	*Oplismenus hirtellus* (L.) P.Beauv.	-	native	TCD:S.J. & T.R. Hodkinson 9405	-	HE573315.1	HE574422.1
Poaceae	*Oplismenus undulatifolius* (Ard.) Roem. & Schult.	South Korea	native	HCCN-PJ008548-PB-274	-	KF163524.1	KF163745.1
Poaceae	*Oplismenus undulatifolius* (Ard.) Roem. & Schult.	South Korea	native	HCCN-PJ008548-PB-120	-	KC164322.1	KF163744.1

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*L*a*	Genbank accession *ma*tK
Poaceae	*Oplismenus undulatifolius* (Ard.) Roem. & Schult.	South Korea	native	HCCN-PJ008548-PB-160	-	KF163523.1	KF163743.1
Poaceae	*Oplismenus undulatifolius* (Ard.) Roem. & Schult.	-	native	G:P.A. Christin 09-2005	-	-	HE574086.1
Poaceae	*Oropetium capense* Stapf	-	native	TCD:Smook 6739	-	HE575831.1	HE575868.1
Poaceae	*Panicum fluviicola* Steud.	-	native		-	HM204476.1	FR657539.1
Poaceae	*Steinchisma hians* (Elliott) Nash & Small	-	naturalised	SI:Zuloaga 6773	-	-	HE574144.1
Poaceae	*Panicum coloratum* L.	-	native	G:G. Besnard 63-2006	-	AM849415.1	HE574090.1
Poaceae	*Panicum maximum* Jacq.	USA	native	J.R. Abbott 23833 (FLAS)	-	GU135169.1	GU135005.1
Poaceae	*Panicum miliaceum* L.	-	naturalised	FR:0004796	-	FR667683	FR667662.1
Poaceae	*Panicum miliaceum* L.	-	naturalised	G. Besnard 65-2006 (G)	-	AM849333	FR822188.1
Poaceae	*Panicum miliaceum* L.	Canada	naturalised	AP237	KSR178-07	HQ590200.1	HQ593379.1

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbcLa*	Genbank accession *matK*
Poaceae	*Panicum parvifolium* Lam.	-	native	SI:F. Zuloaga s.n	-	HE573415	HE574104
Poaceae	*Panicum schinzii* Hack.	-	native	FR:0004794	-	FR667685.1	FR667665.1
Poaceae	*Panicum subalbidum* Kunth	-	native	MNHN:J.N. Labat et al. 3974	-	HE575853.1	HE575883.1
Poaceae	*Parapholis incurva* (L.) C.E.Hubb.	-	naturalised	NMW603	POWNA512-10	JN893508.1	JN896017.1
Poaceae	*Parapholis incurva* (L.) C.E.Hubb.	-	naturalised			-	DQ786931.1
Poaceae	*Paspalum dilatatum* Poir.	-	naturalised	T.R. Hodkinson 128 (TCD)	-	AM849371	HE574118.1
Poaceae	*Paspalum dilatatum* Poir.	Azores	naturalised	BM 2008/112	-	HM850238	-
Poaceae	*Paspalum distichum* L.	-	naturalised	BM<GBR-LONDON>:H. Schaefer 2008/366	-	FN870399.1	FN908063.1
Poaceae	*Paspalum distichum* L.	Italy	naturalised	-	-	-	KC584935.1
Poaceae	*Paspalum notatum* Flüggé	-	naturalised	BM 2008/814	-	-	HM850548.1
Poaceae	*Paspalum quadrifarium* Lam.	-	naturalised	Chase 22020 (K)	-	AM849332.1	HE574121.1

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*L*a*	Genbank accession *mat*K
Poaceae	*Paspalum urvillei* Steud.	-	naturalised	BRU:Christin 158	-	-	HF558516.1
Poaceae	*Paspalum urvillei* Steud.	-	naturalised	BM 2008/534	-	-	HM850549.1
Poaceae	*Paspalum vaginatum* Sw.	-	naturalised	G:P.A. Christin 07-2008	-	HE573426.1	HE574122.1
Poaceae	*Paspalum vaginatum* Sw.	-	naturalised	-	-	-	HM850550.1
Poaceae	*Pennisetum clandestinum* Hochst. ex Chiov	-	naturalised	BM<GBR-LONDON>:H. Schaefer 2008/11	-	FN870400.1	FN908064.1
Poaceae	*Pennisetum glaucum* (L.) R.Br.	-	naturalised	NSL 114290 (Berkeley)	-	-	HE574123.1
Poaceae	*Pennisetum purpureum* Schumach.	Costa Rica	naturalised	BioBot05074	MHPAD124-08	JQ593410	JQ588780.1
Poaceae	*Pennisetum purpureum* Schumach.	Costa Rica	naturalised	BioBot11015	MHPAD2559-10	JQ593411	JQ588781.1
Poaceae	*Pennisetum purpureum* Schumach.	-	naturalised	J.R. Abbott 23653 (FLAS)	-	-	GU134990.1
Poaceae	*Pennisetum setaceum* (Forssk.) Chiov.	-	naturalised	J.R. Abbott 24732 (FLAS)	-	GU135184.1	GU135021.1

Family	**Scientific name**	**Country sampled**	**Origin**	**Voucher number**	**BOLD accession**	**Genbank accession *rbcLa***	**Genbank accession *mat*K**
Poaceae	*Pennisetum setaceum* (Forssk.) Chiov.	-	naturalised	-	-	-	HE574125.1
Poaceae	*Pennisetum villosum* R.Br. ex Fresen.	-	naturalised	BM<GBR-LONDON>:H. Schaefer 2008/367	-	-	FN908065.1
Poaceae	*Pentameris glacialis* N.P.Barker	South Africa	native	Linder, H.P. 5498 (BOL)	-	-	EU400752.1
Poaceae	*Pentameris oreophila* N.P.Barker	South Africa	native	Linder, H.P. 7802 (Z)	-	-	EU400753.1
Poaceae	*Pentaschistis airoides* (Nees) Stapf	South Africa	native	Galley, C. 81 (Z)	-	EU400670.1	EU400755.1
Poaceae	*Pentaschistis aurea* (Steud.) McClean	South Africa	native	Galley, C. 47 (Z)	-	EU400671.1	EU400756.1
Poaceae	*Pentaschistis tysonii* Stapf	South Africa	native	Linder, H.P. 6812 (BOL)	-	DQ913320.1	EU400758.1
Poaceae	*Perotis patens* Gand.	-	native	BRU:S.H. Taylor 24	-	FR821340.1	FR821323.1
Poaceae	*Phalaris arundinacea* L.	Canada	naturalised	AP482	KSR345-07	HQ590203	HQ593381.1
Poaceae	*Phalaris arundinacea* L.	Wales	naturalised	NMW3326	POWNA3075-12	JN893609	JN896095.1

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*La	Genbank accession *mat*K
Poaceae	*Phalaris arundinacea* L.	-	naturalised	USDA-NPGS PI 578797/K. Hilu 5606 (VPI)	-	-	AF164396.1
Poaceae	*Phalaris canariensis* L.	-	naturalised	-	UZ 338.07	-	JX438068.1
Poaceae	*Phragmites mauritianus* Kunth	-	native	K:MS Vorontsova 332		-	HF558517.1
Poaceae	*Poa annua* L.	-	naturalised	04-PMP-18625	GRASS389-07	-	-
Poaceae	*Poa annua* L.	Wales	naturalised	NMW6151	POWNA1347-12	JN891183	-
Poaceae	*Poa annua* L.	Canada	naturalised	08-DPYC-06	GRII045-08	-	-
Poaceae	*Poa bulbosa* L.	Canada	naturalised	06-JMS-0702	GRASS757-07	-	-
Poaceae	*Poa bulbosa* L.	Canada	naturalised	06-JMS-0344	GRASS534-07	-	-
Poaceae	*Poa bulbosa* L.	Canada	naturalised	07-JMS-0821	GRASS957-07	-	-
Poaceae	*Poa bulbosa* L.	Wales	naturalised	NMW3342	POWNA2041-12	JN892190	JN895061.1
Poaceae	*Poa pratensis* L.	Wales	naturalised	NMW3354	POWNA1655-12	JN892391	JN894696.1
Poaceae	*Poa pratensis* L.	-	naturalised	AP093	KSR067-07	HQ590213	HQ593389

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*La	Genbank accession *ma*tK
Poaceae	*Poa pratensis* L.	Canada	naturalised	Saarela_372	GRASS1387-10	-	-
Poaceae	*Poa pratensis* L.	Azores	naturalised	BM 2008/800		-	HM850516.1
Poaceae	*Poa trivialis* L.	Canada	naturalised	Saarela_1267	GRASS1373-10	-	-
Poaceae	*Poa trivialis* L.	Wales	naturalised	NMW6146	POWNA1513-12	JN891433	JN894512.1
Poaceae	*Poa trivialis* L.	Portugal	naturalised	BM 2008/121	-	HM850270	HM850517.1
Poaceae	*Pogonarthria squarrosa* (Roem. & Schult.) Pilg.	-	native	Snow, Burgoyne, McKibbin, & Bronkhorst 7023 (MO)	-	JN681701.1	JN681640.1
Poaceae	*Polypogon monspeliensis* (L.) Desf.	-	naturalised	06-JMS-0681A	GRASS852-07	-	-
Poaceae	*Polypogon monspeliensis* (L.) Desf.	-	naturalised	BM 2008/642	-	HM850275	HM850519.1
Poaceae	*Polypogon monspeliensis* (L.) Desf.	-	naturalised	USNH305585	-	-	DQ146823.1
Poaceae	*Polypogon viridis* (Gouan) Breistr.	-	naturalised	BM 2008/125	-	HM850276.1	HM850520.1

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*La	Genbank accession *mat*K
Poaceae	*Polypogon viridis* (Gouan) Breistr.	-	naturalised	USNH2432658	-		DQ146824.1
Poaceae	*Pseudopentameris macrantha* (Schrad.) Conert	South Africa	native	Linder, H.P. 5470 (BOL)	-	DQ887122.1	EU400761.1
Poaceae	*Puccinellia angustata* (R.Br.) E.L.Rand & Redfield	Canada	native	Gillespie_5786_CAN	-	KC483711.1	KC475545.1
Poaceae	*Puccinellia angustata* (R.Br.) E.L.Rand & Redfield	Canada	native	Gillespie_10298_CAN	-	KC483710.1	KC475544.1
Poaceae	*Puccinellia angustata* (R.Br.) E.L.Rand & Redfield	Canada	native	Elven_2396-99_CAN	-	KC483709.1	KC475543.1
Poaceae	*Puccinellia angustata* (R.Br.) E.L.Rand & Redfield	Canada	native	Gillespie_6158_CAN	-	KC483708.1	KC475541.1
Poaceae	*Puccinellia distans* L. Parl.	Canada	naturalised	04-PMP-18389	GRASS169-07	-	-
Poaceae	*Puccinellia distans* L. Parl.	Canada	naturalised	07-JMS-1009	GRII024-08	-	-

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*L*a*	Genbank accession *ma*tK
Poaceae	*Puccinellia distans* L. Parl.	wales	naturalised	NMW3359	POWNA1780-12	JN892403	JN894821.1
Poaceae	*Puccinellia fasciculata* (Torr.) E.P.Bicknell	-	naturalised	-			AM234588.1
Poaceae	*Rostraria cristata* (L.) Tzvelev	-	naturalised	BM 2008/131	-	HM850311	HM850523.1
Poaceae	*Rostraria cristata* (L.) Tzvelev	-	naturalised	UZ 9.07	-	-	JX438078.1
Poaceae	*Rostraria cristata* (L.) Tzvelev	-	naturalised	-	-	-	AM234670.1
Poaceae	*Rottboellia cochinchinensis* (Lour.) Clayton	-	uncertain origin	TCD:Hodkinson 544	-	FN870921.1	FR832826.1
Poaceae	*Sacciolepis africana* C.E.Hubb. & Snowden	-	native	G: P. Poilecot 3548	-	FR821348.1	FR821332.1
Poaceae	*Sacciolepis indica* (L.) Chase	South Korea	native	HCCN-PJ008548-PB-279	-	KF163797.1	KF163818.1
Poaceae	*Sacciolepis indica* (L.) Chase	-	native	TCD:A.T. & S.S. 654	-	AM887870.1	HE574133.1
Poaceae	*Schismus barbatus* (L.) Thell.	South Africa	native	Verboom, G.A. 503 (BOL)	-	EU400676	EU400767.1

Family	**Scientific name**	**Country sampled**	**Origin**	**Voucher number**	**BOLD accession**	**Genbank accession *rbc*L*a***	**Genbank accession *mat*K**
Poaceae	*Schismus barbatus* (L.) Thell.	-	native	TCD:S.J. & T.R. Hodkinson 9467	-	-	HE574432.1
Poaceae	*Tribolium pleuropogon* (Stapf) Verboom & H.P.Linder	South Africa	native	Verboom, G.A. 628 (BOL)	-	GQ471705.1	GQ471577.1
Poaceae	*Setaria megaphylla* (Steud.) T.Durand & Schinz	Azores	native	BM:H. Schaefer 2008/378	-	FN870408.1	FN908072.1
Poaceae	*Setaria pumila* (Poir.) Roem. & Schult	South Korea	native	HCCN-PJ008548-PB-310	-	KF163547.1	KF163785.1
Poaceae	*Setaria pumila* (Poir.) Roem. & Schult	South Korea	native	HCCN-PJ008548-PB-172	-	KF163546.1	KF163784.1
Poaceae	*Setaria pumila* (Poir.) Roem. & Schult	South Korea	native	HCCN-PJ008548-PB-127	-	KC164338.1	KF163783.1
Poaceae	*Setaria pumila* (Poir.) Roem. & Schult	South Korea	native	HCCN-PJ008548-PB-255	-	KF163543.1	KF163776.1
Poaceae	*Setaria italica* (L.) P.Beauv.	Canada	naturalised	06-JMS-0764	GRASS807-07	-	-
Poaceae	*Setaria italica* (L.) P.Beauv.	-	naturalised	PI315090	-	-	HE574137.1

Family	**Scientific name**	**Country sampled**	**Origin**	**Voucher number**	**BOLD accession**	**Genbank accession *rbcLa***	**Genbank accession *mat*K**
Poaceae	*Setaria parviflora* (Poir.) M.Kerguelen	-	naturalised	BM<GBR-LONDON>:H. Schaefer 2008/647	-	FN870409.1	FN908073.1
Poaceae	*Setaria sphacelata* (Schumach.) Stapf & C.E.Hubb. ex Moss	-	native	MNHN:G. Besnard 74-2006	-	HE577880.1	HE586090.1
Poaceae	*Setaria verticillata* (L.) P.Beauv.	-	naturalised	BRU:S.H. Taylor 4	-	FR821349.1	HE574140.1
Poaceae	*Sorghum halepense* (L.) Pers.	Azores	naturalised	BM 2008/713	-	HM850375	HM850524.1
Poaceae	*Sorghum halepense* (L.) Pers.	-	naturalised	BRU:Christin 160	-	-	HF558520.1
Poaceae	*Sorghum halepense* (L.) Pers.	-	naturalised	MIB:ZPL:03559	-	-	HE967003.1
Poaceae	*Sphenopus divaricatus* (Gouan) Rchb.	-	naturalised	-	-	-	HE646589.1
Poaceae	*Sphenopus divaricatus* (Gouan) Rchb.	-	naturalised	-	-	-	DQ786943.1

Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession ***rbc*****La**	Genbank accession ***mat*****K**
Poaceae	*Sporobolus africanus* (Poir.) Robyns & Tournay	-	native	BM 2008/148	-	HM850383	HM850525.1
Poaceae	*Sporobolus festivus* Hochst. ex A.Rich.	-	native	T. Renaud et al. 192-2003 (G)	-	AM849383.1	HE573974.1
Poaceae	*Sporobolus virginicus* (L.) Kunth	-	uncertain origin	G:P.A. Christin 05-2008	-	HE573440.1	HE573975.1
Poaceae	*Stenotaphrum secundatum* (Walter) Kuntze	-	uncertain origin	BM 2008/151	-	HM850387	HM850526.1
Poaceae	*Stenotaphrum secundatum* (Walter) Kuntze	-	uncertain origin	TCD:Hodkinson 570	-	EF125139.1	FR832839.1
Poaceae	*Stipa capensis* Thunb.	-	native	-	-	-	AM234576.1
Poaceae	*Stipagrostis brevifolia* (Nees) De Winter	South Africa	native	MO:Davidse 33296	-	HE573442.1	HE573955.1
Poaceae	*Stipagrostis zeyheri* (Nees) De Winter	-	native	MO:Davidse 33372	-	-	HE573958.1
Poaceae	*Tenaxia disticha* (Nees) N. P. Barker & H. P. Linder	-	native	V.R. Clark et al. 355 (G)	-	AM849413	HE573985.1

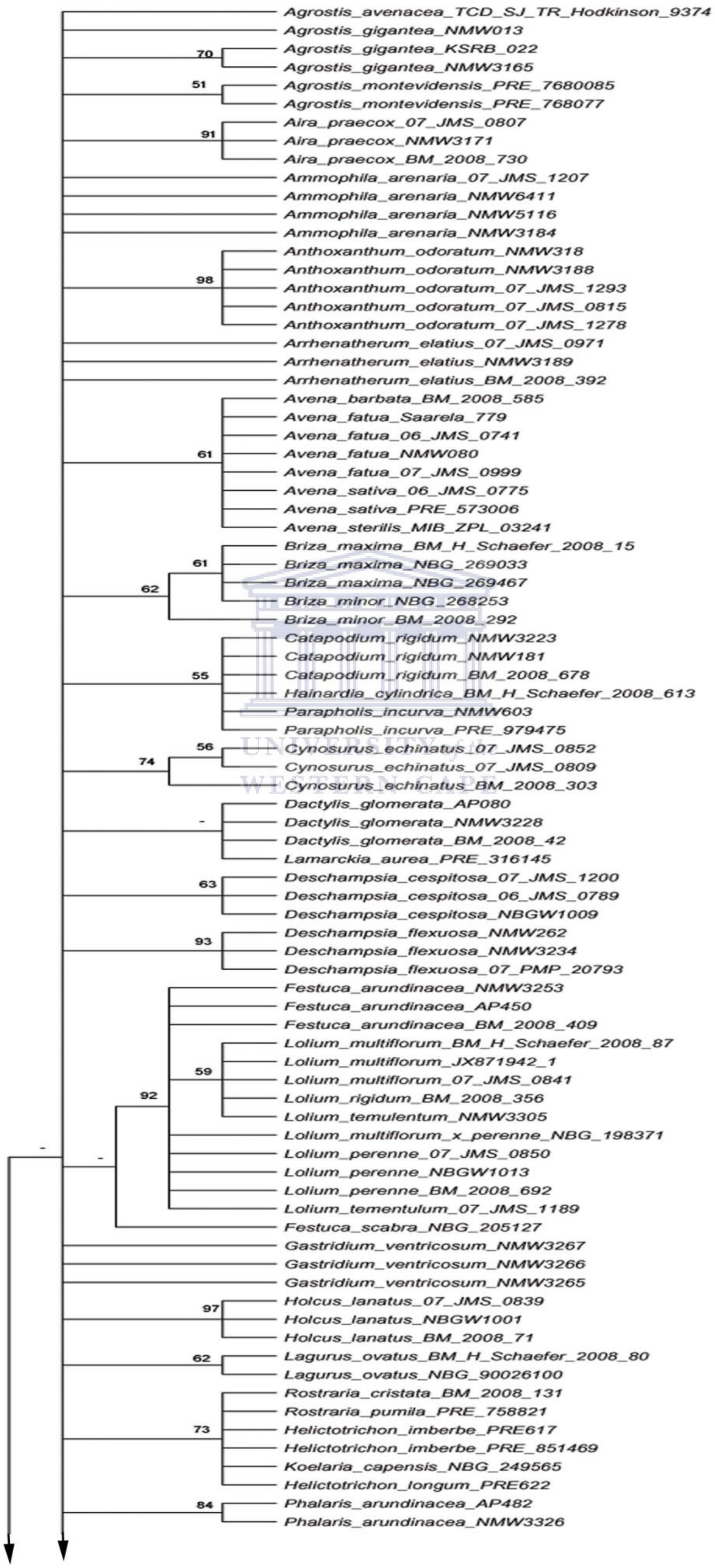
Family	**Scientific name**	**Country sampled**	**Origin**	**Voucher number**	**BOLD accession**	**Genbank accession *rbc*La**	**Genbank accession *mat*K**
Poaceae	*Tenaxia stricta* (Schrad.) N.P.Barker & H.P.Linder	South Africa	native	vd Niet, T. 15 (Z)	-	GQ471692	GQ471563.1
Poaceae	*Tenaxia stricta* (Schrad.) N.P.Barker & H.P.Linder	-	native	V.R. Clark & G. Coombs 270 (G)	-	AM849414	HE573986.1
Poaceae	*Tetrachne dregei* Nees	South Africa	native	PI 300136/K. Hilu 5624 (VPI)	-	-	AF312363.1
Poaceae	*Tetrapogon tenellus* (Roxb.) Chiov.	Ethiopia	native	Gilbert et al. 7640 (MO)	-	-	AF312336.1
Poaceae	*Themeda triandra* Forssk.	-	native	BRU:S. H. Taylor 14	-	HE573359.1	HE574017.1
Poaceae	*Tragus berteronianus* Schult.	South Africa	uncertain origin	N. Barker 1128, Pretoria Botanical Gardens	-	-	AF144591.1
Poaceae	*Tragus racemosus* (L.) All.	-	native	K:Chase 19533	-	AM887881.1	HE573977.1
Poaceae	*Tribolium brachystachyum* (Nees) Renvoize	South Africa	native	Verboom, G.A. 593 (BOL)	-	EU400677.1	EU400768.1
Poaceae	*Tricholaena monachne* (Trin.) Stapf & C.E.Hubb.	-	native	G:G. Besnard 20-2006	-	HE577881.1	HE586091.1

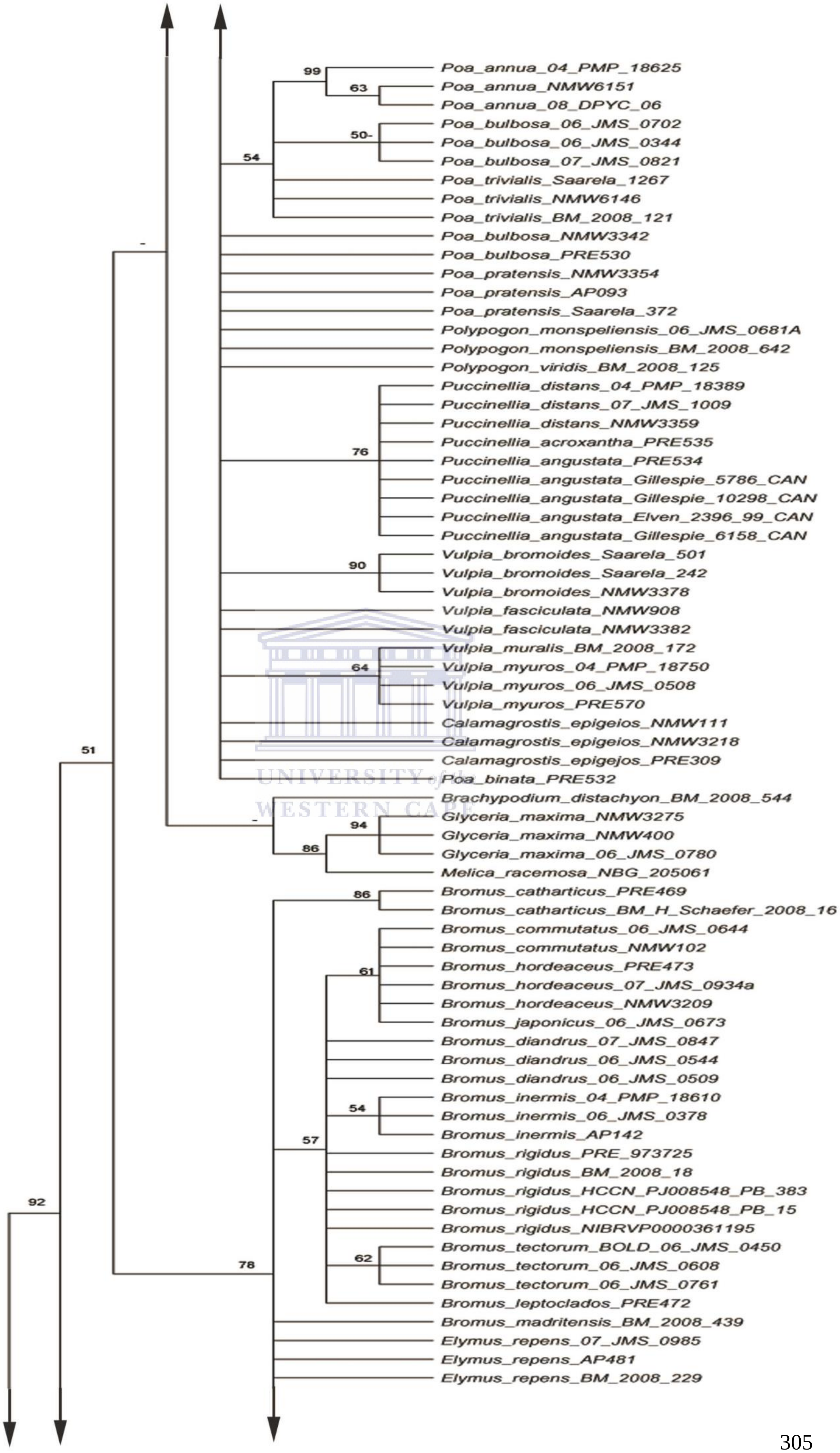
Family	Scientific name	Country sampled	Origin	Voucher number	BOLD accession	Genbank accession *rbc*La	Genbank accession *mat*K
Poaceae	*Trichoneura grandiglumis* (Nees) Ekman	-	native	TCD:Smook 6681	-	FN870947.1	HE575871.1
Poaceae	*Trichoneura grandiglumis* (Nees) Ekman	South Africa	native	USDA-NPGS PI 365064	-	-	AF144595.1
Poaceae	*Tripogon minimus* (A.Rich.) Hochst. ex Steud.	-	native	G:T. Renaud 08-2005	-	AM849342.1	HE573978.1
Poaceae	*Triraphis ramosissima* Hack.	South Africa	native	Seydel 4278 (US)			JF729157.1
Poaceae	*Urochloa panicoides* P.Beauv.	-	native	TCD:S.J. & T.R. Hodkinson 9425	GBVT3782-13	HE573318.1	HE574426.1
Poaceae	*Vulpia bromoides* (L.) Gray	Canada	naturalised	Saarela_501	GRASS1391-10	-	-
Poaceae	*Vulpia bromoides* (L.) Gray	USA	naturalised	Saarela_242	GRASS1382-10	-	-
Poaceae	*Vulpia bromoides* (L.) Gray	Wales	naturalised	NMW3378	POWNA2467-12	JN892830	JN895528.1
Poaceae	*Vulpia fasciculata* (Forssk.) Samp.	Wales	naturalised	NMW908	POWNA830-10	JN891424.1	JN894510.1

Family	*Scientific name*	*Country sampled*	*Origin*	*Voucher number*	*BOLD accession*	*Genbank accession rbcLa*	*Genbank accession matK*
Poaceae	*Vulpia fasciculata (Forssk.) Samp.*	*Wales*	*naturalised*	*NMW3382*	*POWNA1687-12*	*JN891711.1*	*JN894722.1*
Poaceae	*Vulpia muralis (Kunth) Nees*	-	*naturalised*	*BM 2008/172*	-	*HM850471*	*HM850528.1*
Poaceae	*Vulpia myuros (L.) C.C.Gmel.*	*Canada*	*naturalised*	*04-PMP-18750*	*GRASS502-07*	-	-
Poaceae	*Vulpia myuros (L.) C.C.Gmel.*	*Canada*	*naturalised*	*06-JMS-0508*	*GRASS888-07*	*JN681648.1*	*HE574415.1*
Sister clades							
Ecdeiocoleaceae	*Ecdeiocolea monostachya F. Muell.*	-	-	*NSW:364828*		*AF148773.1*	*DQ257528.2*
Joinvilleaceae	*Joinvillea ascendens Gaudich. ex Brongn. & Gris*	-	-	*NSW:612727*	-	*DQ307446.1*	*DQ257534.2*
Restionaceae	*Baloskion tetraphyllum (Labill.) B.G.Briggs & L.A.S.Johnson*	-	-	*NSW:365050 or Herb NSW 365050*	-	*AF148761.1*	*DQ257501.2*
Restionaceae	*Elegia squamosa Mast.*	-	-	*Linder, Hardy, and Moline 7387*	-	*AY881453.1*	*AY881526.1*

Family	*Scientific name*	*Country sampled*	*Origin*	*Voucher number*	*BOLD accession*	*Genbank accession rbcLa*	*Genbank accession matK*
Early diverging grass lineages not found in South Africa							
Poaceae	*Anomochloa marantoidea Brongn.*	-		*L. Clark 1299 (ISC)*	-	*EF423008.1*	*AF164381.1*
Poaceae	*Pharus latifolius L.*	-		*BioBot06145*	-	*JQ593419.1*	*JQ588787.1*
Poaceae	*Puelia olyriformis (Franch.) Clayton*	-		*Clayton 1060 (MO)*	-	*HQ604036.1*	*HQ604000.1*
Poaceae	*Streptochaeta angustifolia Soderstr.*	-		*J.I. Davis 757*	-	*HQ182451*	*HQ180887.1*
Poaceae	*Streptochaeta angustifolia Soderstr.*	-		*Clark1304ISC*	-	*EF423009.1*	*AF164382.1*

Appendix 2A. MP strict consensus tree of family Poaceae from *rbcLa* data with bootstrap values plotted above branches. – indicates no bootstrap support.

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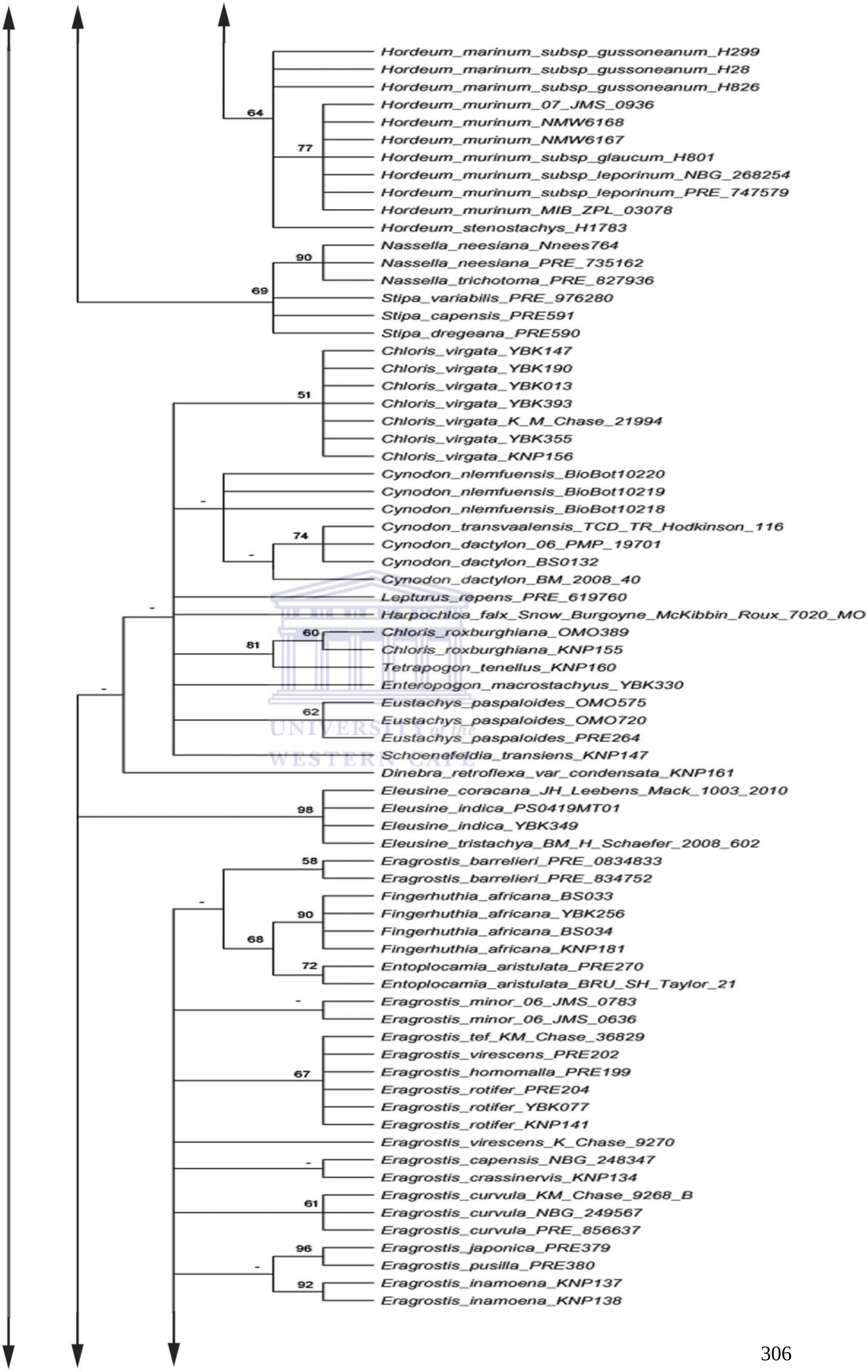
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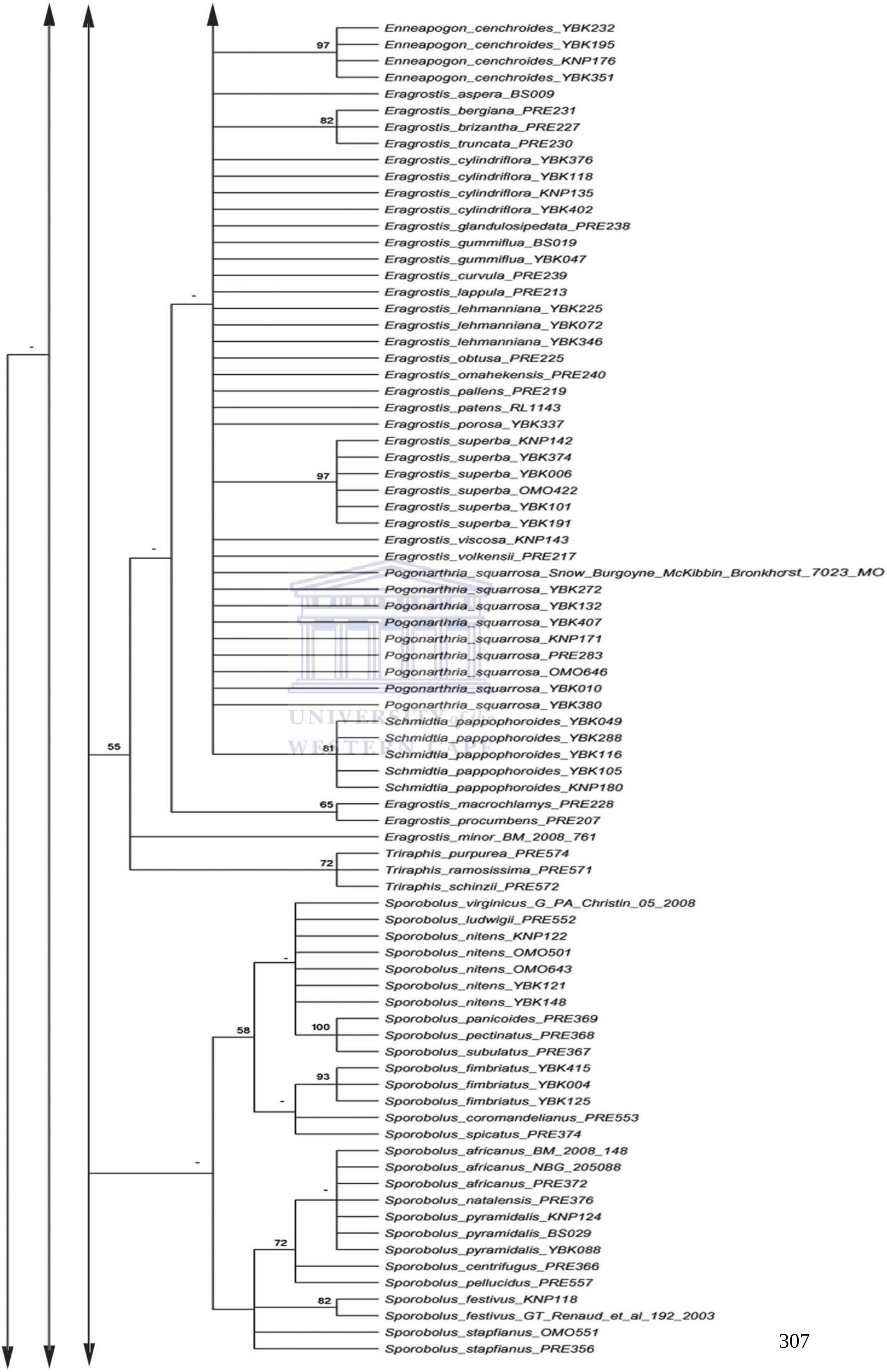
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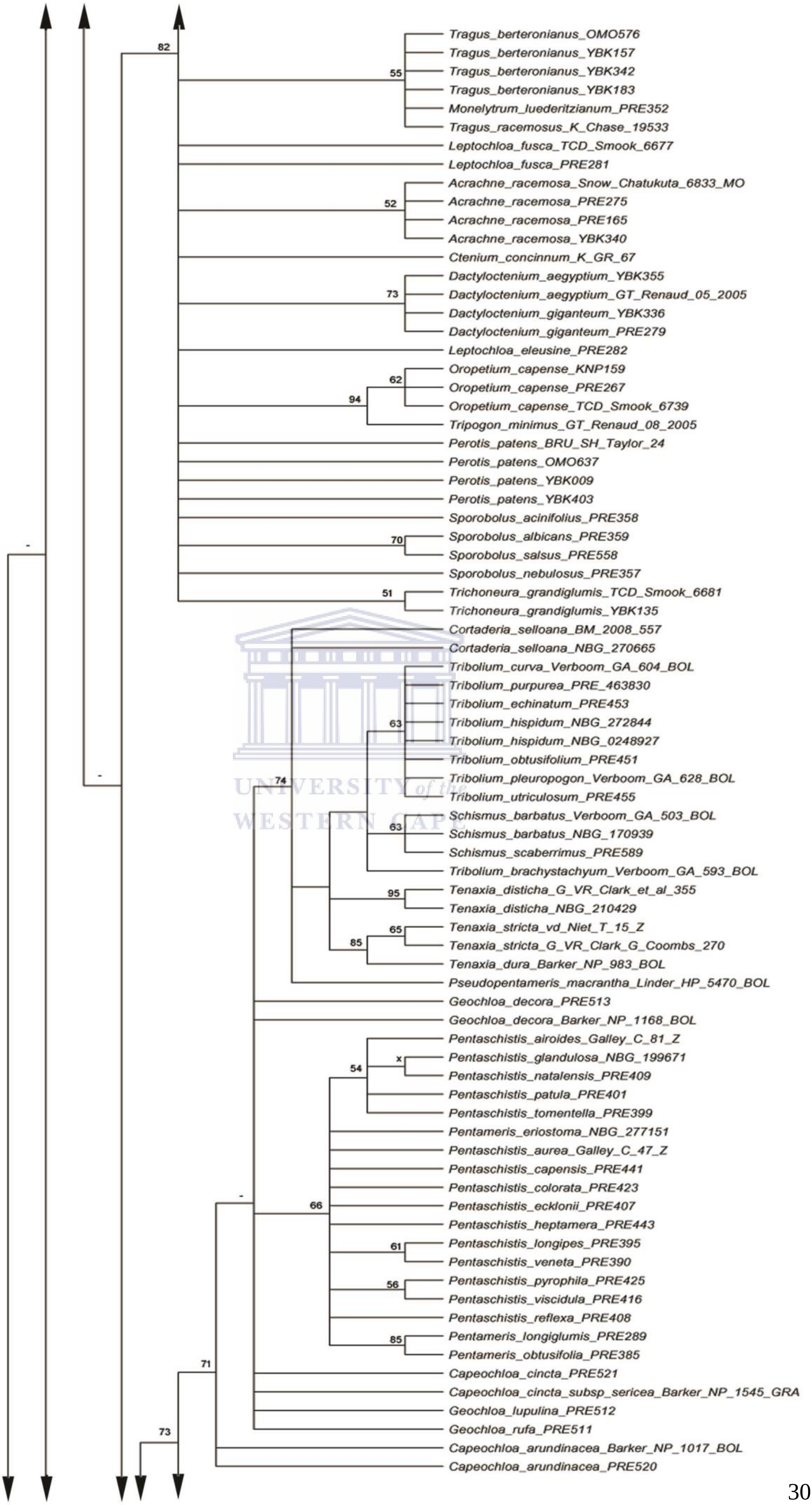
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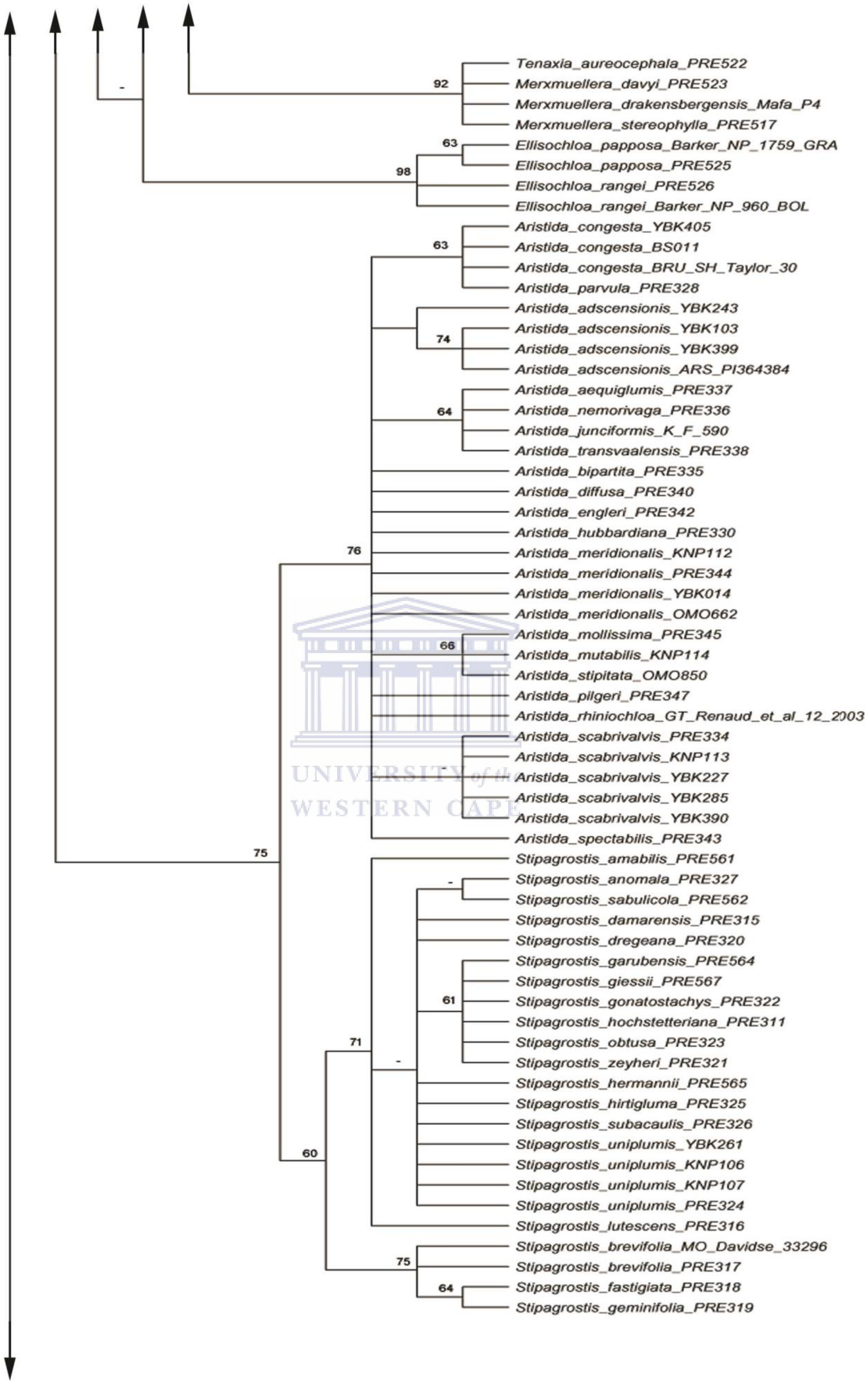
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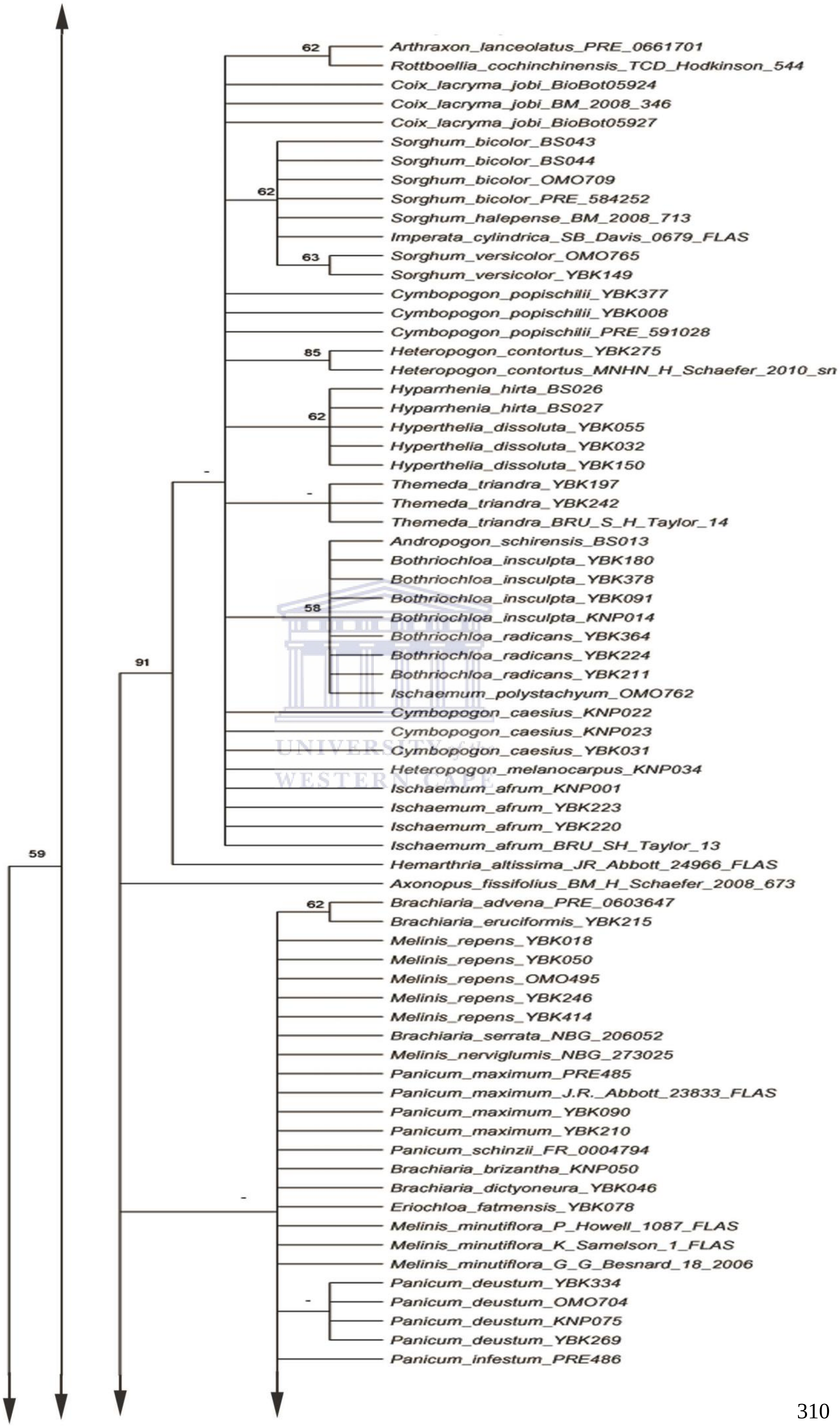
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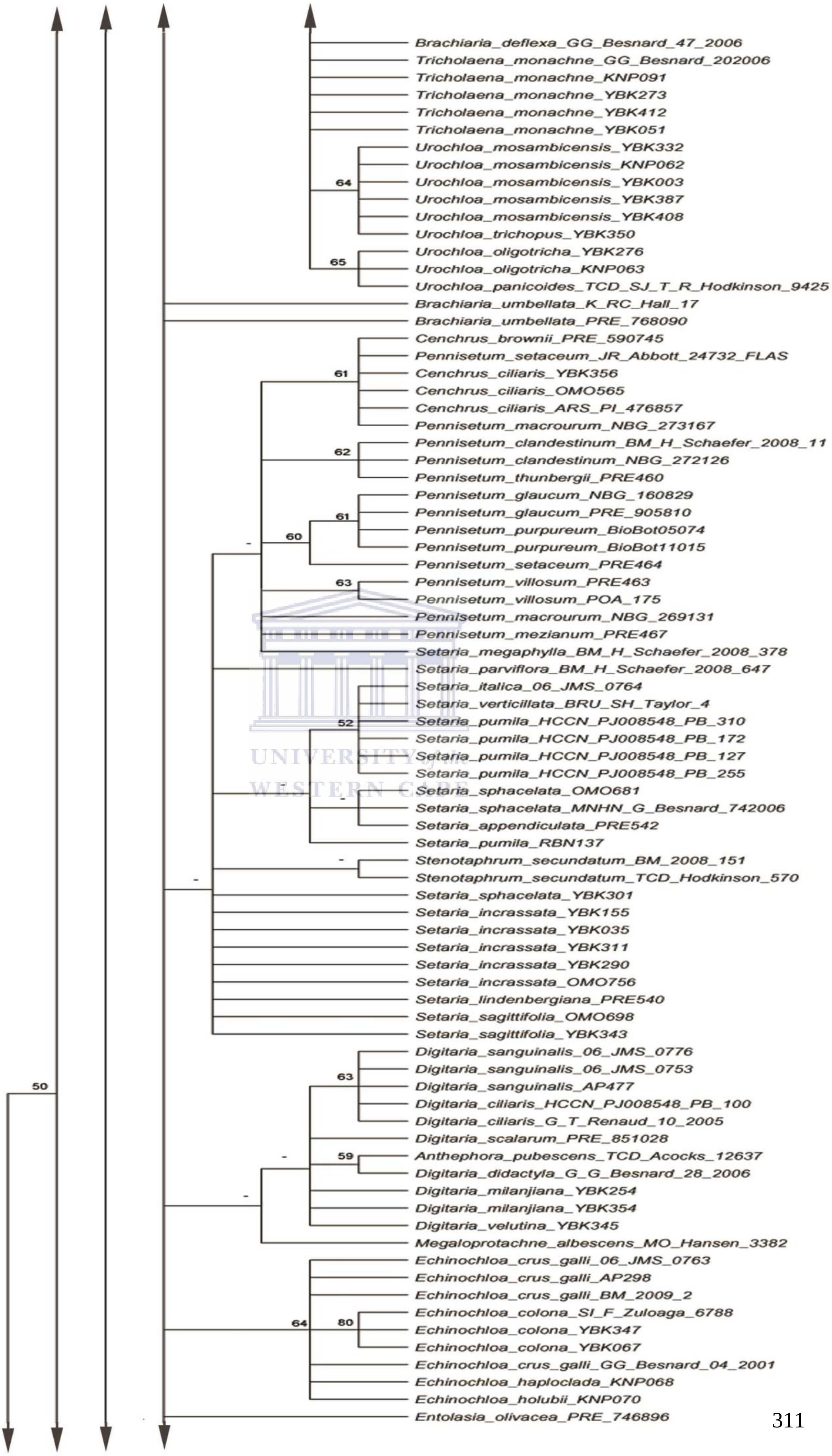
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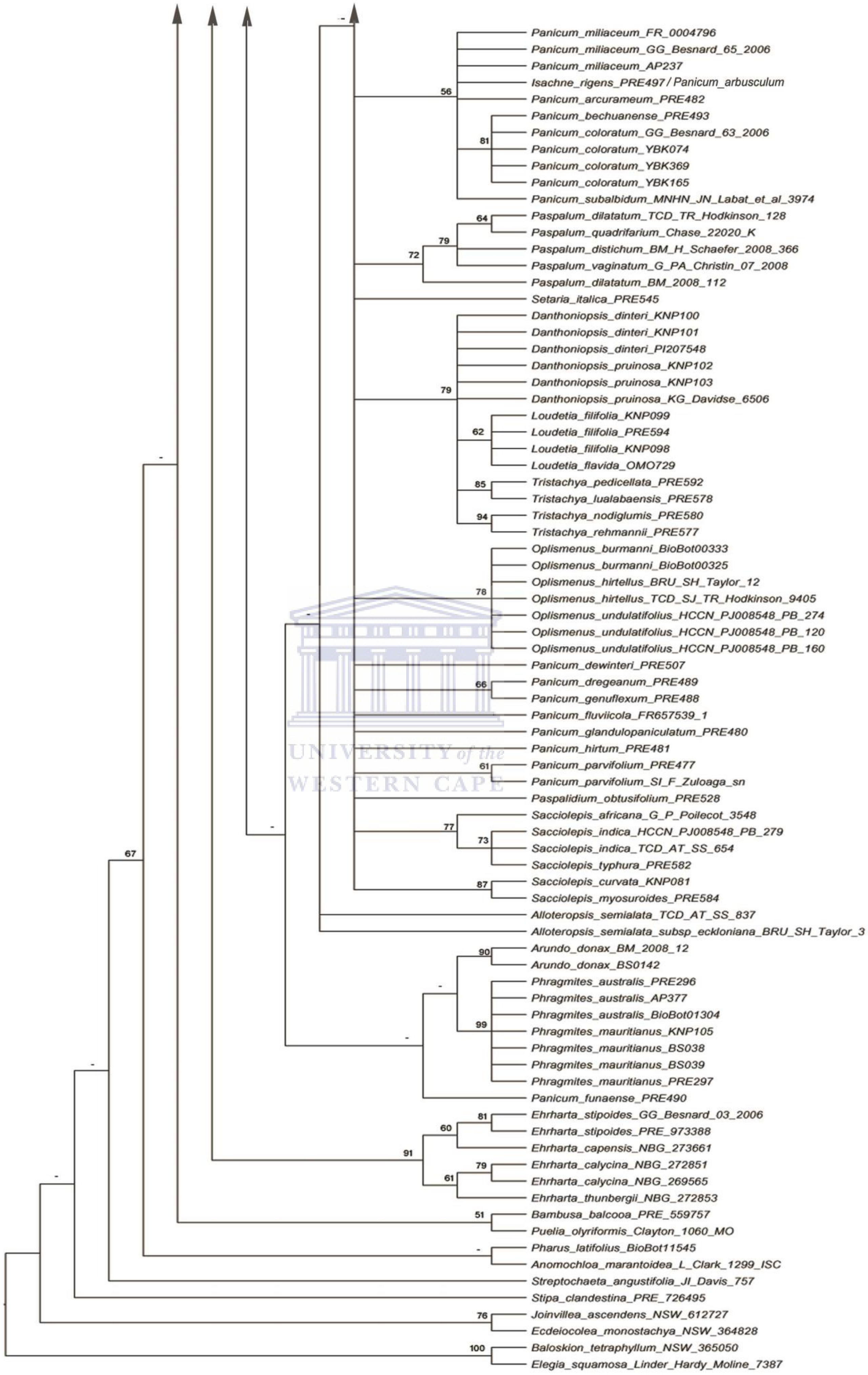
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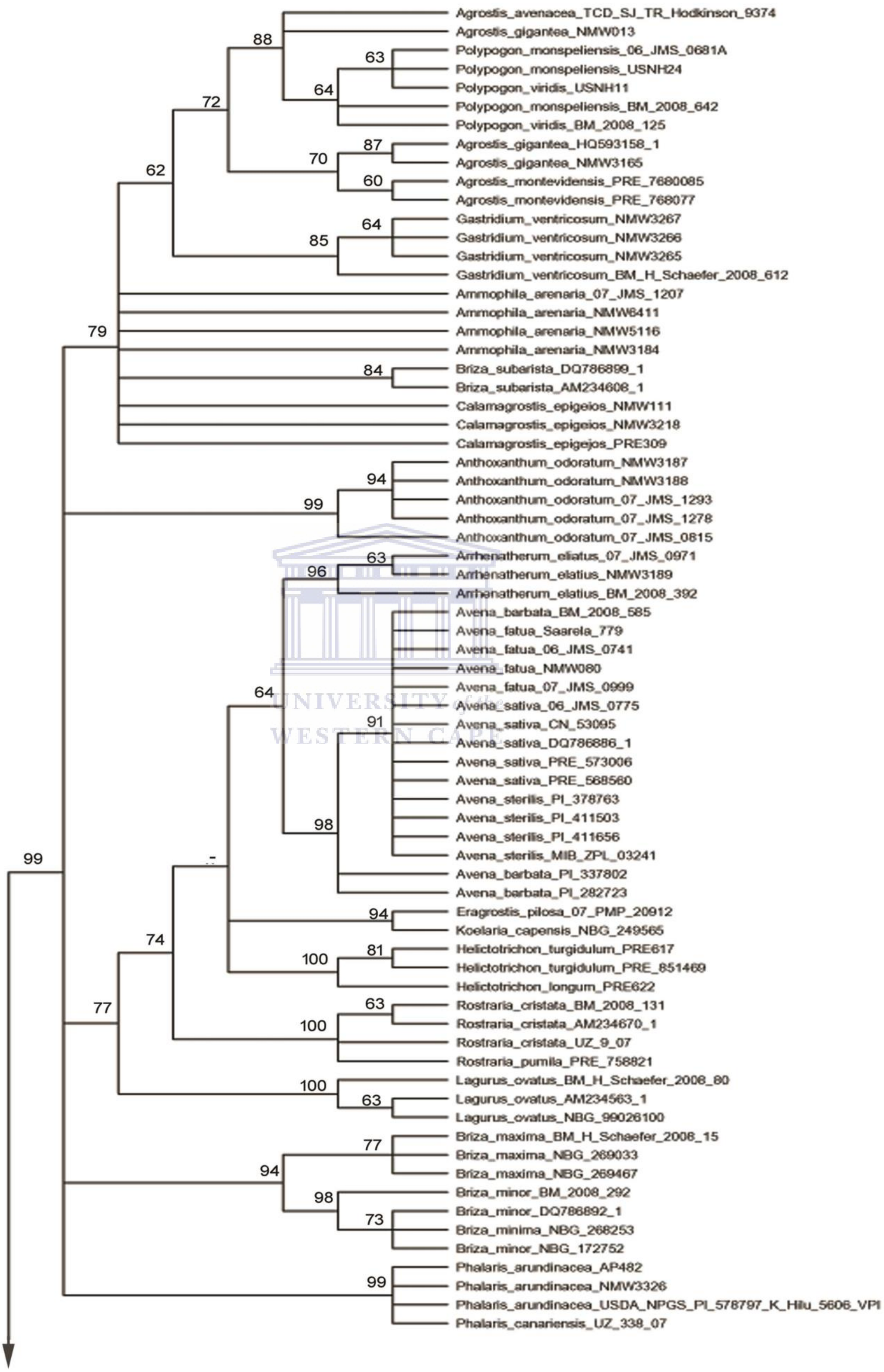
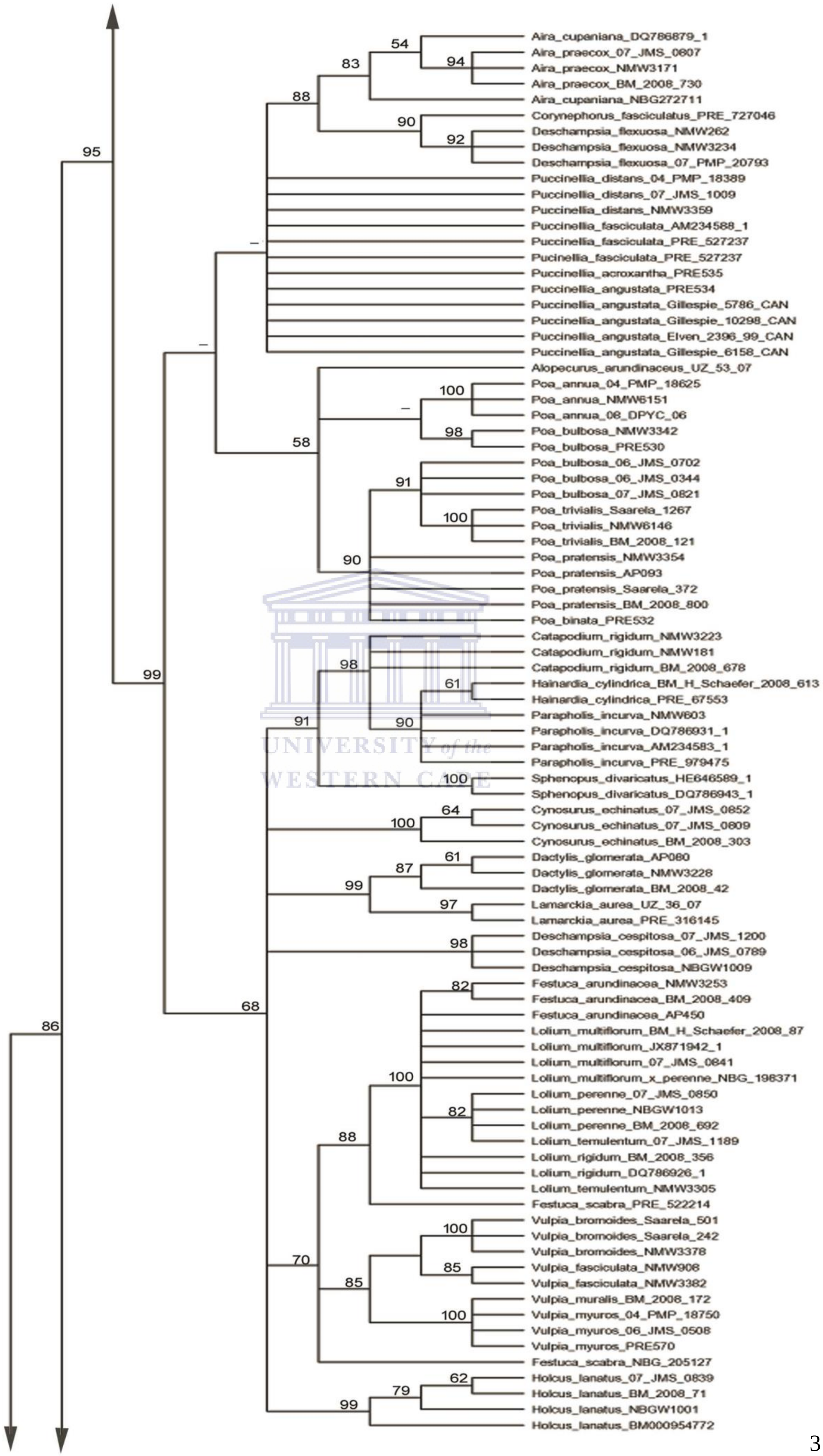
Appendix 2B. MP strict consensus tree of family Poaceae from *matK* data with bootstrap values plotted above branches. – indicates no bootstrap support.

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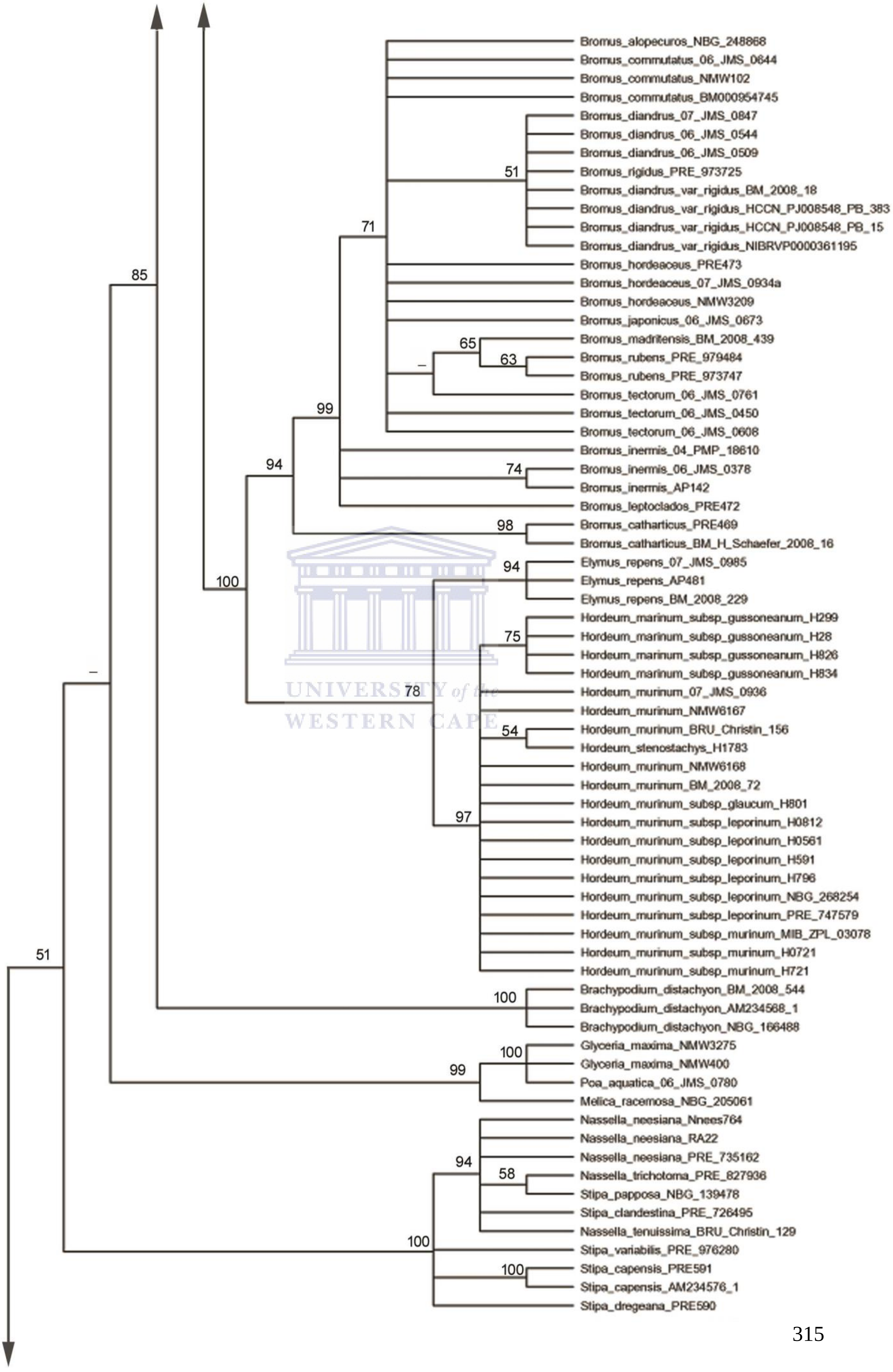
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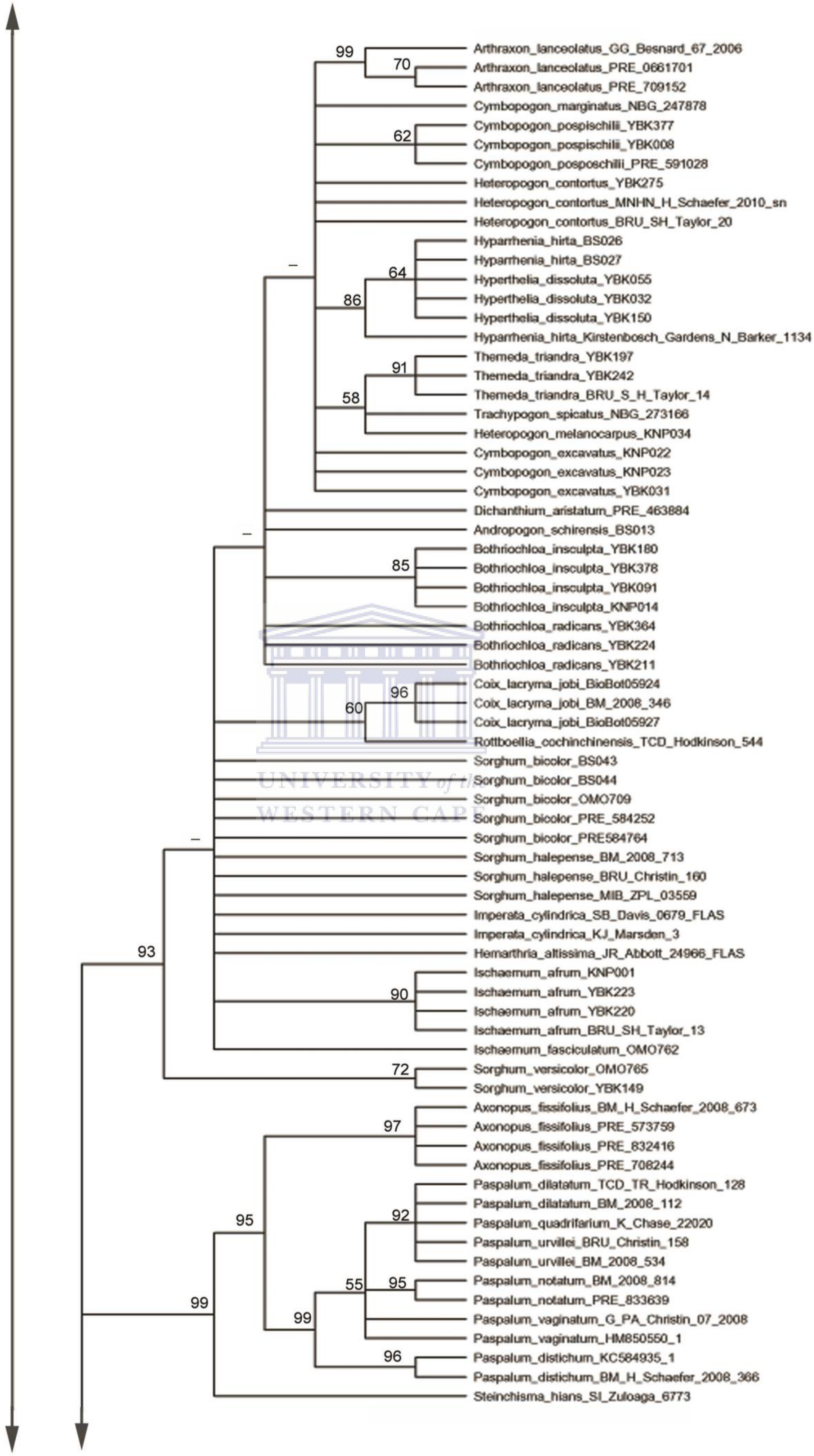
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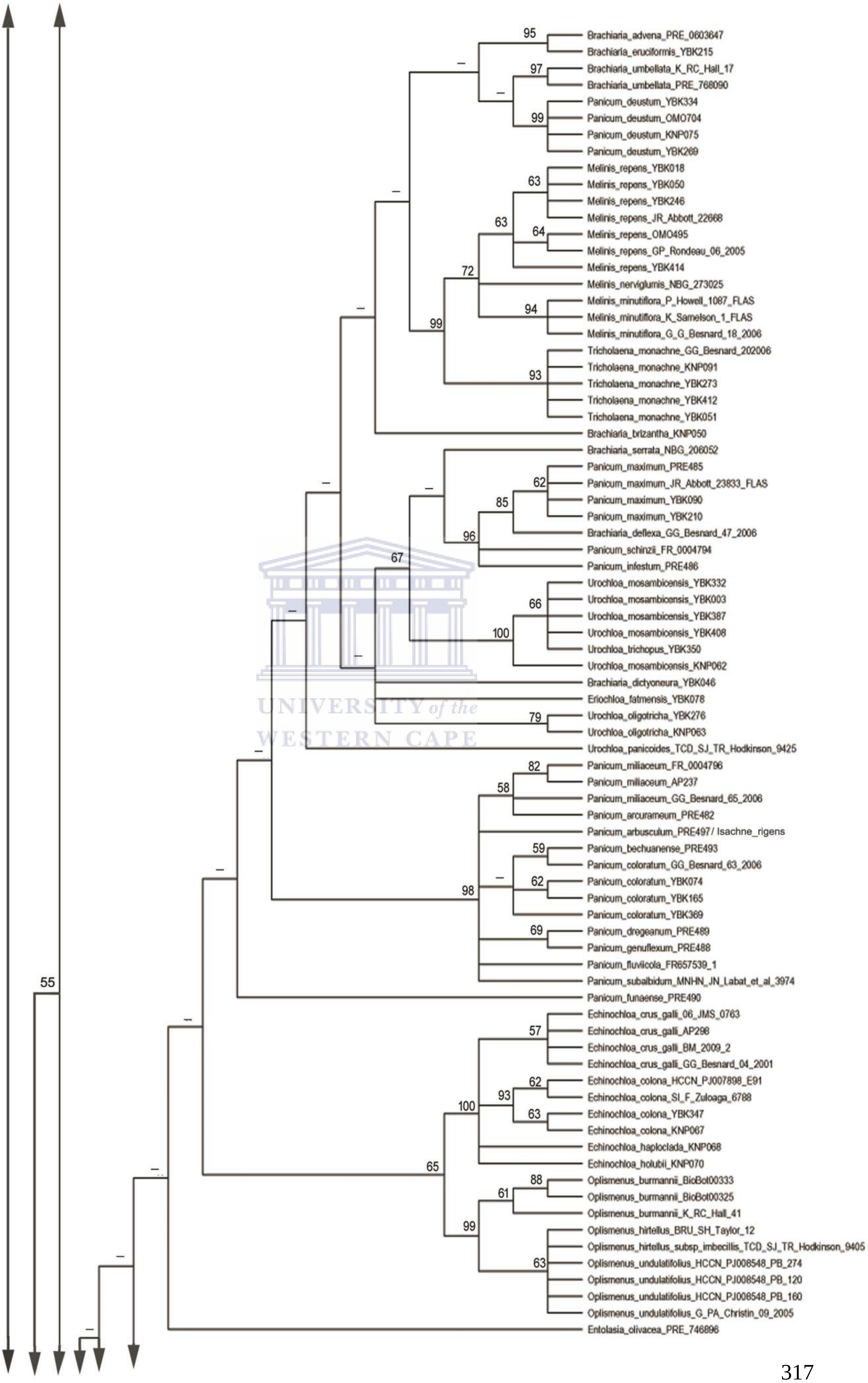
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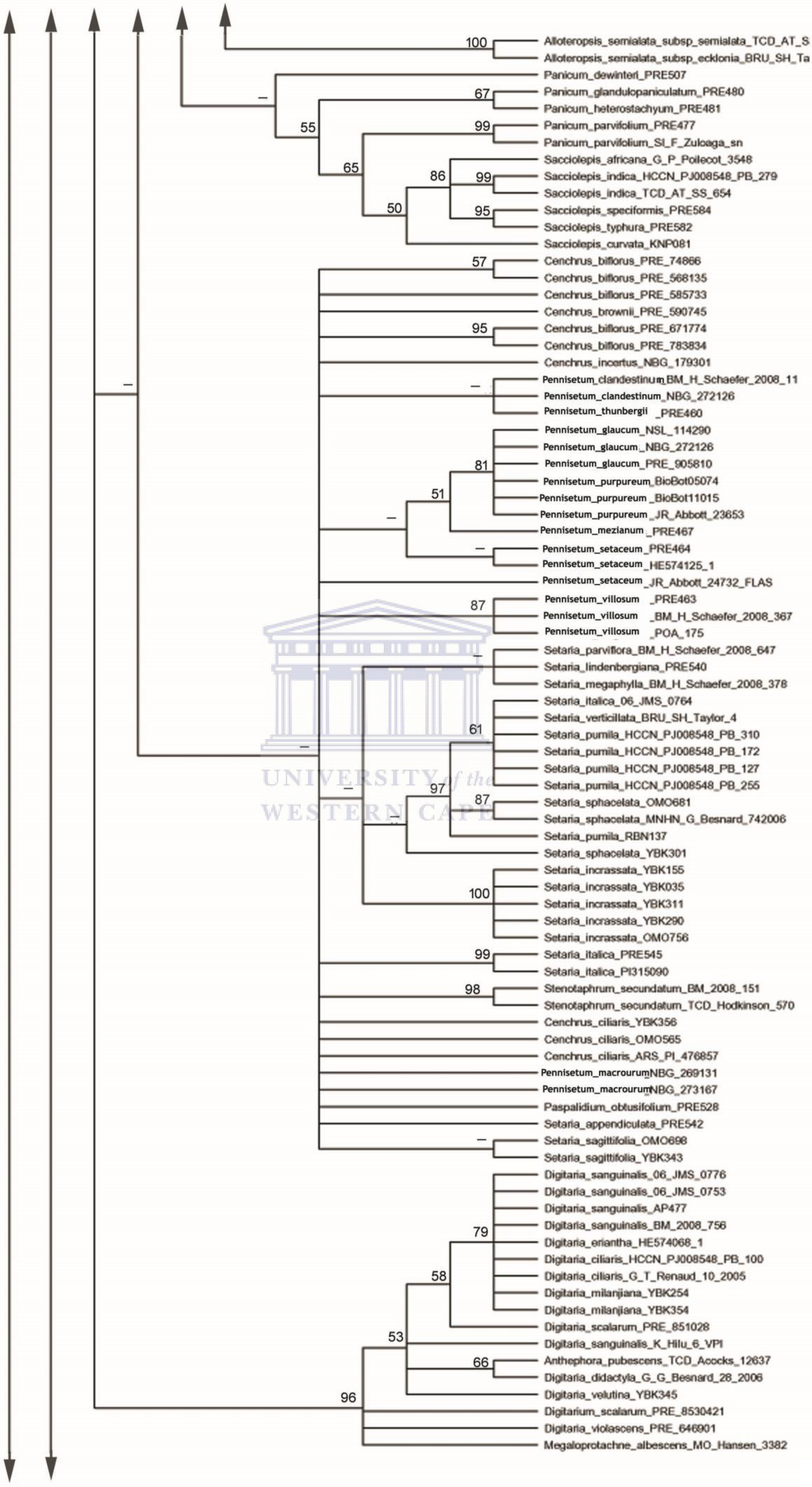
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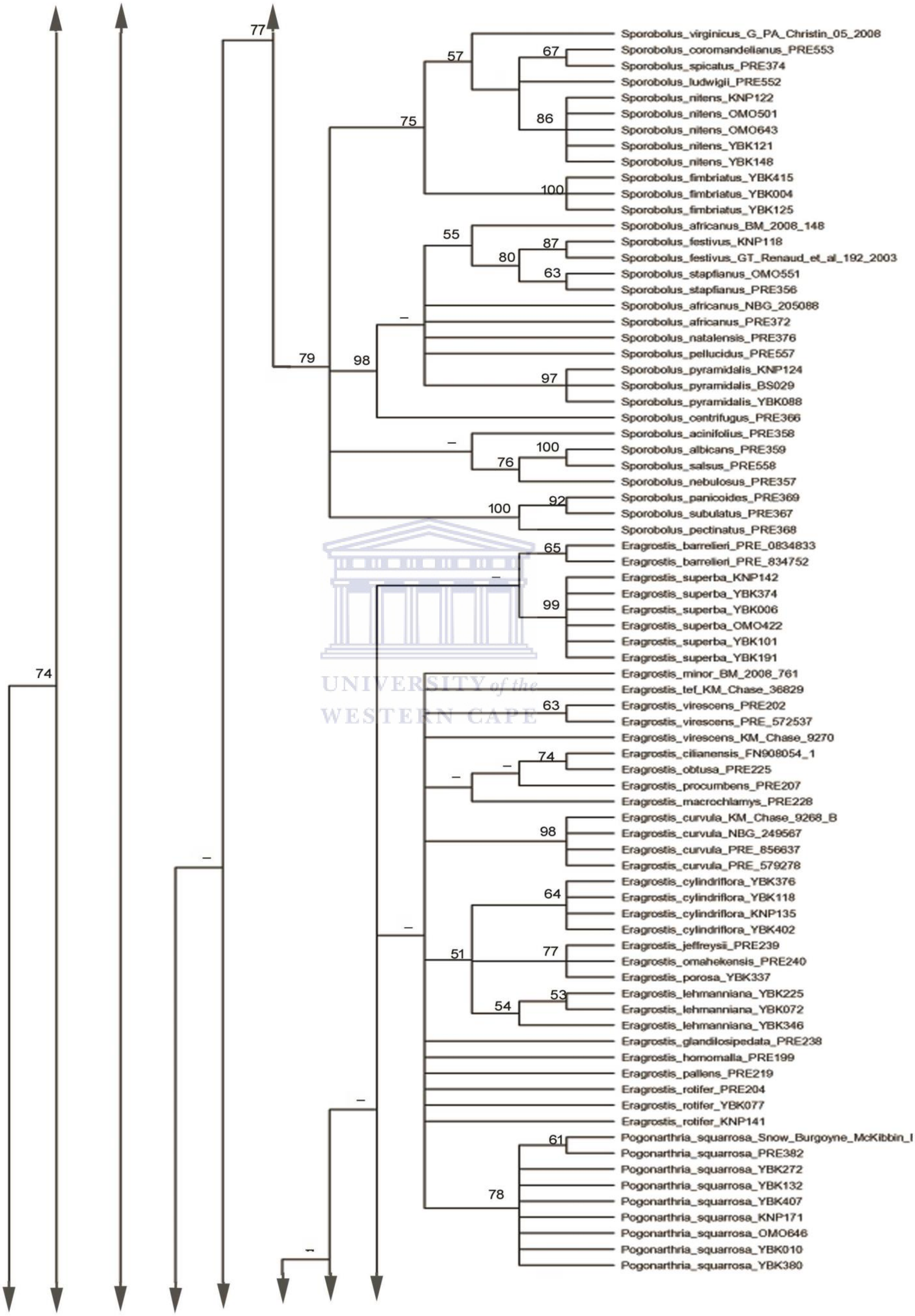
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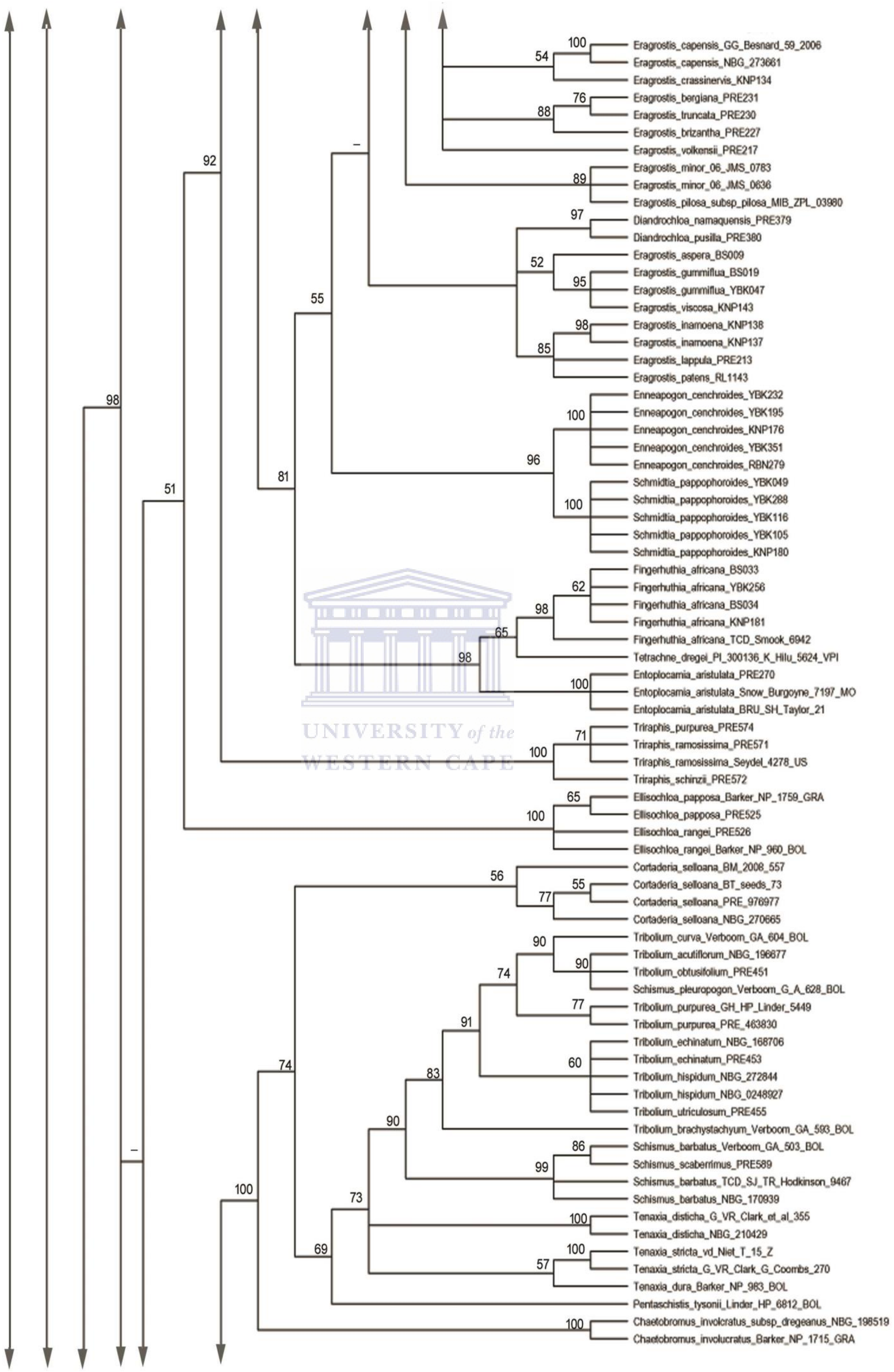
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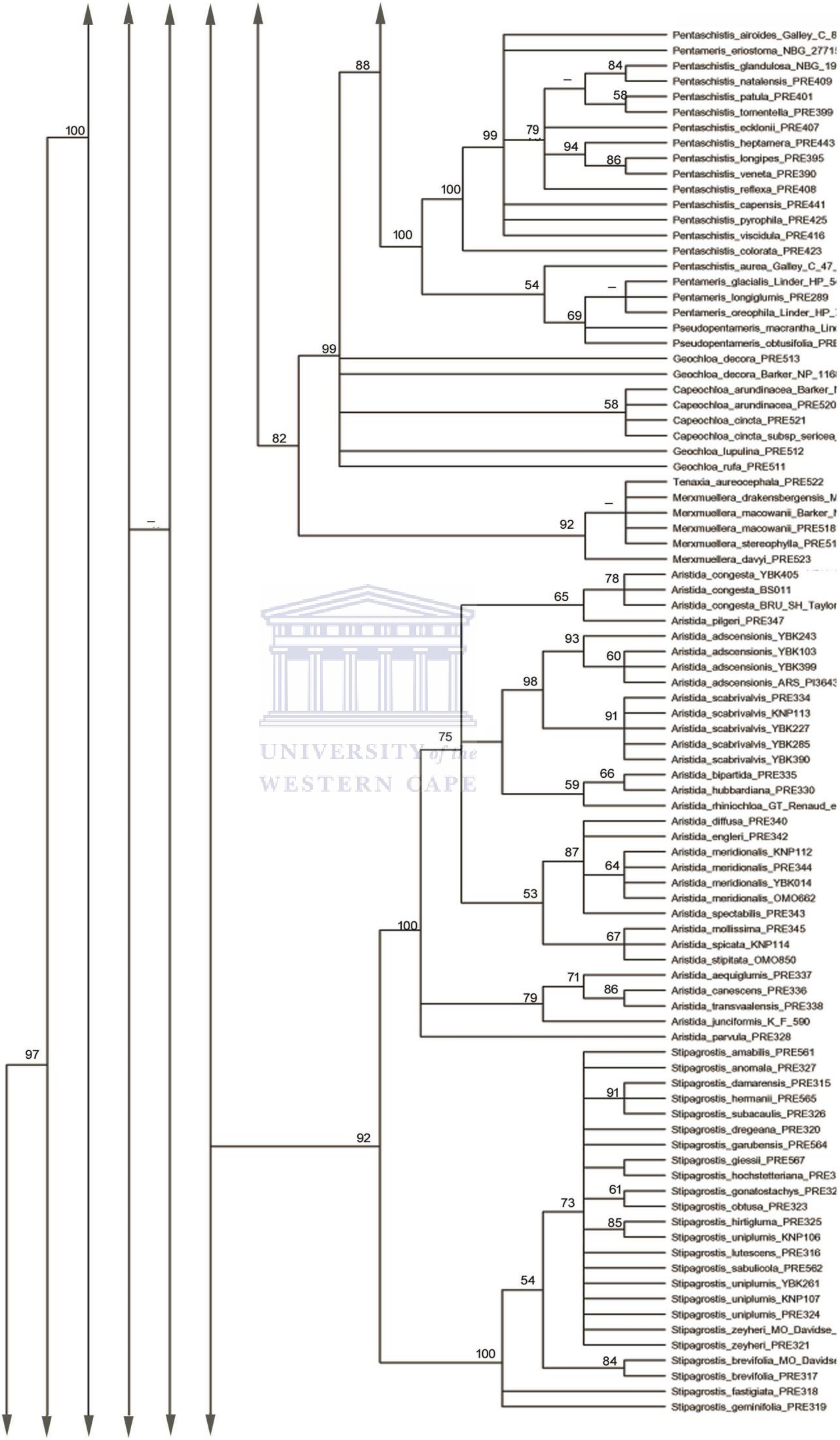
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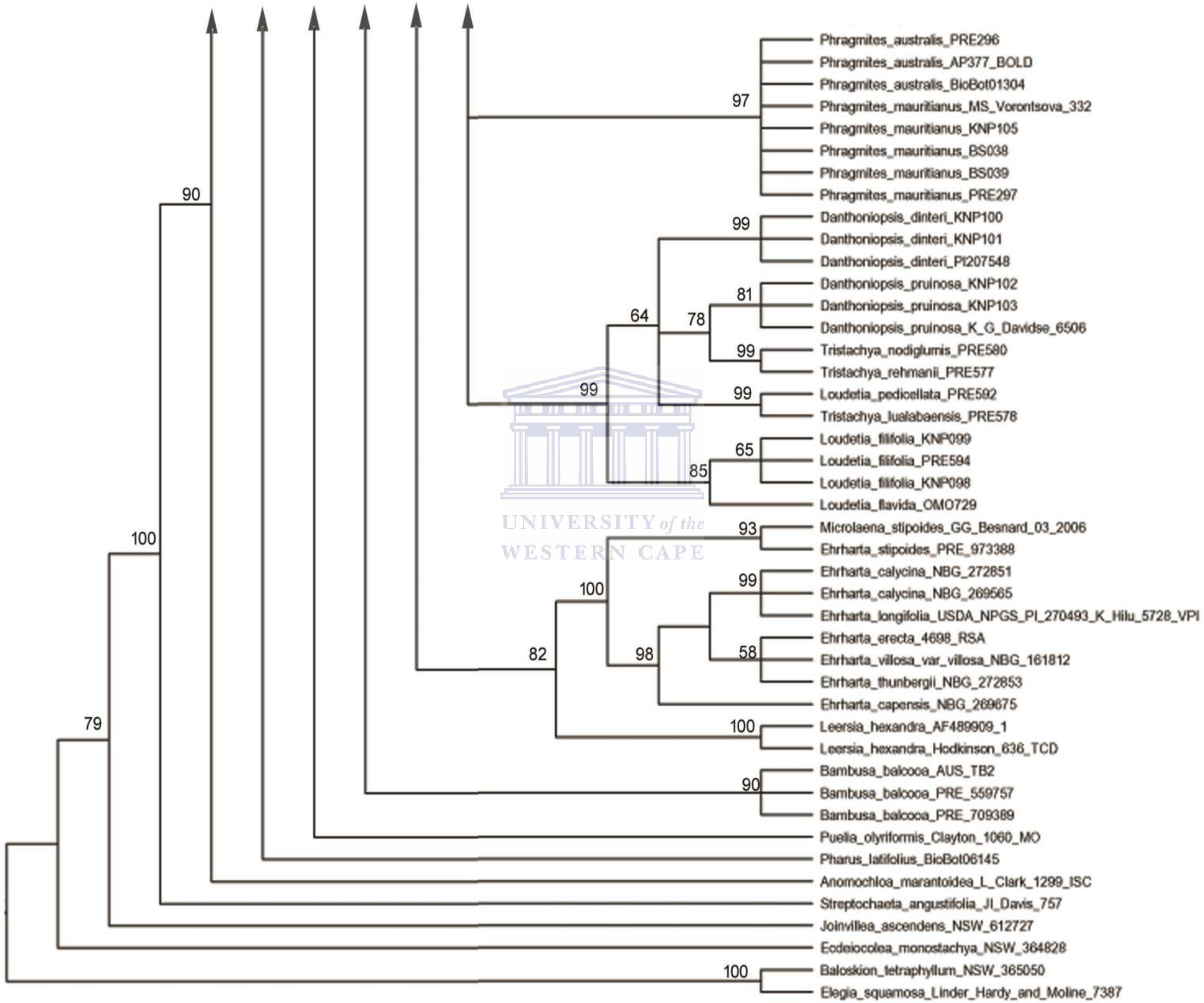
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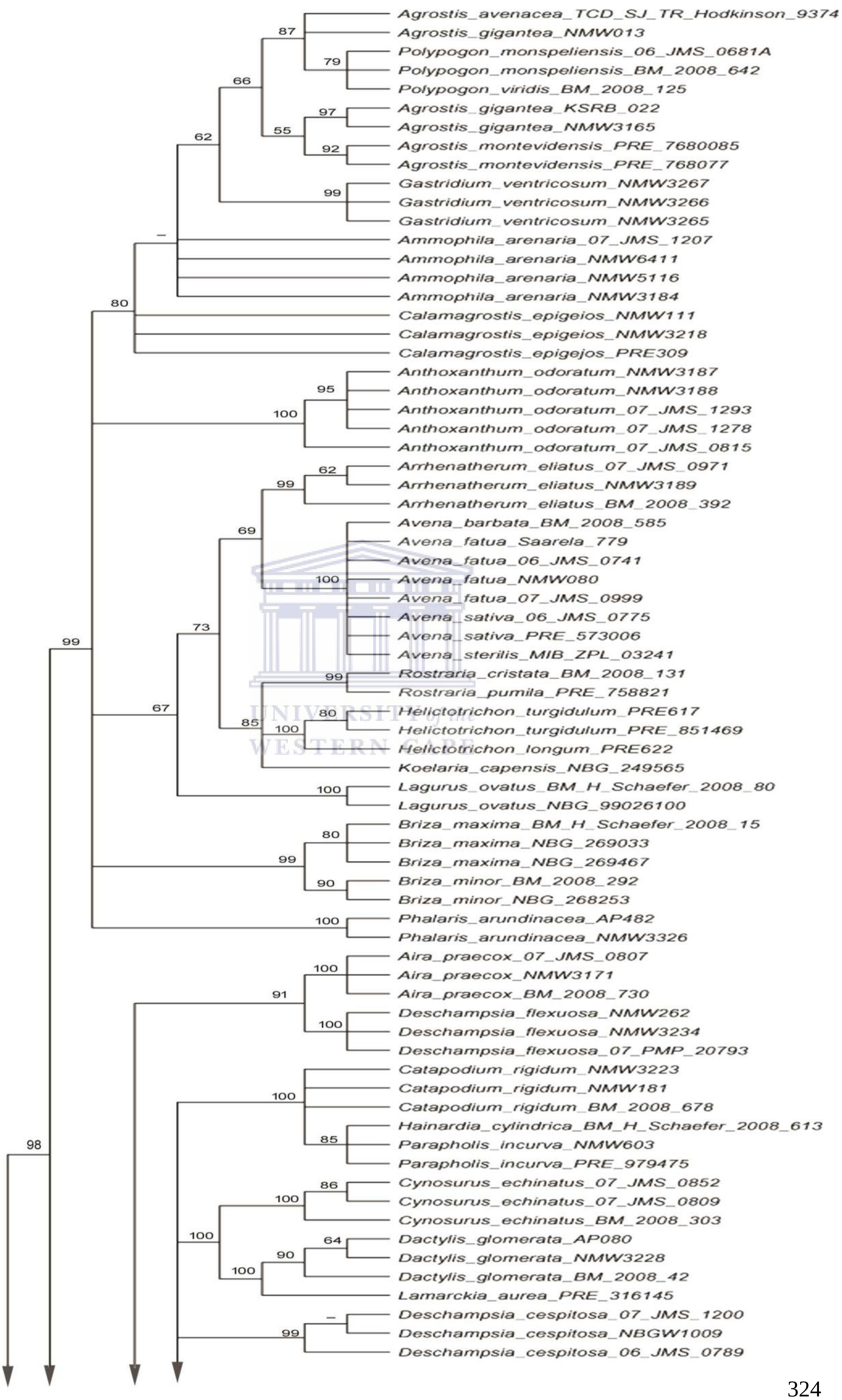
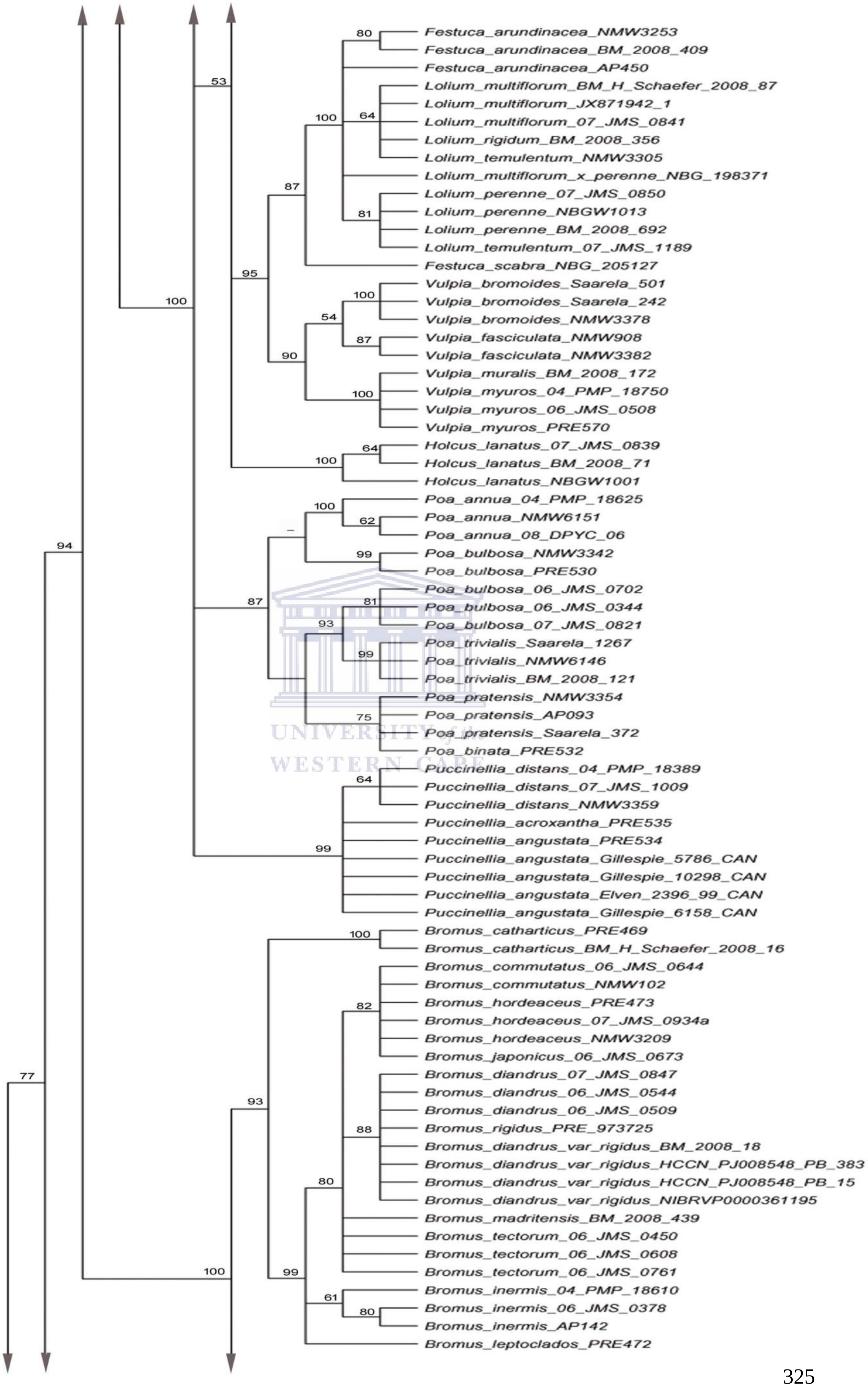
Appendix 2C. MP strict consensus tree of family Poaceae from *rbcLa* + *matK* data with bootstrap values plotted above branches. – indicates no bootstrap support.

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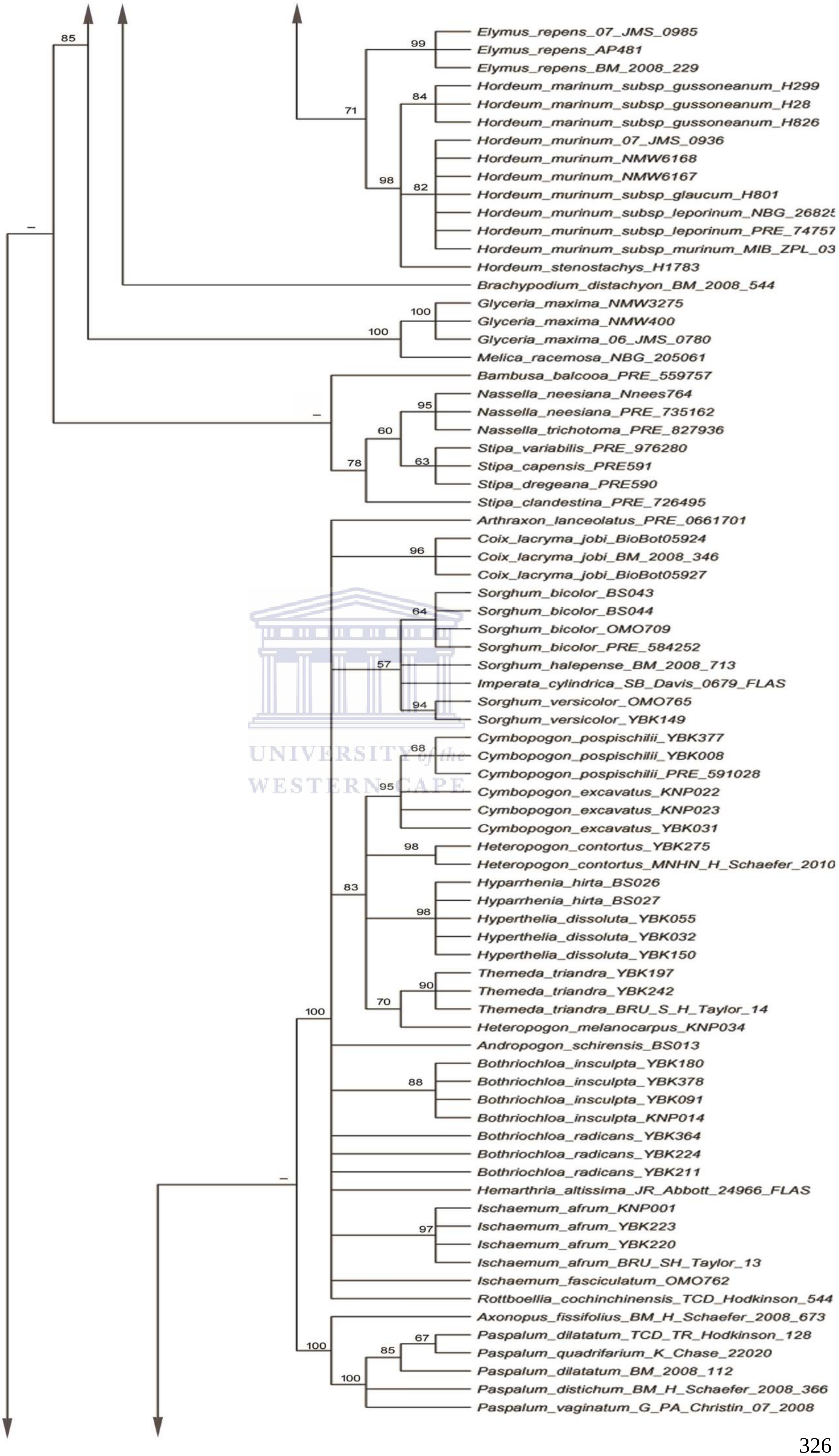
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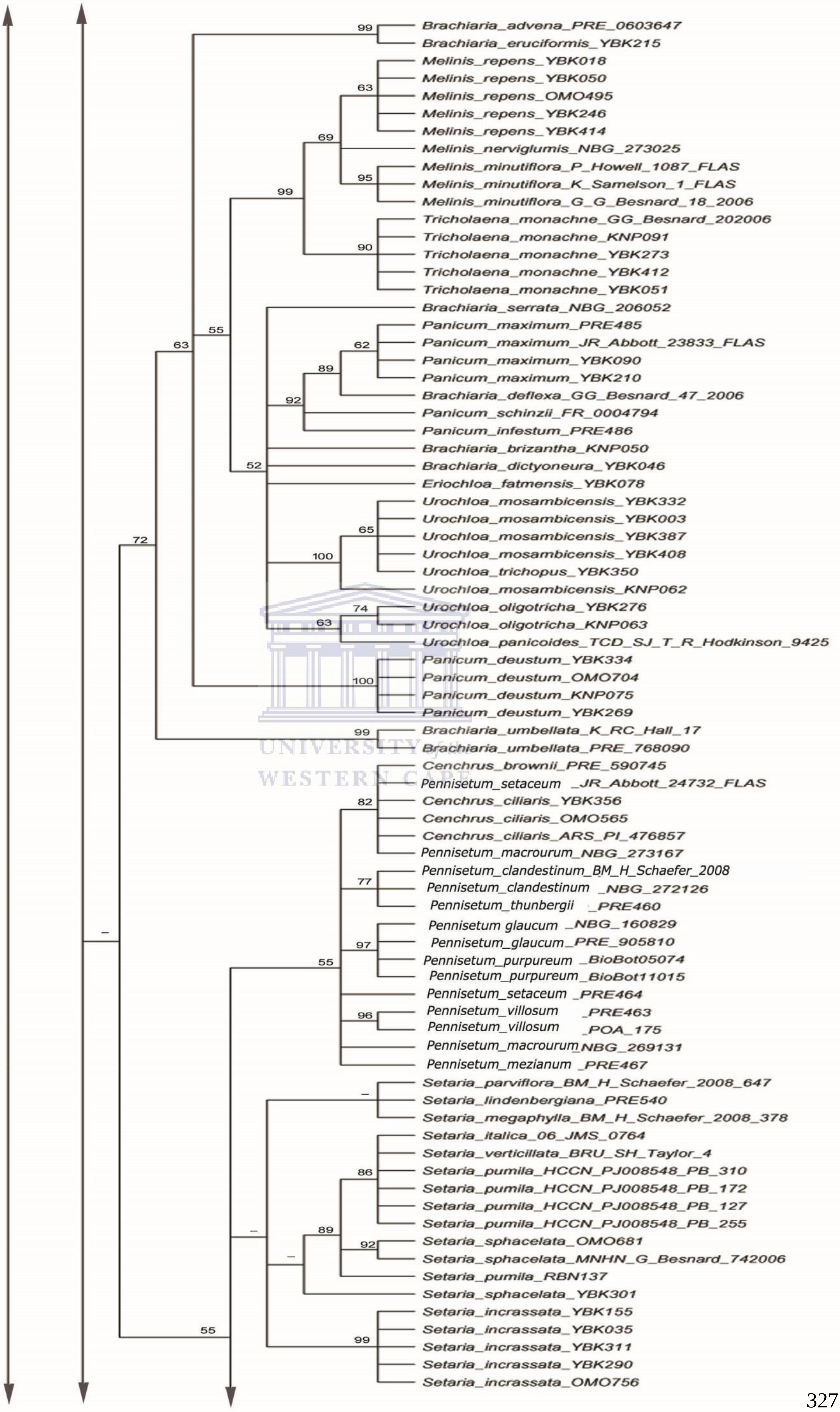
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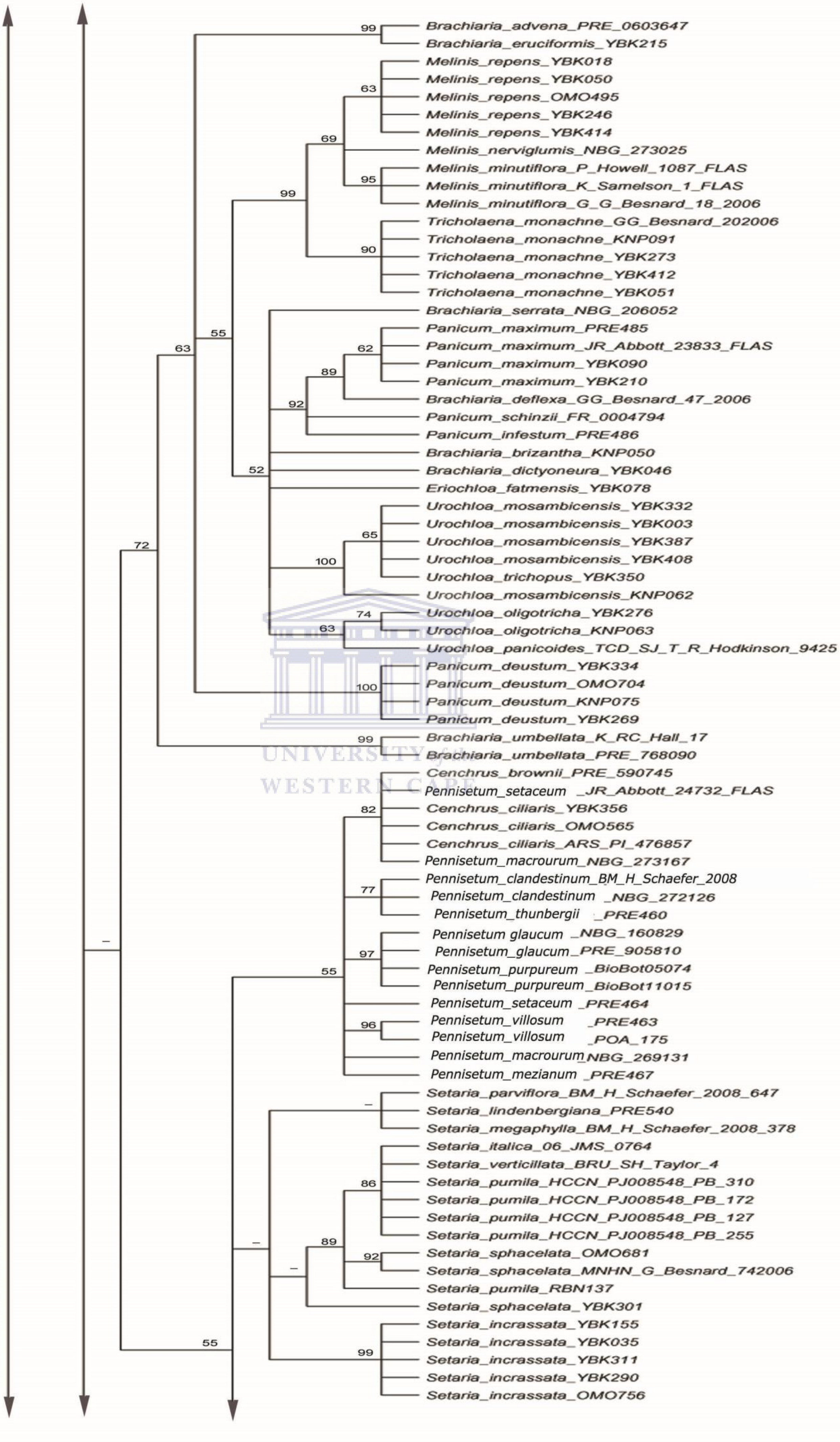
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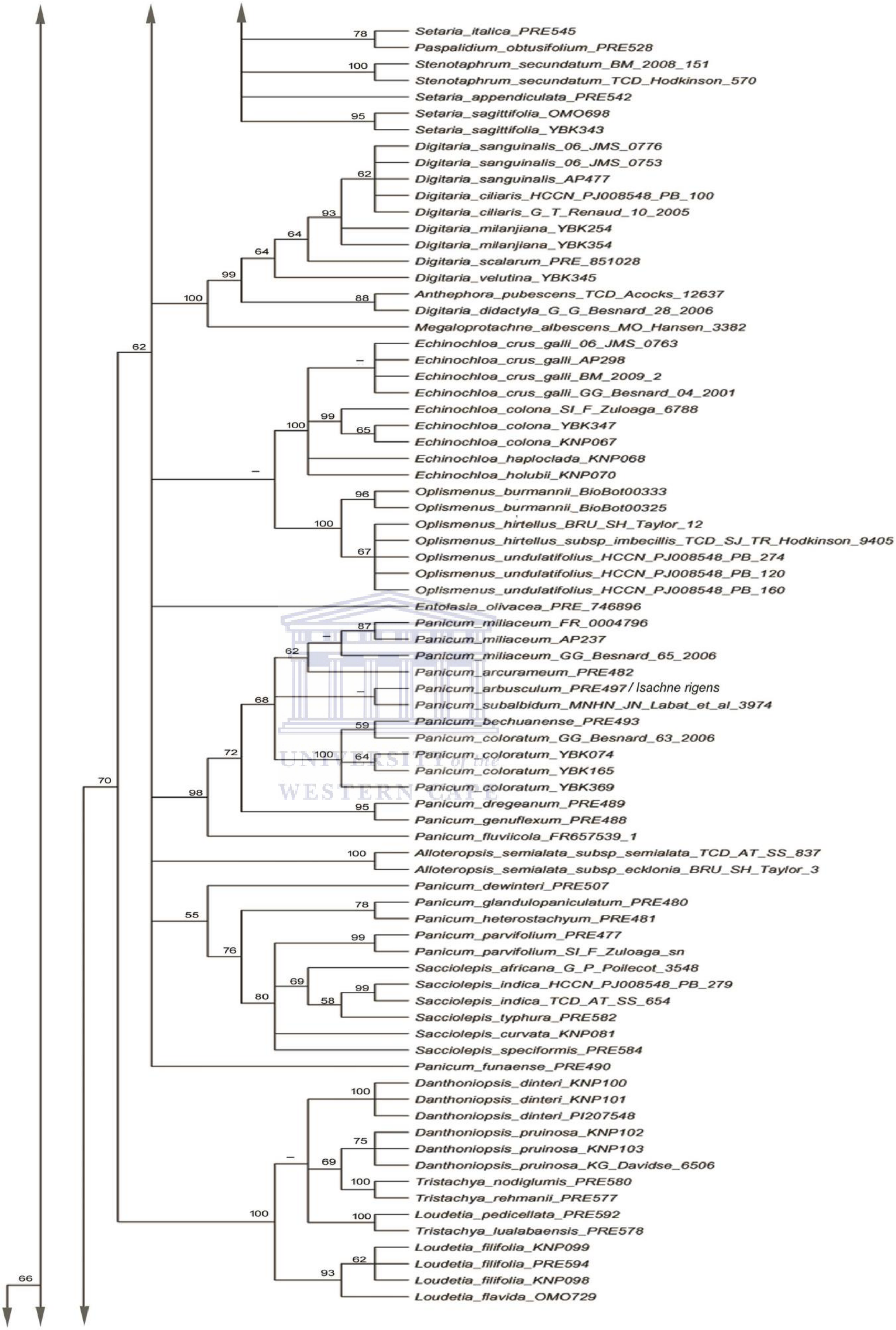
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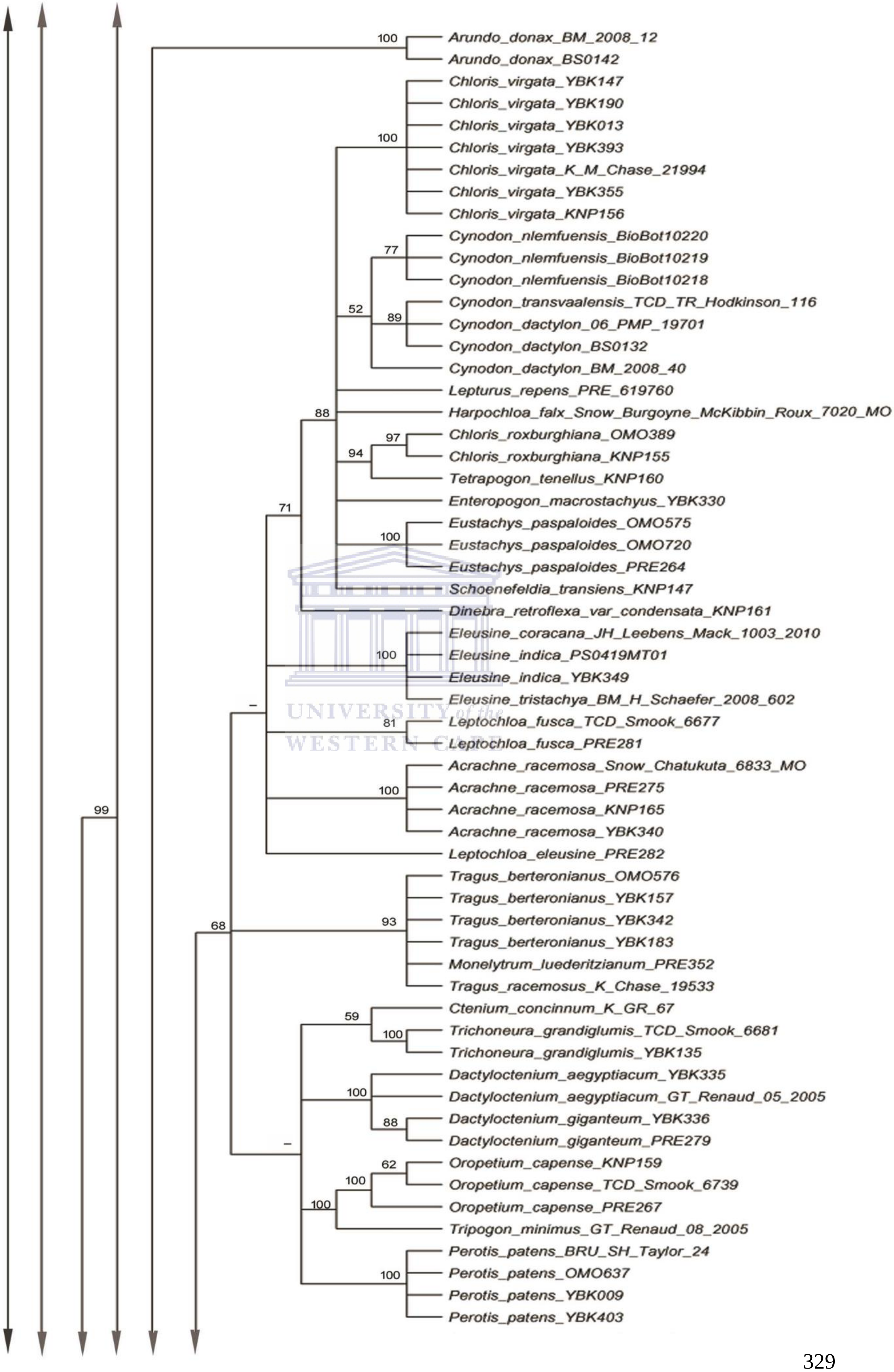
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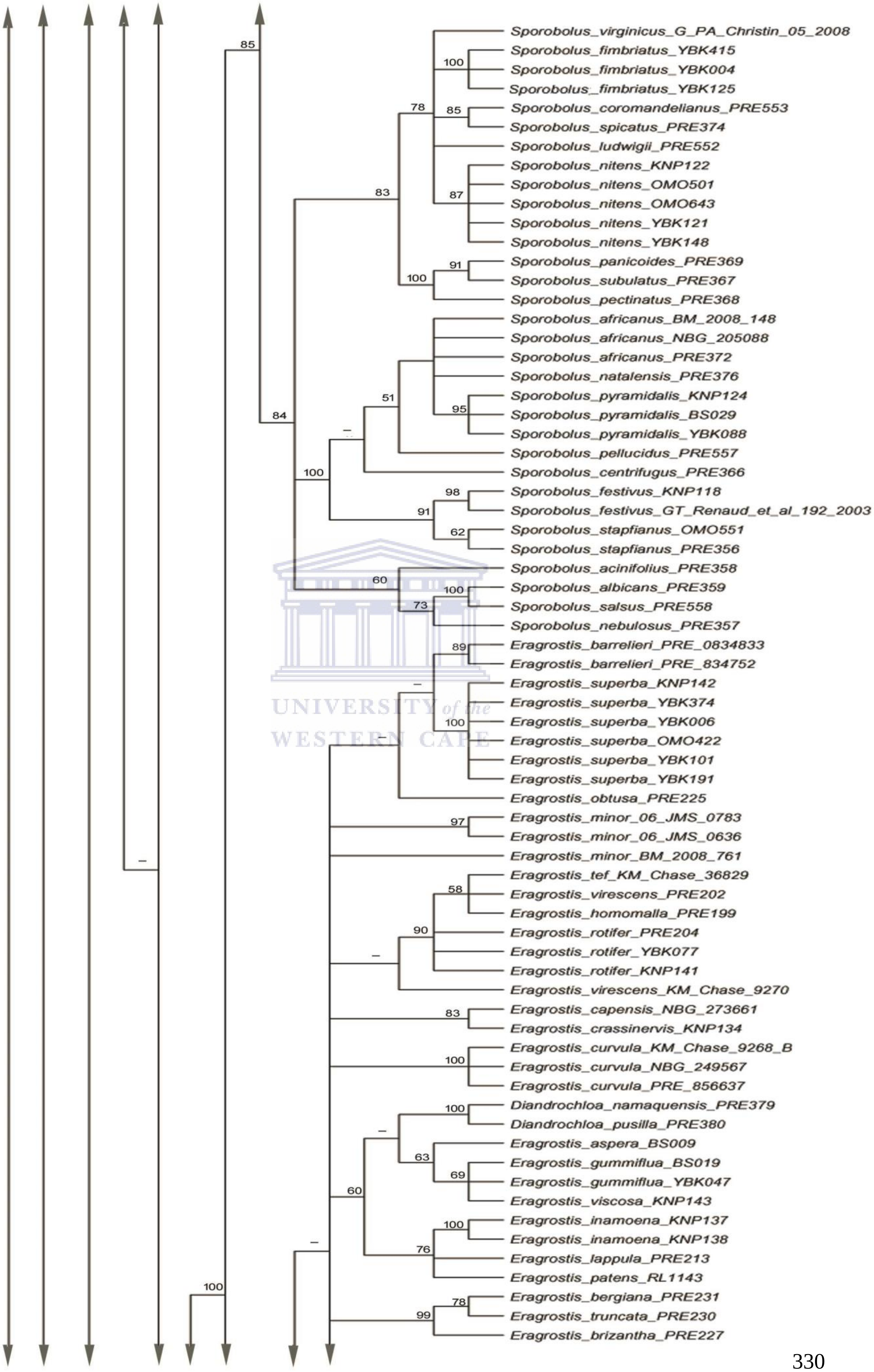
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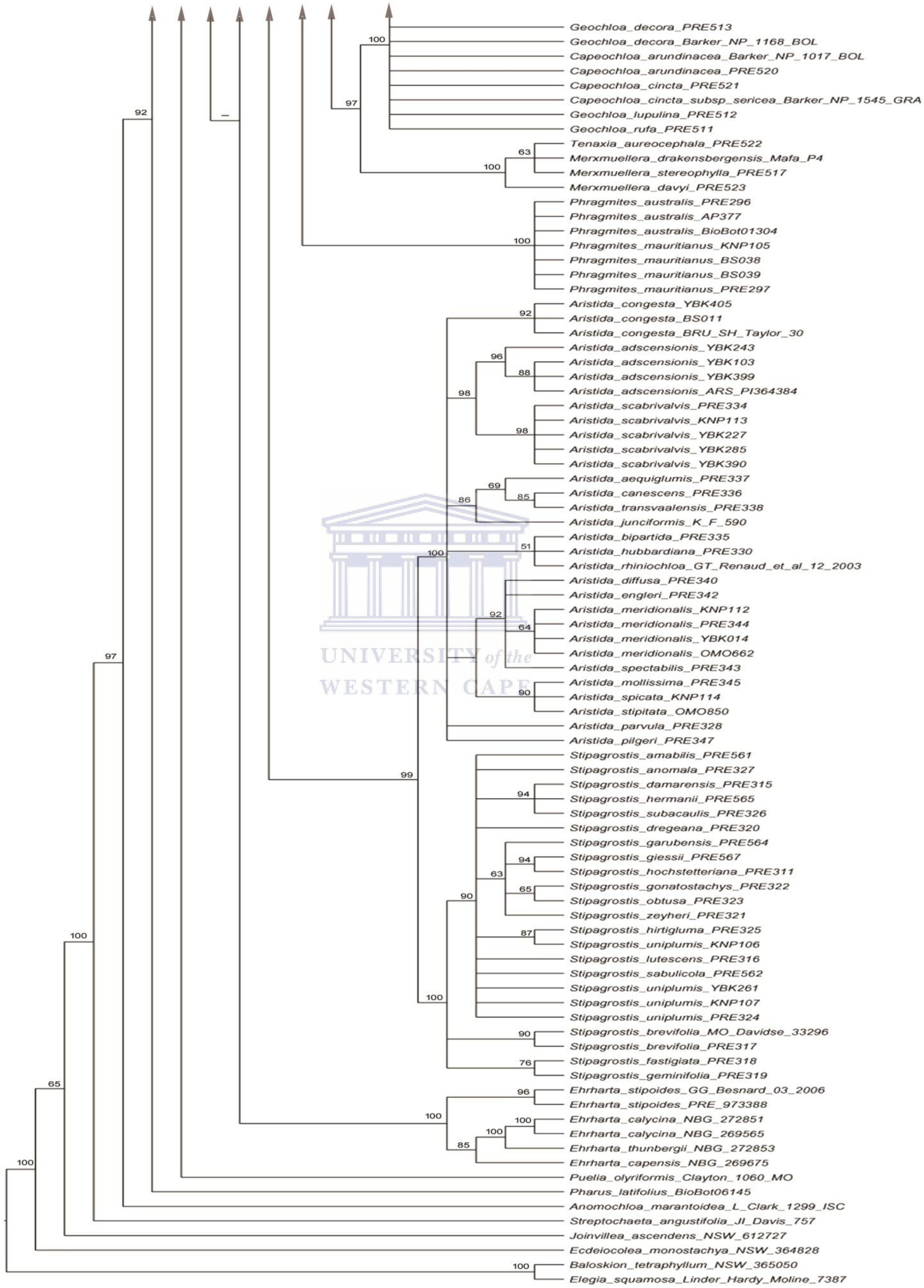
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