Areas of neo- and palaeo-endemism in southern Africa revealed by phylogenetic measures of biodiversity in *Bradypodion* and *Helichrysum*

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A research report submitted to the Faculty of Science, University of the Witwatersrand, in partial fulfilment of the requirements for the Degree of Masters of Science by Coursework and Research Report.

**June 2017**

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Declaration

I declare that this research report is my own, unaided work. It is being submitted for the Degree of Master of Science (by Coursework and research report) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

(Michelle Rumbidzai Mahove)

5th day of June 2017 in Braamfontein, Johannesburg
Abstract

The threats imposed on biodiversity by anthropogenic factors and climate change could cause major biodiversity loss that will have major detrimental effects on the livelihoods of humans and ecosystems. With time and resource constraints plaguing the conservation field it is of utmost importance that biodiversity assessments are prioritised and conducted effectively. Species richness and endemism have been used in biodiversity assessments for many years now but due to their shortcomings, phylogenetic metrics were developed. These phylogenetic metrics offer an advantage in that they include the evolutionary history and relatedness of taxa in the analysis of biodiversity, consequently providing in-depth information on the structure of a taxa and how it relates to other taxa.

Phylogenetic metrics also allow the differentiation between areas of neo- and palaeo-endemism, an important facet in the conservation field. Southern Africa is species diversity and endemism rich and conservation of the region is very important. Therefore, this study incorporated the use of phylogenetic metrics as well as a new metric for differentiating neo- and palaeo-endemism, CANAPE, with the aim of assessing the value of these phylogenetic metrics in identifying hotspots for biodiversity conservation in the biodiverse southern African region. The study also aimed to identify areas of neo- and palaeo-endemism in the region so as to maximise on their conservation. The widespread, everlasting daisy genus, *Helichrysum* (Asteraceae, Gnaphalieae) and the endemic dwarf chameleon genus, *Bradypodion* (Chamaeleonidae) were utilised for this study.

First, phylogenetic metrics and species metrics were calculated for both genera and hotspots were inferred. The hotspots revealed by phylogenetic metrics were then assessed for overlap with the hotspots revealed by species metrics. Next, the CANAPE analysis was run so as to identify the areas of neo- and palaeo- endemism in southern Africa. A phylogenetic diversity (PD) dissimilarity analysis was then conducted to analyse the clustering of the significantly high endemic areas on the phylogenetic tree. Lastly, all the hotspots identified were assessed for representation in the protected areas of southern Africa so as to check how well they are being conserved.

Phylogenetic metrics were largely congruent with the species metrics for both genera with the phylogenetic metrics revealing additional information about the taxa and its history. *Bradypodion* hotspots were mainly located in the Knysna area (South Africa), Walvis Bay
(Namibia) as well as in the Drakensberg region (South Africa) where it is likely that different niches have led to the proliferation of this genus. The results showed more Helichrysum hotspots distributed throughout the region with important areas being Sekhukhuneland, Wolkberg region, Drakensberg region, and the Maputaland-Pondoland centre of diversity in South Africa, as well as southern Namibia.

Some of the phylogenetic metrics did not reveal any useful information for Bradypodion, possibly due to the small genus size. However, using the large Helichrysum genus revealed more information about the hotspots of phylogenetic diversity and endemism in southern Africa. Protection of the hotspots of neo- and palaeo-endemism still requires attention since some of the major hotspots are not located in protected areas. Such areas where conservation is still lacking were identified by the study. Plans to include the hotspots not covered by protected areas need to be prioritised to avoid the loss of speciation hubs, valuable species, and the rich evolutionary history contained within them.

It can be concluded that phylogenetic metrics do reveal additional information that is important to conservation but care must be taken when making conclusions based on taxa of different sizes.

**Keywords:** Bradypodion, CANAPE, Helichrysum, neo- and palaeo-endemism, phylogenetic metrics, southern Africa
Dedication

I dedicate this research to my life partner Tawana Musewe without whom I would not have made it this far. His love and encouragement kept me going even when I felt discouraged. The technical support he gave me and his patience through it all have been overwhelming. To the Lord Almighty, I say thank you for giving me the grace to accomplish this in the time I did.
Acknowledgements

I would like to thank my supervisors Professor Glynis Goodman-Cron and Dr. Kelsey Glennon for their continued guidance and support throughout this year. Their unwavering support and motivation is greatly appreciated. I am grateful to Rendani Nenguda, Hedwig Black and my fellow master’s students for their assistance and tireless support throughout this year. Many thanks to Krystal Tolley of the South African National Biodiversity Institute for the Bradypodium sequences and other resources shared. Dr. Nicola Bergh, Dr, Santi Andrès-Sanchez, Mercè Galbany Casals and Glynis Goodman-Cron are thanked for the Helichrysum sequences that enabled the generation of a phylogeny. Many thanks to Shawn Laffan who helped me with many of the Biodiverse analyses, without his help I would not have been able to complete this project. I would also like to thank the curator at the National Botanical Research Institute (NBRI) in Windhoek for the additional Helichrysum distribution data. Many thanks to Jonah Choiniere who helped me analyse the Helichrysum sequences in TNT. I would also like to appreciate Renee Reddy for always being ready to help me with any resource I needed during the course of this study.

To my parents, Jephita and Priscilla Mahove, I say thank you for always believing in me, I am so grateful for your support and encouragement throughout this year. Finally, I would like to thank my brothers, Jesse and Emmanuel for their love, patience and understanding throughout this year.
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Chapter 1

Introduction

Biodiversity, the variety of life, from genes to populations is currently threatened (Sarkar et al. 2006; Fenker et al. 2014). The threat is mainly due to climate change and anthropogenic factors such as poaching, land degradation, habitat fragmentation and over-usage (Myers et al. 2000; Devictor et al. 2012; Jantz et al. 2015; Arnan et al. 2016). Loss of biodiversity has a detrimental effect on the function of ecosystems, as well on the livelihood of people due to the loss of or reduction of ecosystem services on which they depend (Davies & Cadotte 2011). Therefore, measuring and quantifying biodiversity forms a very important part of the conservation field (Arnan et al. 2016).

Quantifying of biodiversity has been useful in identifying areas of high conservation priority (Ceballos & Ehrlich 2006). Areas harbouring a wide variety of species are especially important for conservation because of the components contained within them. Such areas that contain a huge variety of species and are also vulnerable to negative anthropogenic effects are then defined as biodiversity hotspots (Myers et al. 2000). Biodiversity hotspots could also be defined as speciose areas that contain narrow-ranging species (Myers et al. 2000; Balletto et al. 2010). With a lot of conservation concepts weighing on the amount of biodiversity in an area, there is a need to measure it efficiently.

A limited amount of resources can be poured into biodiversity conservation and the measurement of biodiversity needs to be efficient (Mittermeier et al. 2011; Lee & Mishler 2014). Efficient measurements can only be conducted using the correct tools, viz. biodiversity metrics. Various of these biodiversity metrics were created to measure biodiversity, e.g. species richness, species diversity, functional, ecological and genetic diversity (Magurran & McGill 2011; Mittermeier et al. 2011). However, species richness (SR) is the most commonly used biodiversity metric (Mittermeier et al. 2011; Brooks et al. 2015).

SR is simply the number of species in a given area (Faith 1992; Magurran & McGill 2011). This metric forms the basis of many ecological hypotheses and theories and was traditionally used to define areas of conservation importance (MacArthur & Wilson 1967; Connell 1978; Gotelli & Colwell 2001). For example, the relationships between SR and area, as well as SR and distance form the basis of the island biogeography theory which aims to
explain the endemism and amount of species found on islands (MacArthur & Wilson 1967; Lomolino et al. 2010). Another example of a hypothesis based on SR is the intermediate disturbance hypothesis which suggests that species diversity is favoured when ecological disturbance is not too high or too low (Townsend et al. 1997; Roxburgh et al. 2004). The SR metric also forms the basis for many other diversity metrics and is a good indicator of biodiversity in many cases (Gotelli & Colwell 2001; Lean & Maclaurin 2016).

However, SR alone is not sufficient in assessing the biodiversity of an area because it gives limited insight into the underlying characteristics of taxa (Lean & Maclaurin 2016). A number of shortcomings are presented when using the SR metric, for example, SR can be susceptible to sampling bias (Davies & Cadotte 2011). This could cause a species rich area to appear species poor thereby giving a poor reflection of the diversity of an area (Smith & Wilson 1996). Another problem with using SR is that there is no species concept that is universally accepted and no congruent definition of what a species is; this lack of congruence could confound measurements of biodiversity (Lee & Mishler 2014; Mishler et al. 2014). Taxonomic uncertainty and changes are also issues that hinder SR from being the ultimate biodiversity metric (Lee & Mishler 2014; González-Orozco et al. 2015).

Species endemism, or endemism, is another commonly used biodiversity metric (Mittermeier et al. 2011). Endemism refers to the geographic restriction of a species (Crisp et al. 2001) and it is central to mapping the distribution of biological diversity. It does this by helping us understand the spatial species component or to identify areas of priority for conservation, such as global biodiversity hotspots (Myers et al. 2000; Crisp et al. 2001; Rosauer & Jetz 2015). Endemism is also a good estimator of extinction risk since geographically restricted species are irreplaceable and are not found anywhere else (Gaston & Fuller 2009; Gudde et al. 2013).

Even though traditional species metrics are useful in quantifying biodiversity they fall short of being the best metrics. For example, SR scores can bias endemism, high SR gives a high endemism score even if the species are wide spread and vice versa (Crisp et al. 2001; Laffan et al. 2013). Traditional biodiversity metrics do not reveal areas where few species represent a significant amount of evolutionary history (Mooers 2007; Yek et al. 2009), these metrics only consider the terminal taxa on the phylogenetic tree and do not account for the relationships among the taxa (Lee & Mishler 2014; Rosauer & Jetz 2015).
Phylogenetic metrics give insight into how species evolved and which ones are important for biodiversity assessments because they reveal the genetic diversity as well as shared characteristics of taxa (Mooers 2007; Lee & Mishler 2014). Traditional species metrics are regarded as surface level metrics because they do not measure the relationships between taxa as well as information on the evolutionary history of taxa (Forest et al. 2007; Lee & Mishler 2014). Therefore, including evolutionary history in biodiversity assessments has become a priority (Mouquet et al. 2012). Conserving evolutionary history is an effective way of prioritising areas for biodiversity protection because variation in the rates of evolution of species has led to the variety of traits and functions of taxa that contribute to biodiversity even up to this day (Forest et al. 2007; Costion et al. 2015).

Phylogenetic metrics were created to try to mitigate the problems associated with traditional biodiversity metrics. A phylogeny is an evolutionary tree based on shared characteristics of taxa that also reveals common ancestry (Eldredge & Cracraft 1980). Including the evolutionary relationships and history of taxa in biodiversity analyses reveals more information as compared to using traditional species metrics alone (Rosauer & Jetz 2015). Evolutionary histories of taxa tell a story about them and reveal how they evolved. Phylogenetic interpretation reveals the branching structure of evolutionary relationships between taxa using analysis of shared characteristics inferred through molecular and morphological data (Crozier et al. 1997; Lean & Maclaurin 2016).

Species metrics depend on sample size and do not include processes such as interspecific interactions and evolutionary information (Noss 1990). Ideally, biodiversity should include the composition, structure and function of an area (Noss 1990). Species metrics provide information on the composition and structure of an area but rarely on the function (Noss 1990). This gap in information is where phylogenetic metrics step in since they provide information on the function and evolutionary processes in an area thereby giving a complete picture of biodiversity.

Measures based on evolutionary history capture aspects of biodiversity missed by species level metrics. Phylogenetic metrics have an advantage in that they can reduce the issues caused by taxonomic uncertainty and changes (Mace et al. 2003). Another advantage is that, unlike traditional metrics, phylogenetic metrics are not limited by geographic boundaries (Rosauer & Jetz 2015). These advantages cause phylogenetic metrics to offer a
more comprehensive and inclusive outlook on biodiversity and give a more integrated approach to conservation (Mace et al. 2003; Davies & Buckley 2011; Daru et al. 2015; Rosauer & Jetz 2015).

Phylogenetic diversity (PD) is one of the commonly used phylogenetic metrics. PD is defined as the sum of branch lengths connecting a set of taxa on a rooted phylogenetic tree (Faith 1992). This metric accounts for shared evolutionary history of taxa (Faith 1992; Strecker et al. 2011) thereby giving more insight into biodiversity assessments. The loss of species that are evolutionary distinct leads to a loss of functions for ecosystems and this loss is sometimes irreversible (Davies & Buckley 2011; Bracken & Low 2012). Using PD to select important areas for conservation will ensure more diversity is captured in protected areas (Forest et al. 2007; Nipperess 2016).

An increase in PD has been suggested to increase evolutionary potential of communities to adapt to changes in their environment (Meynard et al. 2011; Sgro et al. 2011; Mouquet et al. 2012). An increase in the ability of taxa to adapt to changes in their environment is becoming more and more essential in the face of the current global change. The increase in temperatures and drastic changes in weather patterns caused by global change put taxa at risk of extinction (Eeley et al. 1999). The ability to adapt to these changes will ensure the survival and proliferation of the taxa. Protection of PD will be beneficial to communities as it will enhance their ability to adapt (Faith 1992).

PD has been used for identifying priority areas for conserving plants (Forest et al. 2007) and animals (Davies & Pedersen 2008). Another study used this metric to investigate ecosystem functioning where PD aided in estimating the functional trait components of a community (Srivastava et al. 2012). In the Cape Floristic Region of South Africa, protection of PD has been shown to lead to greater protection of feature diversity (Forest et al. 2007) and it has also been used to identify regions of significant evolutionary history in the world (Sechrest et al. 2002; Vandergast et al. 2008). Jetz et al. (2012) used PD to assess the global diversity of birds and concluded that consideration of phylogenetics causes a shift from diversity being all about species to an inclusion of features and characters shared by taxa (Laity et al. 2015).

Numerous case studies have demonstrated the value of PD for providing a more effective assessment of biodiversity (Faith 1992; Forest et al. 2007; Diniz-Filho et al. 2013).
PD is important for nature conservation because it can be linked to extinction of species (Purvis et al. 2000), biotic invasions (Winter et al. 2009) as well as ecosystem functioning (Srivastava et al. 2012). High PD values reveal the presence of phylogenetically distinct taxa and could indicate areas that are refugia (Schmidt-Lebuhn et al. 2015), while low PD values reveal phylogenetically close taxa that could have arisen due to local radiations and local speciation (Donoghue 2008; Da Silva et al. 2010).

Incorporating phylogenetic information into biodiversity analysis reveals information on the processes leading up to the current spatial patterns of diversity and endemism (Molina-Venegas et al. 2016). Endemism is also usually studied at the species level which we have already seen to be limited, so a phylogenetic metric of endemism known as phylogenetic endemism (PE) was created (Rosauer et al. 2009). PE, is the sum of branch lengths weighted by the proportion of the range of each branch that is found in the area; in other words, range weighted PD (Rosauer et al. 2009; Laity et al. 2015). PE reveals units of PD that have restricted ranges and provides evolutionary and genetic information to aid in the formulation of conservation policy.

Phylogenetically endemic species contain distinct genetic information and contribute to biodiversity and it is very essential to include this information in analyses (Crozier et al. 1997; Gudde et al. 2013). Unlike, species endemism, PE incorporates all the connecting branches of the tree and not just species, thereby providing a more comprehensive outlook on the rarity of a clade (Rosauer et al. 2009). High PE is found where long branches on the phylogenetic tree are restricted to a small geographic range (Rosauer & Jetz 2015). Areas of high PE are very important to conserve as they harbour evolutionary history that is at risk of extinction (Jetz et al. 2012; Rosauer & Jetz 2015).

PE has been used to reveal areas of mammal endemism in Australia (Rosauer & Jetz 2015) as well as to reveal biodiversity hotspots for trees in southern Africa (Daru et al. 2015). Other uses of the PE metric include the assessment of extinction risks of various taxonomic groups (González-Orozco et al. 2015) as well as studying the PE of Malagasy lemuriformes (order of primates) in an effort to determine the best course of action in their conservation (Gudde et al. 2013). Palaeo-climate, primary productivity, isolation and dispersal ability have been attributed to affect levels of PE found in an area (Rosauer & Jetz 2015).
A very useful application of PE is the differentiation between areas of neo- and palaeo-endemism (Jordan et al. 2016; Molina-Venegas et al. 2016). Palaeo-endemics are taxa that persist in restricted areas but were once widespread, for example the *Sequoiadendron*, a genus of evergreen trees that was once widespread across North America and the Northern Hemisphere and is now restricted to a small area of California (Stebbins & Major 1965). Palaeo-endemics are of high value because they contain relict genetic and evolutionary distinctness and heritage (Pepper et al. 2011; González-Orozco et al. 2015). Palaeo-endemics have been found to occur where the environment has retained features that support them and have been lost elsewhere (Jordan et al. 2016).

Most palaeo-endemics are extant representatives of past species that lived under historical climates (Molina-Venegas et al. 2016). Areas where the climate remained stable enough to support species have a high concentration of palaeo-endemics (Anacker 2011). In the Mediterranean, hotspots of palaeo-endemics are thought to have been shaped by climate, tectonic activities and geomorphology (Molina-Venegas et al. 2016). Glaciation events would have given rise to refugia that protected species that have now become palaeo-endemics. A split of a genus due to tectonic plates could also give rise to palaeo-endemics, the species that would end up in unfamiliar territory could die off and the remaining population would become palaeo-endemics.

Areas that contain a lot of palaeo-endemics are important for conservation because of the irreplaceability of the taxa (Jordan et al. 2016). Areas of palaeo-endemism should be prioritised in protected areas so as to avoid the loss of evolutionary rich areas and the associated taxa with valuable evolutionary information (González-Orozco et al. 2015; Molina-Venegas et al. 2016). On the other hand, neo-endemics are newly formed taxa that are restricted to specific geographic areas (Mishler et al. 2014). Areas of neo-endemism are cradles of speciation that give rise to unique species and should also be conserved (Molina-Venegas et al. 2016). Neo-endemics need to be protected because they are range-restricted lineages that could give a clue about the evolutionary potential of lineages (González-Orozco et al. 2015). Other causes of speciation that could lead to neo-endemics being formed in a particular area are ecological divergence as well as a barrier to migration that leads to allopatric speciation (Barraclough & Herniou 2003).
Neo-endemics could have risen due to species diversification caused by tectonic upliftment as well as mountain building (Molina-Venegas et al. 2016). Another major cause could possibly be palaeo-climatic histories (the variation in climate over many periods of earth’s history) of regions and countries (Pepper et al. 2011). Specialisation due to adaptation to substrates and climate has also given rise to neo-endemics (Molina-Venegas et al. 2016). In the Cape Floristic Region (CFR) of South Africa, topographical complexity, edaphic complexity, pollinator specialisation, fire and short dispersal distances are documented to be responsible for the massive speciation that has caused the area to be a major area of endemism (Linder 2003; Linder & Hardy 2004; Barraclough 2006).

The CFR contains high diversity and a variety of neo- and palaeo-endemics (Goldblatt & Manning 2002). The area has a total of 8920 angiosperm species with 69.5% of them endemic to the region, with the Asteraceae being the most speciose plant family (Goldblatt & Manning 2002; Linder & Hardy 2004; Schnitzler et al. 2011). This region forms one of the areas of floristic endemism in southern Africa as well as being an area of high chameleon diversity (Van Wyk & Smith 2001a; Tolley et al. 2006). Within the CFR, an area of particularly high diversity and endemism is the Cape Peninsula (Helme & Trinder-Smith 2006). There are an estimated 158 plant species endemic to the Cape Peninsula, making up 7% of the total flora of the Peninsula (Linder 2003; Helme & Trinder-Smith 2006).

The Drakensberg Alpine Centre (DAC) is another area of high floral diversity and endemism within southern Africa spanning across South Africa and Lesotho (Van Wyk & Smith 2001a; Carbutt & Edwards 2003). It contains an estimated total of 2618 species and of these, 334 are endemic and 595 near endemic (Carbutt & Edwards 2006). Asteraceae, Scrophulariaceae, and Iridaceae make up most of the endemic and near endemic taxa and the genera with the most endemic and near endemic taxa are Helichrysum and Senecio (Carbutt & Edwards 2006).

The Maputaland, Pondoland, and Albany areas of southern Africa which span the eastern coast of southern Africa and extend inland towards the Great Escarpment also harbour a wide variety of species. They harbour a total of 1900 endemic plant species (Van Wyk & Smith 2001a; Mittermeier et al. 2011). The region also contains 62 endemic and 60 near endemic small vertebrate species with chameleons and lizards having the highest representation (Perera et al. 2011).
The rich amount of phylogenetic history that is characteristic of southern Africa can be attributed to the level of climatic stability the region has experienced, especially in the Cape (Cowling et al. 2009), over the years as compared to other regions of the world (Dynesius & Jansson 2000; Padayachee & Proches 2016). Southern African taxa show a rich history of evolutionary information which can reveal mechanisms underlying present day biodiversity patterns (Tankard et al. 2012).

**Rationale**

All the areas of high diversity and endemism need to be prioritised in conservation plans to avoid loss of important species and services. There is a need for effective biodiversity metrics to be utilised in quantifying the biodiversity of areas. Phylogenetic metrics offer an improvement over traditional biodiversity metrics because they incorporate the evolutionary relationships of taxa. Such metrics have been shown to give more accurate assessments of biodiversity (Diniz-Filho et al. 2013; González-Orozco et al. 2015). However, these phylogenetic metrics are relatively recently developed and there is a need for an assessment of their effectiveness in identifying hotspots of biodiversity for conservation.

Conservation in southern Africa is a priority and there are a lot (1 697) of protected areas (Duffy 2006). However, many of these areas were decided upon using SR and species endemism and there is a need for an assessment of whether areas of high SR correlate with areas of high PD. SR can be a good surrogate of PD but areas of high PD and SR do not always correlate (Tucker & Cadotte 2013; Tucker et al. 2016). For example, in southern Africa, areas of high tree SR and PD were found to be incongruent. There might be areas of conservation priority (high PD) that are being ignored in the current protected areas and so there is a need to assess if we are accurately conserving phylogenetic rich areas.

Even though there are many endemics in southern Africa, there is still a lack of information on areas of neo- and palaeo-endemism, hence there is a need for an assessment of these areas of endemism especially with regards to conservation. This study will incorporate the use of phylogenetic diversity and endemism measures as well as a new approach that differentiates between areas of neo- and palaeo-endemism as proposed by Mishler et al. (2014). This study will provide important information on whether incorporating phylogenetics into biodiversity metrics reveals more information as well as whether areas of
high species diversity overlap with areas of high phylogenetic variability. Results from this study will aid in assessments of whether we are efficiently conserving important areas.

The aim of this study is to assess the value of biodiversity indices that incorporate phylogeny in identifying hotspots of diversity and endemism that should be prioritised in conservation efforts in southern Africa as well as to identify areas of neo- and palaeo-endemism using the small dwarf chameleon genus, *Bradypodion* and the large plant genus, *Helichrysum*. The difference in size and extent of distribution of the two genera provides an opportunity for assessing the effects of genus size and distribution on biodiversity studies.

To achieve this aim, the objectives are to:

1. Calculate measures of diversity for each genus with and without incorporating phylogenetic information.
2. Compare hotspots inferred by phylogenetic diversity and/or endemism measures to those inferred by traditional biodiversity measures.
3. Identify areas of neo- and palaeo-endemism using CANAPE for each genus.
4. Compare the identified centres of neo- and palaeo-endemism and discuss the implications for conservation.
Chapter 2

Introduction

The quantification of biodiversity is an integral part of ecological and conservation studies (Scheiner 2012; Arnan et al. 2016). Due to the increasing threat on biodiversity it is of paramount importance that we focus on its conservation. There are usually time and resource constraints in the conservation field, and there is need for prioritisation of biodiversity assessments (Myers et al. 2000). Prioritising conservation efforts requires assessments of areas that need more effort and these areas are inferred using various aspects of their biodiversity. In order for conservation to occur efficiently, there needs to be an effective assessment of the biodiversity and endemism in areas of high concern (Ferrier 2002; Pressey et al. 2013).

With this decrease in biodiversity, selecting the appropriate biodiversity metrics becomes very essential. Traditionally, species richness (number of species in a specified area) and endemism (geographic restrictedness of taxa) were primarily used in biodiversity assessments but their limitations led to new innovations (Faith 1992; Pressey et al. 2013). Researchers began to suggest biodiversity metrics that include evolutionary distinctiveness as these were regarded to be more ‘accurate’ (Sarkar et al. 2006; Costion et al. 2015; Laity et al. 2015). These metrics, also known as phylogenetic metrics, incorporate the phylogenies of the taxa in the analysis and are not just surface level metrics.

A commonly used phylogenetic metric is phylogenetic diversity (PD) which measures the diversity of taxa on a phylogeny using branch lengths (Faith 1992). Several studies have demonstrated the value of PD in providing a more comprehensive assessment of biodiversity e.g., (Faith 1992; Forest et al. 2007; Diniz-Filho et al. 2013; Mishler et al. 2014; Tucker et al. 2016). This is because phylogenetic metrics incorporate the evolutionary history and relatedness of taxa when analysing biodiversity (Strecker et al. 2011). Another commonly used phylogenetic metric is phylogenetic endemism (PE) which measures the units of PD that have restricted ranges (Rosauer et al. 2009; Mishler et al. 2014). Areas of high PD and PE are very important to conserve as they harbour important evolutionary history that is at risk of extinction (Jetz et al. 2012; Rosauer & Jetz 2015).

There are two types of endemic species: neo-endemics—recently diverged and restricted to specific areas, and palaeo-endemics—old species that were more widespread in
the past and are now restricted to a local region (Schmidt-Lebuhn et al. 2015; Jordan et al. 2016; Ma et al. 2016). PE is useful in differentiating between these areas of neo- and palaeo-endemism (Rodrigues et al. 2005b; Forest et al. 2007). Areas of neo-endemism are cradles of speciation that give rise to unique species and should be conserved (Molina-Venegas et al. 2016) and areas that harbour palaeo-endemics could reveal historical climatic stability and could have acted as refugia for taxa (Molina-Venegas et al. 2016).

Since areas of endemism carry components of biodiversity that are not widely represented, they are very important to conservation (Myers et al. 2000). This has led to the use of endemic species to assign conservation priority to one area over another (Myers et al. 2000; Tucker et al. 2016). Global biodiversity hotspots are traditionally characterised based on species richness, endemism, and expert opinion (Myers et al. 2000; Ceballos & Ehrlich 2006). More recently, hotspots have been identified based on complementarity of species richness and endemism (Küper et al. 2004). By incorporating phylogenetic information into conservation decisions, we should be able to more accurately identify regions of unusually high diversity and evolutionary distinctness (Krupnick & Kress 2003; Isaac et al. 2007; Winter et al. 2013).

Southern Africa harbours an enormous variety of species and a considerable number of them are endemic to the region (Van Wyk & Smith 2001a). Some areas of endemic importance are the Cape Floristic Region (CFR), the Cape Peninsula region, the Drakensberg Alpine Centre (DAC), Maputaland, Pondoland and Albany hotspots (Van Wyk & Smith 2001a; Carbutt & Edwards 2006; Perera et al. 2011). These areas of endemic importance contain many endemic species owing to various factors. One of the reasons for this high level of endemism in southern Africa is its relatively stable climatic history (Dynesius & Jansson 2000; Padayachee & Procheș 2016). Southern African taxa show a rich history of evolutionary information and can reveal mechanisms underlying present day biodiversity patterns (Tankard et al. 2012).

The rich diversity and endemism of southern Africa needs to be adequately conserved to avoid loss of important taxa. This requires that biodiversity assessments be accurately conducted, thereby putting emphasis on the biodiversity metrics that will be used in the assessments. Even though phylogenetic metrics have been suggested to be the most accurate metrics (Diniz-Filho et al. 2013; González-Orozco et al. 2015), they are relatively recently
developed and should be assessed for effectiveness in identifying areas for conservation. There is also need for more studies that compare the effectiveness of these phylogenetic metrics as compared to traditional ones. Areas of neo- and palaeo-endemism are also poorly studied in southern Africa and more studies are required in this area of conservation ecology.

Therefore, this study incorporates the use of the measures of phylogenetic diversity and endemism as well as a new approach that differentiates between areas of neo- and palaeo-endemism as proposed by Mishler et al. (2014). This study thus provides valuable information on whether incorporating phylogenetics into biodiversity metrics reveals more information on the biodiversity and endemism of an area. It also provides information on whether areas of high species diversity overlap with areas of high phylogenetic variability. Results from this study will aid in assessments of whether we are effectively conserving important areas of diversity and endemism in southern Africa.

The aim of this study is to assess the value of biodiversity indices that incorporate phylogeny in identifying hotspots of diversity and endemism that should be prioritised in conservation efforts in southern Africa as well as to identify areas of neo- and palaeo-endemism using the dwarf chameleon genus, Bradypodion and the everlasting daisy genus, Helichrysum. These two taxa were selected because the difference in their size and extent of distribution provides an opportunity for assessing the effects of genus size and distribution on biodiversity studies.

To achieve this aim, the objectives are to:

1. Calculate measures of diversity for each genus with and without incorporating phylogenetic information.
2. Compare hotspots inferred by phylogenetic diversity and/or endemism measures to those inferred by traditional biodiversity measures.
3. Identify areas of neo- and palaeo-endemism using CANAPE for each genus.
4. Compare the identified centres of neo- and palaeo-endemism and discuss the implications for conservation in southern Africa.
Method

Study region

This study focuses on southern Africa, a region incorporating all the countries in the southernmost part of Africa. It includes the countries south of the Kunene, Zambezi and Limpopo Rivers (Goldblatt 1978; Werger 1978): Botswana, Lesotho, Namibia, South Africa and Swaziland (Goldblatt 1978). Southern Africa covers an area of ca. 2.5 million km$^2$ (Hilton-Taylor 1996) and is bordered by the Indian Ocean and the Atlantic Ocean to the east and the west, respectively (McKenna 2010).

![Map of southern Africa](http://goo.gl/MScrvB)

Figure 1. Map of southern Africa highlighting the five southern African countries included in this study (http://goo.gl/MScrvB).

Southern Africa is home to a great diversity of plants, with about 24 000 species of vascular plants (Goldblatt 1978; Linder & Hardy 2004; Raimondo et al. 2009; Charters 2010). More than 60% of these plants are endemic to the region making it one of biological importance (Davis 1994; Van Wyk & Smith 2001b). Nested in southern Africa are three of Africa’s nine biodiversity hotspots: the Cape Floristic region, Maputaland-Pondoland and the Succulent Karoo (Van Wyk & Smith 2001a). These hotspots are areas of high species richness and endemism and have been identified as important areas for conservation (Myers et al. 2000).
The southern African region is not only biodiverse in floral terms, but it is also home to a wide variety of mammals, birds, reptiles and amphibians (Jürgens et al. 2010). For example, southern Africa has the richest reptile diversity in Africa exceeding that of North America and Western Europe combined (Alexander & Marais 2007). There are about 500 reptile species recorded in southern Africa with 250 of them being lizard species (Branch 2001; Alexander & Marais 2007; Bates et al. 2014). Over 75% of this reptile fauna occurring in the region is endemic. The richness of plants and animals in southern Africa provides an ideal opportunity to study diversity measures and their value for recognising areas for conservation.

Study taxa

In this study, one reptile genus and one plant genus are used to evaluate the incorporation of phylogeny in biodiversity indices for the identification of hotspots of diversity and endemism; viz., a small, endemic reptile genus of dwarf chameleons, Bradypodion (Chamaeleonidae) and the large, widespread everlasting daisy genus Helichrysum (Asteraceae, Gnaphalieae). Bradypodion was chosen due to its endemicty to southern Africa, while Helichrysum is widespread but also with many species endemic to the region. The two genera also differ in terms of size; Bradypodion is a small genus (17 species), while the very large genus Helichrysum includes approximately 245 species in southern Africa. The difference in size and extent of distribution of the two genera provides an opportunity for assessing the effects of genus size and distribution on biodiversity studies.

Bradypodion, commonly known as the dwarf chameleon, is endemic to southern Africa (Branch 2001). Bradypodion comprises 17 currently recognised species, with one species (B. setaroi) occurring in both South Africa and Mozambique (Tolley et al. 2004b; 2006). Eight species were currently discovered as distinct and are yet to be described (K. Tolley pers. comm.). Dwarf chameleons are widespread in all biomes except for the Karoo, but are mainly found in the moister eastern regions of southern Africa (Tolley et al. 2006). They occur in a wide range of habitats including montane and lowland rainforest, grasslands and shrubby thicket (Branch 2001). These dwarf chameleons are easily distinguishable from the common chameleons due to their small size, usually <15 cm in total length while other chameleons range between 20 and 30 cm. Also, the presence of a gular crest of enlarged scales is a unique distinguishing character of this genus (Figure 2B). All dwarf chameleons
are viviparous (give birth to live young) and have between 5 and 15 young per litter (Bates et al. 2014).

*Helichrysum* comprises ~600 species worldwide with c. 245 species found in southern Africa (Hilliard 1983; Germishuizen 2006; Galbany-Casals et al. 2009; Galbany-Casals et al. 2014). These plants can be annual, biennial or perennial herbs or shrubs with variously shaped leaves and discoid capitula (Figure 2A) with papery involucral bracts (Hilliard 1983). Many are used for medicinal, food, ornamental and/or spiritual purposes (Tadesse & Reilly 1995). *Helichrysum* species occur mainly in the forest, grassland, and fynbos biomes of southern Africa (Hilliard 1983). Due to the widespread nature of this genus yet with some highly endemic species within the study region, it is an appropriate study taxon.

![Figure 2](http://plantinfo.co.za/wp-content/uploads/2015/11/1373995778_helichrysum-bracteatum.jpg)

**Figure 2.** Samples of species of the study genera showing in A. the discoid capitula of *Helichrysum bracteatum* (http://plantinfo.co.za/wp-content/uploads/2015/11/1373995778_helichrysum-bracteatum.jpg) and B. the gular crest of enlarged scales as shown on an individual of *Bradypodion transvaalense* (Photo credit: V. Mounier).

**Objective 1: Calculation of diversity measures with and without phylogenies**

**Species metrics**

Distribution data collection and processing

*Bradypodion* distribution data was obtained from the Animal Demography Unit (ADU) and the Global Biodiversity Information Facility (GBIF), which provide open, free access to biodiversity data. Pretoria Computerised Information System (PRECIS) distribution data (Gibbs Russell 1985) for *Helichrysum* was obtained from the South African National Biodiversity Institute (SANBI) and from the Namibian National Botanical Research Institute.
The PRECIS data are a compilation of about 20,933 records from various national herbaria [viz. Kirstenbosch (NBG), Pretoria (PRE), Durban (NH) and the South African Museum (SAM)]. Additional *Helichrysum* data were obtained from the C.E. Moss Herbarium, University of the Witwatersrand, Johannesburg (J).

Data downloaded from online databases were verified for accuracy of the locality information. To do this, localities obtained from the database were projected in ArcMap 10.1 (ArcGIS 2012), creating species distribution maps for each species. These maps were then used to check for congruence between the locality data and published *Bradypodion* and *Helichrysum* distribution maps and habitat information for each species. *Bradypodion* distribution maps were obtained from Branch (2001) as well as the Reptile Atlas of southern Africa (Bates *et al.* 2014), while *Helichrysum* distribution maps and information were obtained from Hilliard (1983). Localities that did not coincide with the published maps and habitat information were excluded from the dataset.

Diversity indices

Using the distribution data of both *Helichrysum* and *Bradypodion*, diversity indices were separately calculated for each genus using the programme Biodiverse v 1.1 (Laffan *et al.* 2010). Biodiverse links visualisations of data distribution in geographic and phylogenetic spaces and allows randomisations for hypothesis testing (Laffan *et al.* 2010). Both species richness (SR) and weighted endemism (WE) were calculated for each genus. Species richness is defined as the total number of species in a quarter degree square (QDS) grid (25 x 25 km), and weighted endemism as species richness inversely weighted by the species ranges (Crisp *et al.* 2001). The results were then exported as float grids into ArcMap 10.1 (ArcGIS 2012) to generate the desired maps.

**Phylogenetic metrics**

Data collection and processing

Newly generated phylogenies were used for this study. DNA sequence data for 54 specimens (25 species) of *Bradypodion* were obtained from K. Tolley for two regions, and Labord’s chameleon *Furcifer labordi* and the Kilimanjaro Blade-horned Chameleon *Kinyongia tavetana* were used to root the tree (listed in Appendix A). The data matrix comprised 1404 base pairs – and comprised two mitochondrial markers: 16S and the NADH-
dehydrogenase subunit 2 (ND2). It was analysed using the Bayesian Markov chain Monte Carlo (MCMC) method in Mr Bayes v. 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) performed on a partitioned combined dataset. The model GTR + I + G was applied as per Tolley et al. (2006). The analyses were run for 10 million generations, sampling every 1000 generations. Two independent runs of four chains (two heated, two cold) were performed and 25% of the trees were discarded as burn-in prior to calculating the posterior probabilities. Log files were analysed in Tracer v 1.5.0 (Rambaut & Drummond 2007) for convergence assessment. The majority rule consensus tree produced was then compared to those from previous studies (Tilbury et al. 2006; Tolley et al. 2006) to ensure that the branching patterns were the same and imported into Mesquite v3.1 (Maddison & Maddison 2001) for analysis using Biodiverse.

For the Helichrysum phylogeny, DNA sequences were obtained from a variety of sources with contributions from Mercé Galbany-Casals (University of Barcelona, Spain), Glynis Cron (University of the Witwatersrand, Johannesburg), Nicola Bergh (NBG, Kirstenbosch), and from Santiago Andrés Sánchez (University of Salamanca, Spain). The data include newly generated sequences as well as sequences already published on GenBank (listed in the Appendix B). The DNA sequence alignment comprised 187 taxa, including 181 species of Helichrysum (five species had more than one subspecies or variety). Eleven placeholder species from outside of southern Africa were included to represent clades as per Galbany-Casals et al. (2014). These placeholders were deleted in Mesquite after the phylogenetic analysis. In total, 170 of the 245 species in South Africa (70%) were included in the analysis.

Two nuclear regions were combined: the external transcribed spacer region (ETS) and the internal transcribed spacer region (ITS) comprising 735 and 765 base pairs, respectively, including gaps in the alignment. There was some missing data due to different sets of primers being used for the ETS region: primers AST1 and 18S-ETS (Markos & Baldwin 2001) were used by Cron – generating sequences of ca. 500 bp, whereas the other sequences were generated with primers ETS1f (Linder et al. 2000) and 18S-ETS, generating a longer sequence – of which only 735 bases were used in this alignment to reduce the amount of missing data. In addition, 14 species were missing one of the regions (seven missing ETS, seven missing ITS) due to inability to amplify these regions.
A Bayesian inference analysis was then undertaken in Mr. Bayes v. 3.2.6 on the CIPRES Science Gateway (www.phylo.org) on the combined nuclear dataset, applying the model GTR + I + G as determined in jModelTest v.0.1.1 (Posada 2008) and employing Akaike Information Criterion (Akaike 1974). Analyses were run for five million generations, sampling every 1000 generations. Two independent runs of four chains (three heated, one cold) were performed with 25% of the trees discarded as burn-in prior to calculating the posterior probabilities. Log files were analysed in Tracer v. 1.5.0 to assess the level of convergence and ensure that the optimal tree was obtained. The majority rule consensus tree file produced was imported into Mesquite v 3.1 (Maddison & Maddison 2001) for analysis using Biodiverse.

Diversity indices

Using the phylogenies and mapped distribution data of both *Bradypodion* and *Helichrysum*, phylogenetic diversity (PD) and phylogenetic endemism (PE) indices were calculated for each genus using the software Biodiverse v. 1.1 (Laffan et al. 2010). A phylogeny is generated based on the taxa in the dataset and then Biodiverse v. 1.1 (Laffan et al. 2010) uses the locality data of the taxa and the generated phylogeny to calculate PD and PE of the taxa and outputs a mapped representation of the metrics. Phylogenetic diversity measures shared phylogenetic history amongst the taxa in a sample (Faith 1992) and phylogenetic endemism incorporates the spatial range of the phylogenetic branch lengths down to the root of the phylogeny (Rosauer et al. 2009). Methodology on how to use Biodiverse has been included in Appendix C.

Values of phylogenetic and traditional species metrics were outputted by Biodiverse v. 1.1 (Laffan et al. 2010) including the maximum and minimum values, thereby giving a range. After calculating the diversity indices and generating maps of species richness, weighted endemism, phylogenetic diversity and phylogenetic endemism hotspots were identified for each genus. Hotspots were defined as the top 2.5% of the grid cells with high values of the indices on each category map. This threshold has been used in other studies and with other taxonomic groups and has shown to be sufficient for delineating hotspots (Myers et al. 2000; Orme et al. 2005; Ceballos & Ehrlich 2006).
Objective 2: Comparison of effectiveness of phylogenetic measures in identifying hotspots

Grid cells identified as ‘hotspots’ using phylogenetic measures phylogenetic diversity (PD) and phylogenetic endemism (PE) were compared to the ‘hotspots’ inferred by the traditional metrics species richness (SR) and weighted endemism (WE). The hotspots were projected onto a map in ArcMap 10.1 (ArcGIS 2012) and the overlap between them was assessed. The overlap between the hotspots was assessed by checking if the hotspots inferred by traditional metrics and those inferred by phylogenetic metrics fell within the same grid cells. Where both hotspots shared the same grid cells it was considered an overlap.

Objective 3: Identification of areas of neo- and palaeo-endemism using CANAPE

Phylogenetic diversity (PD) and phylogenetic endemism (PE) raw values are not very helpful in differentiating between types of endemic areas, and there is need for a null model. Following Mishler et al. (2014), two derived metrics were calculated for each grid cell using Biodiverse (Laffan et al. 2010), viz. relative phylogenetic diversity (RPD) and relative phylogenetic endemism (RPE). RPD is the PD measured on the actual tree divided by the PD measured on the comparison tree while RPE is the PE measured on the actual tree divided by the PE measured on the comparison tree. To obtain a comparison tree, 999 replicates of random taxon assignment to grid cells were generated without replacement in the programme Biodiverse (Laffan et al. 2010). RPD and RPE were then used to compare the PD and PE observed on the actual tree in the numerator to that of a comparison tree in the denominator.

A randomisation test of the diversity indices was then conducted in Biodiverse (Laffan et al. 2010) to identify grid cells that show values significantly different from that expected from a random assemblage of the same number of taxa. The diversity values obtained from the randomisation test formed the null distribution that was used to compare the distribution of values from the actual diversity indices.

To identify hotspots of neo- and palaeo-endemism, a two-step procedure called Categorical Analysis of Neo- and Palaeo-Endemism (CANAPE) (Mishler et al. 2014) was used for both genus. First, in order to establish that a grid cell is a hotspot of endemism, all cells that did not show significantly high values for either the numerator or denominator of RPE were excluded (one tailed, $\alpha = 0.05$). From the grid cells remaining, the statistical significance of RPE was examined by creating a normal distribution of the grid cells using
the p-value. Grid cells were classified as hotspots of neo-endemism if they fell into the 2.5% lower tail, indicating significantly young rare clades or as hotspots of palaeo-endemism if they fell into the 2.5% higher tail, indicating significantly old rare clades, and of mixed endemism if they fell in the middle of the distribution.

**Objective 4: Comparison of the identified centres of endemism and implications for conservation**

The identified centres of statistically significant centres of endemism were compared using phylogenetic diversity dissimilarity, as described by Mishler *et al.* (2014). This analysis was implemented using the ‘phylo_Jaccard’ measure, which uses a weighted average linkage from cluster analysis in the program Biodiverse (Mishler *et al.* 2014).

Additionally, the hotspots identified using various indices were assessed for representation in protected areas. The hotspots were overlaid on a GIS layer of protected areas in southern Africa and the proportion of hotspots in protected areas visually assessed. The GIS layer was acquired from the UNEP World Conservation Monitoring Centre (UNEP-WCMC 2012); this database is a GIS inventory of all protected and conservation areas in the world. These data provide an indication as to whether we are sufficiently protecting the hotspots inferred by the two genera, *Helichrysum* and *Bradypodion*. The data also enables us to assess whether the outputs from PE and PD analyses are equally reliable for both the small reptile genus dataset and large plant genus dataset and whether information from either one or both of these genera can be used generally for other reptiles and plants, respectively.

**Results**

*Bradypodion*

The final locality data set comprised 492 records after data cleaning and the removal of inaccurate records. These records represent 25 species found in southern Africa, with 17 described species and eight recently discovered ones. *Bradypodion* species occur in all of the southern African countries included in the study with the exception of Botswana (Figure 3). Most of the species are found only in South Africa, with only three found in the other countries. Namibia has two species, *B. pumilum* and *B. ventrale*, co-occurring on the western coast near Walvis Bay (Figure 3). In Lesotho, one species *B. dracomontanum* is located right by the border with South Africa in the Drakensberg mountains (Figure 3).
Figure 3. Distribution map of the dwarf chameleon genus *Bradypodion* with localities for all 25 species in southern Africa shown.

The South African species are mainly restricted to the eastern and northern regions of the country and most of these species are coastal or associated with the Great Drakensberg Escarpment which extends from the Eastern Cape along the Lesotho- KwaZulu-Natal border into Mpumalanga (Figure 3). The two most speciose provinces in South Africa are the Eastern Cape and KwaZulu-Natal with nine species each (Figure 3). The Western Cape Province contains six species with a concentration of *B. pumilum* in the Cape Peninsula (Figure 3). Only one species occurs in the Free State Province, *B. dracomontanum*, on the border with Lesotho (Figure 3). Similarly the Northern Cape Province has only one species, *B. occidentale*, occurring by the coast, north of Port Nolloth (Figure 3). Gauteng and North West Province have no recorded presence of *Bradypodion*.

In the Eastern Cape Province, the *Bradypodion* species are concentrated mainly along the coast with a few species inland. A number of species co-occur in KwaZulu-Natal (KZN)
with many of them concentrated in the Drakensberg Region and KZN Midlands (Figure 3). *Bradypodion transvaalense* is represented by the most collections and is the most widespread species, found only in the Limpopo and Mpumalanga provinces of South Africa (Figure 3). *Bradypodion transvaalense* is found in the mountains and grasslands of Sekhukhuneland as well as the Wolkberg.

*Bradypodion* phylogeny

A resolved Bayesian consensus tree was generated for *Bradypodion* (Figure 4). The phylogeny has two main clades diverging from the base, the smaller (clade A) of the two clades contains five species mainly from the Western Cape and the larger clade (clade B) is further divided into two clades (Figure 4). These two clades contain nine and eleven species each.

![Figure 4](image)

Figure 4. The Bayesian majority consensus tree for the dwarf chameleon genus *Bradypodion* with two outgroups *Kinyongia tavetana* and *Furcifer labordi*. Species in clade X, located in the Eastern Cape Province of South Africa are shown in the red box and species in clade Y, mostly located in the KwaZulu-Natal Province of South Africa are shown in the blue box.
There is some geographic structure to the phylogeny with parts of two clades containing species found in the same geographic location. Clade X contains species mainly located in the Eastern Cape Province of South Africa near East London and Queenstown (Figure 4, red box). Clade Y includes species mostly located in the KwaZulu-Natal Province in the Drakensberg region and near St Lucia and Richards Bay (Figure 4, blue box).

Species metrics

Species richness for *Bradypodion*, as calculated using Biodiverse v1.1 (Laffan et al. 2010), was found to be highest in the Knysna District of the Western Cape where three *Bradypodion* species are found in the area (Figure 5). At Walvis Bay in Namibia as well as some localities in Eastern Cape and KwaZulu-Natal there were species richness scores of two, showing that there are two species of *Bradypodion* found in the area. In most parts of southern Africa there was only one species per QDS (quarter degree square: 25 x 25 km) and thus a low species richness (Figure 5).

**Figure 5.** Map showing the species richness for the dwarf chameleon genus *Bradypodion* in southern Africa.
Weighted endemism was more variable across the region with the highest endemism (2) in KwaZulu-Natal, South Africa and the second highest in the Knysna district of the same country (Figure 6). Areas with medium endemism scores of between 0.1 and 1.2 are scattered in the Eastern Cape and KwaZulu-Natal Midlands (Figure 6). The rest of the region has relatively low mean endemism (WE) with scores of below 0.05. Namibia, Lesotho and Swaziland had relatively low endemism scores with scores ranging between 0.04 and 0.1 (Figure 6).

**Figure 6.** Map showing weighted endemism for the dwarf chameleon genus *Bradypodion* in southern Africa.

**Phylogenetic metrics**

Phylogenetic diversity (PD) and endemism (PE) were also calculated for *Bradypodion* using Biodiverse v 1.1 (Laffan *et al.* 2010). From the analysis it can be seen that areas with
the highest PD are located in the Knysna area of South Africa and Walvis Bay, Namibia (Figure 7). The Drakensberg region and the area near Richards Bay in KwaZulu-Natal had medium PD values ranging between 0.3 and 0.35 (Figure 7). Areas with low levels (PD lower than 0.3), are scattered across South Africa with a concentration in the Western Cape, in the Cape Peninsula and near Saldanha Bay (Figure 7).

**Figure 7.** Map showing phylogenetic diversity for the dwarf chameleon genus *Bradydion* in southern Africa.

In contrast, PE was highest in KwaZulu-Natal in the Nyembe area as well as in the Eastern Cape, with centres of high PE near Port St Johns and in the Entumeni Nature Reserve (Figure 8). The Cape Floristic Region as well as the Maputaland Centre of diversity located in northern KwaZulu-Natal between Swaziland and the coast contain medium PE with values ranging between 0.01 and 0.06 (Figure 8). The rest of the southern African region had low PE with values lower than 0.06.
Figure 8. Map showing phylogenetic endemism for the dwarf chameleon genus *Bradypodion* in southern Africa.

*Comparison of the hotspots inferred by the different metrics and their representation in protected areas*

Hotspots of SR, WE, PE and PD were determined by selecting the top 2.5% cells in each category. There are a total of 84 quarter degree grid cells containing *Bradypodion* species and of these cells, only two with the highest values of SR, WE, PD and PE were selected as hotspots. The hotspot inferred by the SR index is located in the Knysna area in the Goukamma Nature Reserve where three species co-occur (Figure 9), whereas the one inferred by the WE index is located in the Entumeni Nature Reserve close to Stanger on the north coast of KwaZulu-Natal (Figure 9).
Figure 9. Protected areas of southern Africa with the species richness and weighted endemism hotspots (top 2.5% of grid cells) for *Bradypodion* indicated.

The species richness hotspot of *Bradypodion* in the Knysna district of South Africa falls within the Goukamma Nature Reserve. This provincial nature reserve managed by Cape Nature encompasses 2 500 hectares of dense coastal forest (Figure 9). The weighted endemism hotspot on the north coast of KwaZulu-Natal is partially protected with a portion of the hotspot not falling within a protected area (Figure 9). There are however, three protected areas that coincide with the hotspot: the large (4000 ha) Ngoya Forest Reserve, the small (750 ha) Entumeni Nature Reserve and the very small (187 ha) Hlinza Forest Nature Reserve – all these reserves are managed by the KwaZulu-Natal Nature Conservation Board.

The hotspot inferred by the PD metric is located in the Knysna area as well as at Walvis Bay in the Erongo province of Namibia (Figure 10). The PE metric revealed a hotspot in the Entumeni Nature Reserve close to Stanger in the north coast of KwaZulu-Natal (Figure 10). *Bradypodion pumilum* located at Walvis Bay is situated at the end of a long branch on
the phylogeny whilst the species found in the Knysna district are situated at the end of short branches.

The PD hotspot in Knysna falls within the Goukamma Nature Reserve along with the SR hotspot (Figure 10), whereas in Namibia the hotspot falls within two protected areas: the Namib-Naukluft National Park and the Dorob National Park (Figure 10). The Namib-Naukluft National Park encompasses part of the Namib Desert as well as the Naukluft mountain range while Dorob National Park extends from south of Walvis Bay to the Ugab River in the north. The phylogenetic endemism hotspot falls within the same protected areas as the WE hotspot in KwaZulu-Natal, South Africa (Figure 10).

**Figure 10.** Protected areas of southern Africa with the phylogenetic diversity and phylogenetic endemism hotspots (top 2.5% of grid cells) for *Bradypodion* indicated.

The hotspots inferred by the SR and PD metrics overlap in the same area, viz. in the Goukamma Nature Reserve of Knysna. However, the PD hotspot in Namibia did not overlap
with the SR hotspot. These two metrics show high levels of overlap in southern Africa which suggests that the two metrics can be used interchangeably with PD giving more information as compared to SR. The hotspots inferred by the WE and PE metrics also overlap in the Entumeni Nature Reserve near Eshowe, KwaZulu-Natal. Consequently there is high overlap between hotspots inferred by traditional metrics as compared to the hotspots inferred by phylogenetic metrics.

**CANAPE (Categorical Analysis of Neo- and Palaeo-Endemism) analysis**

![Map showing areas of neo- and palaeo-endemism](image)

**Figure 11.** Map showing the areas of neo- and palaeo-endemism in the dwarf chameleon genus, *Bradypodion* in southern Africa. The white areas contain no records; grey cells are not significant. The red cells indicate areas that contain significantly lower RPE than expected from the random sampling of a null tree, called ‘centres of neo-endemism’. The purple values indicate grid cells that are a mix of neo- and palaeo-endemism.

From the CANAPE analysis, nine grid cells of significantly high endemism were identified. The analysis inferred hotspots of neo-endemism in one area, in the central
Drakensberg region – the mountain range that forms the border between KwaZulu-Natal (South Africa) and Lesotho (Figure 11). There were no hotspots of palaeo-endemism discovered in southern Africa for Bradypodion. However, there were scattered hotspots of mixed endemism inferred on the eastern parts of South Africa (Figure 11). These areas of mixed endemism contain both neo- and palaeo-endemics. Grid cells of mixed endemism are mainly scattered along the eastern coastline with two grid cells slightly inland in the Eastern Cape and one in northern KwaZulu-Natal (Figure 11). Some areas of mixed endemism are located in the mountains of the Ngome Forest Nature Reserve, Entumeni Nature Reserve, forests of Umzimvubu and in the mountainous Baviaanskloof Nature Reserve (Figure 11).

Comparisons of areas of endemism (PD-dissimilarity)

The cluster analysis conducted using PD-dissimilarity did not reveal any organised clustering due to the small number of cells with significantly high endemism (Figure 12).

**Figure 12.** Map and cluster analysis showing phylogenetic similarity relationships among centres of endemism for Bradypodion. The cluster analysis used PD-dissimilarity and a phylo-jaccard metric with weighted link-average linkage. Areas that cluster closely, indicating that they share many branches of their phylogenetic subtrees, are shown in the same colour.
Helichrysum

Helichrysum distribution

The final dataset comprised of 20 324 records representing 245 species of Helichrysum species found in southern Africa. A total of 1 613 records were excluded from the dataset, as these records comprised localities outside of the study area or had no localities provided.

Helichrysum species were found to occur across the whole study area with a concentration in South Africa (Figure 13). Botswana had the least recorded occurrences of species with a few scattered around the country in a random manner. In Namibia, species are mainly located towards the western side of the country (Figure 13). Helichrysum species occur all through Lesotho and Swaziland with the exception of a few patches of the countries (Figure 13).

Figure 13. Distribution map of Helichrysum (Asteraceae, Gnaphalieae) showing the occurrence of 245 species in southern Africa.
Similar to *Bradypodion*, *Helichrysum* species’ occurrences are concentrated in South Africa (Figure 13). However, unlike *Bradypodion*, *Helichrysum* species are found in every province and not only by the coast and along the Great Escarpment (Figure 13). There are slightly more species occurring in the east of the country, especially in KwaZulu-Natal, Mpumalanga and Eastern Cape Provinces. In the Northern Cape Province, species distribution is patchy (Figure 13).

*Helichrysum* phylogeny

The *Helichrysum* consensus tree, rooted using *Filago pyramidata*, was not fully resolved due to missing data and/or lack of phylogenetic signal. The phylogenetic tree is shown in Appendix D. *Helichrysum daysanthum* branches off the phylogeny quite early and forms its own clade. All the other species fall into one major clade that subdivides into six clades. These clades contain between three and twenty species in each of them. There is however no clearly defined geographic structure possibly due to the incomplete phylogeny.

Species metrics

![Map showing the species richness for Helichrysum (Asteraceae, Gnaphalieae) in southern Africa.](image)

**Figure 14.** Map showing the species richness for *Helichrysum* (Asteraceae, Gnaphalieae) in southern Africa.
The same metrics (species richness and weighted endemism) were calculated for *Helichrysum* as was done for *Bradypodion* using the software Biodiverse v 1.1 (Laffan *et al.* 2010).

*Helichrysum* species richness peaks in the mountains and grasslands of Sekhukhuneland and the Wolkberg in Mpumalanga and Limpopo, as well as along the Greater Drakensberg escarpment in Mpumalanga and KwaZulu-Natal (Figure 14). Notably, the highest species richness (110 species) found in one area is in the Central Drakensberg escarpment by the border between Lesotho and KwaZulu-Natal, as well as in the mountains separating Limpopo and Mpumalanga Province (Figure 14). Other areas with medium species richness (between 50 and 80 species) are found in Gauteng, the Barberton area in the Makhonjwa Mountains, the Albany area in the Eastern Cape, and the Cape Floristic Region (Figure 14). All the other provinces and neighbouring countries have relatively low species richness (less than 40 species).

**Figure 15.** Map showing weighted endemism for *Helichrysum* (Asteraceae, Gnaphalieae) in southern Africa.
Weighted endemism follows a similar distribution to that of species richness in that it peaks in the grasslands of Sekhukhuneland and in the Drakensberg Alpine Centre (Figure 15). The Albany area, the Wolkberg as well as the Greater Drakensberg mountain range have medium weighted endemism with values ranging between ten and six (Figure 15). The rest of the study area has relatively low *Helichrysum* weighted endemism.

**Phylogenetic metrics**

As with the other metrics, centres of high PD were mainly in South Africa. Areas with the highest PD are centred in the Drakensberg Alpine Centre of endemism, the grasslands of Sekhukhuneland, some areas around Johannesburg, as well as the Magaliesberg in the North West Province (Figure 16). Moderate PD was found to be occur in the Barberton centre of endemism and the Wolkberg as well as in KwaZulu-Natal Midlands (Figure 16). Other areas with moderate PD are parts of the Eastern and Western Cape Provinces, as well as sections of Namibia, with values ranging between 0.9 and 0.4.

**Figure 16.** Map showing phylogenetic diversity for *Helichrysum* (Asteraceae, Gnaphalieae) in southern Africa.
In contrast, PE was highest in two places, the Northern Drakensberg escarpment and the grasslands of Sekhukhuneland (Figure 17). The Wolkberg and Magaliesberg areas had moderate PE with values ranging between 0.1 and 0.05 (Figure 17). The rest of the region had relatively low PE with values less than 0.02.

**Figure 17.** Map showing phylogenetic endemism for *Helichrysum* (Asteraceae, Gnaphalieae) in southern Africa.

Comparison of the hotspots inferred by the different metrics and representation in protected areas

Hotspots of SR and WE were determined by selecting the top 2.5% cells in each category. Of the 1,670 quarter degree grid cells containing *Helichrysum* species, only 42 with the highest values of SR and WE were selected as hotspots. The hotspots inferred by the species richness and weighted endemism metrics are located in Sekhukhuneland, the Wolkberg, Barberton area as well as in the Drakensberg Alpine Centre (Figure 18 and 19).
However, the weighted endemism metric reveals an additional hotspot area in the Rustenburg district, North West Province (Figure 19).

The species richness and weighted endemism hotspots of *Helichrysum* fall within five protected areas. These are the Songimvelo, Barbeton, Makobulaan, Kudu Nature Reserves, and the Kruger to Canyons Biosphere Reserve in the Limpopo and Mpumalanga Provinces (Figure 18 and 19). The *Helichrysum* hotspot located in the Drakensberg Alpine Centre includes the Sehlabathebe National Park, a part of the Maloti-Drakensberg World Heritage Site, and falls within the Ukhahlamba-Drakensberg Park in Lesotho and South Africa (Figures 18 and 19).

**Figure 18.** Protected areas of southern Africa with the species richness hotspots (top 2.5% of grid cells) for *Helichrysum* (Asteraceae, Gnaphalieae) indicated.

Weighted endemism reveals an additional hotspot in the Rustenburg district and this hotspot falls within the Magaliesberg Biosphere Reserve (Figure 19). The hotspots are mainly found in protected areas but there are a few hotspots that are unprotected such as the ones
inferred by weighted endemism in the KwaZulu-Natal Province (Figure 19). These areas do not fall within a protected area but are in close proximity of other nature reserves.

Figure 19. Protected areas of southern Africa with the weighted endemism hotspots (top 2.5% of grid cells) for *Helichrysum* (Asteraceae, Gnaphalieae) indicated.

The hotspots inferred by the phylogenetic diversity metric are located in the grasslands of Sekhukhuneland, the Wolkberg, Drakensberg Alpine Centre, grasslands of Johannesburg, Magaliesberg, as well as in the Tugela and Stanger areas in the KwaZulu-Natal Province (Figure 20). Phylogenetic endemism (PE) infers hotspots in the same areas as phylogenetic diversity, with the exception of the Tugela area and an addition of another hotspot in the Cradock area of the Eastern Cape Province (Figure 21). PE also infers another hotspot in the Western Cape Province in the Cederberg Mountain Catchment Area (Figure 21).
Figure 20. Protected areas of southern Africa with the phylogenetic diversity hotspots (top 2.5% of grid cells) for Helichrysum (Asteraceae, Gnaphalieae) indicated.

The PD and PE hotspots of Helichrysum are fairly well protected in southern Africa and fall within five protected areas. These are: the Magaliesberg Biosphere Reserve, De Onderstepoort Nature Reserve, Songimvelo Nature Reserve, Barbeton Nature Reserve, Vhembe Biosphere Reserve as well as the Kruger to Canyons Biosphere Reserve in the Limpopo and Mpumalanga Provinces (Figure 20). The Helichrysum hotspot located in the Drakensberg Alpine Centre falls within the Ukhahlamba-Drakensberg Park (World heritage site) in Lesotho and South Africa and also in the Sehlabatebhe National Park (Figure 20).

The additional two hotspots inferred by PE in the Cradock area (Eastern Cape Province) and in the Cederberg area (Western Cape Province) are partially located in protected areas (Figure 21). The Cradock hotspot is partially (25%) located in the Mountain
Zebra National Park while the Cederberg hotspot is completely located in the Koue Bokkeveld Mountain Catchment Area (Figure 21).

Figure 21. Protected areas of southern Africa with the PE hotspots (top 2.5% of grid cells) for *Helichrysum* (Asteraceae, Gnaphalieae) indicated.

The hotspots inferred by the SR and PD metrics overlap in the same area in the Northern Drakensberg escarpment, Barberton area, Wolkberg, and the Sekhukhuneland grasslands. There are some hotspot areas inferred by PD that did not overlap with hotspots of SR, such as the Tugela area, Magaliesberg, and the Johannesburg hotspots. There are high levels of overlap between the hotspots inferred by the two metrics in southern Africa. Hotspots inferred by the WE and PE metrics also overlap in the northern Drakensberg Alpine Centre, Stanger, Magaliesberg, Barberton area, Wolkberg and the Sekhukhuneland forests. The only hotspot areas where SR and PD do not overlap are the Cradock, Cederberg and Johannesburg hotspots. Therefore, results show that there is high overlap between hotspots inferred by traditional metrics as compared to the hotspots inferred by phylogenetic metrics.
CANAPE (Categorical Analysis of Neo- and Palaeo-Endemism) analysis

From the CANAPE analysis, 98 grid cells of significantly high *Helichrysum* endemism were identified with hotspots of both neo- and palaeo-endemism inferred. Hotspots of neo-endemism were found on the border of Free State and Northern Cape as well as on the border between North West and Northern Cape provinces (Figure 22). Other areas found to contain neo-endemics are the Otjiwarongo District in northern Namibia as well as the Grahamstown area in the Eastern Cape (Figure 22). Lesotho and Swaziland contained no grid cells that had significantly high endemism (Figure 22).

*Figure 22.* Map showing the areas of neo- and palaeo-endemism in *Helichrysum* (Asteraceae, Gnaphalieae) in southern Africa. The white areas contain no records; beige cells are not significant. The red cells indicate hotspots of neo-endemism while the blue cells indicate hotspots of palaeo-endemism. The purple values indicate grid cells that are a mix of neo- and palaeo-endemism and the dark purple indicates where a lot of neo- and palaeo-endemics occur in one area.
Hotspots of palaeo-endemism are more widespread than the neo-endemism ones with a concentration in southern Namibia (Figure 22). There are a lot of hotspots of mixed endemism (purple cells) for *Helichrysum* in southern Africa as these are widespread over the region. Northern Namibia has many hotspots of mixed endemism as well as some scattered areas of the great escarpment (Figure 22). Areas that contain a large concentration of both neo- and palaeo-endemics are referred to as hotspots of ‘super endemism.’ In southern Africa there are such areas (dark purple cells) scattered in the region in areas such as the DAC, Wolkberg, grasslands of Sekhukhuneland, Barberton, Magaliesberg and areas in the Western Cape Province (Figure 22).

Comparisons of areas of endemism (PD-dissimilarity)

**Figure 23.** Map and cluster analysis showing phylogenetic similarity relationships among centres of endemism for the everlasting daisy genus, *Helichrysum* (Asteraceae, Gnaphalieae). Areas that cluster closely indicate that they share many branches of their phylogenetic trees and are indicated by the same colour.

The cluster analysis implemented using PD-dissimilarity (Figure 23) revealed that the grid cells cluster geographically to some extent. The north-eastern (red) grid cells and southern (blue) grid cells of southern Africa are grouped together in the dendrogram (Figure 23). Southern and northern Namibia (green and purple) grid cells also cluster together in the analysis (Figure 23). The rest of the grid cells do not reveal any particular geographic
clustering. Interestingly, the greatest diversity of phylo-clusters is present in the eastern and north-eastern regions of southern Africa, as well as south-west Namibia (Figure 23).

**Analysis of representation of endemic hotspots in protected areas**

Assessments of the level of protection of endemic hotspots as inferred using the CANAPE analysis revealed that only one hotspot of neo-endemism is protected in the Otjiuuo Communal Conservancy in Namibia (Figure 24). Eight hotspots of super endemism are fairly well protected in South Africa with the rest not contained within a protected area. Interestingly, the palaeo-endemic hotspots are more protected than all the other hotspots (Figure 24). However, only a few hotspots of mixed endemism are protected in Namibia (Figure 24).

**Figure 24.** Protected areas of southern Africa with the hotspots of endemism as inferred by the CANAPE analysis for *Helichrysum* (Asteraceae, Gnaphalieae) indicated. The grid cells that had insignificant endemism values have been excluded from this figure to allow better representation.
Discussion

Bradypodion distribution

*Bradypodion* is mainly distributed along the eastern side of South Africa along the Great Escarpment. This explains why there were no records of dwarf chameleons found in Botswana, a generally arid country. The mapped distribution indicated that there are two dwarf chameleon species in Namibia, *B. ventrale* and *B. pumilum*, however these two species are known to be restricted to the Eastern Cape and Western Cape Provinces, respectively (Tolley et al. 2006). Bates et al. (2014) provide a possible explanation as to why this would be so; they allude to situations where dwarf chameleon species are introduced to new territories outside of their natural ranges.

Many species were found to be mainly distributed along the south-west and eastern coast, specifically in coastal forests and this is because many dwarf chameleons species are forest dwellers (Tolley et al. 2011; Bates et al. 2014). For example, *Bradypodion kentanicum* and *B. thamnobates* which are both forest dwarf chameleons, are distributed along the Eastern Cape and KwaZulu-Natal coasts respectively (Tolley et al. 2006). Hotspots for dwarf chameleons were reported to be in the KwaZulu-Natal Midlands as well as in the Maputaland biodiversity hotspot (Bates et al. 2014), where eleven dwarf chameleon species were found in this study.

The Cape Peninsula has a high concentration of *Bradypodion pumilum*, a species restricted to the Western Cape, specifically the CFR (Bates et al. 2014). The CFR contains high topographical heterogeneity, varied rainfall patterns as well as a wide variety of nutrient-poor soils thereby providing a high number of habitats and ecological communities (Cowling et al. 1996; Cowling et al. 2009). The concentration of *B. transvaalense* in the Mpumalanga and Limpopo provinces is also interesting. This species is found in the forests patches of the grasslands of Sekhukhuneland as well as the Wolkberg, a region of floristic endemism that was recently included as a hotspot for vertebrates (Perera et al. 2011). The forest patches provide suitable habitat for these forest dwellers (Bates et al. 2014).

Species metrics

Species richness, a metric for assessing the number of species in an area, forms the basis for numerous biological studies and has been utilised in assigning conservation priority
to areas for many decades now. *Bradypodion* species richness was found to be highest in the Knysna area of South Africa where three species are found in an area of 1 250 km². Tolley et al. (2006) note the same *Bradypodion* high species richness in this area. The Knysna district is a temperate coastal climatic area and its montane forests sustain various organisms (Geldenhuys 1991), including a variety of *Bradypodion* species.

Dwarf chameleons are highly dependent on vegetation as their survival is dependent on camouflage and stealth to obtain food, as well as for protection from predators (Tolley et al. 2004a). The Knysna coastal forests provide the much needed dense vegetation as well as a variety of habitats for these chameleons, which explains the high *Bradypodion* species richness in the area (Tolley et al. 2004a; Tolley et al. 2006). The forest has a mosaic of closed canopy habitats, with forest patches and riverine thickets that can be taken advantage of by the chameleons. Much of the Knysna forest occurs on gentle to moderate slopes, ranging from 5 m to 1 220 m above sea level (a.s.l.) with a mean altitude of 240 m a.s.l. (Geldenhuys 1991).

As noted previously, two species, *B. pumilum* and *B. ventrale*, co-occur at Walvis Bay in Namibia. This is mystifying since both these species are reported to be endemic to South Africa, *B. pumilum* is restricted to the Western Cape where its preferred habitat is dense vegetation, fynbos, and renosterveld while the habitat generalist, *B. ventrale* is endemic to the Eastern and Western Cape Provinces (Tolley & Burger 2007). It is highly unlikely this is a natural disjunction – i.e., that these species would have naturally dispersed to Namibia, or that they are relictual – i.e., remaining from a previous, more widespread distribution. It is then more likely that they were introduced to Namibia. The rest of the grid cells in the region with species richness scores of ‘2’ are mainly in coastal forests thereby reflecting the habitat preference of many *Bradypodion* species.

Southern African dwarf chameleons are endemic to the study region (Tolley et al. 2004b; Branch & Tolley 2010; Bates et al. 2014). Weighted endemism was highest in KwaZulu-Natal with the second highest score in Knysna. Areas with high species richness coincided with areas of high weighted endemism. The Knysna area is also home to the Knysna dwarf chameleon, *Bradypodion damaranum*, which is restricted to this region. This region falls into the Cape Floristic Region (CFR) of endemism which could explain the high endemicity (Linder & Hardy 2004). The shifting and fragmentation of the landscape brought
on by climatic fluctuations has been noted as the main reason for the high endemism in the CFR (Midgley et al. 2003; Born et al. 2007) and the same processes are believed to have given rise to the high endemism of the dwarf chameleons (Tolley et al. 2006).

The area with high weighted endemism in KwaZulu-Natal falls within the Entumeni forests. The province has a wide variety of chameleon species including many of Bradypodion (Tilbury & Tolley 2009). Divergences within Bradypodion in the Late Miocene can be linked to the high weighted endemism of the province (Tolley et al. 2008; Tilbury & Tolley 2009). The divergence in this province resulted in various Bradypodion species becoming endemic to the region (Raw 2001; Tolley et al. 2006).

Southern Africa has experienced climatic shifts over the years where periods of warm and wet conditions led to the expansion of forests, while the dry and cool periods caused contraction of forests (Eeley et al. 1999; Lawes et al. 2007). This expansion and contraction of forests led to extinction filtering (Balmford 1996), a scenario where the change in climate and vegetation acts as a filter allowing some species to survive while others go extinct (Eeley et al. 1999; Tilbury & Tolley 2009). These conditions then led to adaptation of species to the various patches of forest in KwaZulu-Natal. The province is also home to the Zululand endemic dwarf chameleon, B. nemorale (Tolley et al. 2004a).

Phylogenetic metrics

Phylogenetic diversity (PD) was also found to be highest in the Knysna area and in Walvis Bay, Namibia. Areas of high PD usually represent long branches and reveal many distantly related taxa (Faith 1992). Species found in areas of high PD have undergone diversification and are very important to conservation as they are platforms for future evolution. Interestingly, the species found in the Knysna area are closely related which confounds the result of high phylogenetic diversity being found in the area. However, the two species at Walvis Bay are distantly related thereby explaining the high PD indicated there. The two species, B. pumilum and B. ventrale are generalists that can survive in a range of habitats (Bates et al. 2014). The high PD at Walvis Bay is not likely to be natural since the species are likely introduced but if it were, the habitat generalist chameleons would be able to co-occur here.
The central Drakensberg and the Entumeni Forests in KwaZulu-Natal had medium levels of PD. This means that there are some distantly related taxa that occur in the same area, as well as some closely related ones, e.g. *B. thamnobates* and *B. melanocephalum* (Tolley *et al.* 2008; Tilbury & Tolley 2009). The radiation of vegetation during the Pleistocene led to the creation of new habitats that the chameleons took advantage of (Tolley *et al.* 2008; Tilbury & Tolley 2009). Extinction filtering (Balmford 1996). The expansion and contraction of forests also allowed for distantly related taxa to co-occur in the area, thereby increasing the PD.

The central Drakensberg likely supports distantly related taxa due to the different niches and habitats characteristic of the area (Carbutt & Edwards 2006). Low PD is associated with short branches on the phylogenetic tree and low levels of diversification (Faith 1992). Therefore, the rest of the region has had low levels of past diversification in *Bradypodion*. This lack of diversification could be due to a barrier to dispersal as well as rarity of migration events. The mountainous regions of the Wolkberg and the Northern Cape most likely created conditions that led to the allopatric speciation of *Bradypodion* thereby giving rise to species in related clades.

Phylogenetic endemism (PE) was highest in the KwaZulu-Natal and Eastern Cape provinces. These are areas where PD is restricted to a specific geographic location Port St Johns and the Entumeni Nature Reserve were found to be centres of high PE. Port St Johns in the Eastern Cape falls within the Pondoland centre of endemism (Van Wyk & Smith 2001a). This centre of endemism contains topography that is characterised by narrow deep gorges where the *Bradypodion* species are found in the afro-temperate forests. This isolation between gorges likely led to the high PE of the area. Tolley *et al.* (2006) note that there was a possible dispersal of *Bradypodion* species from the CFR into the Eastern Cape which then diversified and became endemic thereby adding to its PE.

In KwaZulu-Natal, the two species occurring the area of high PE, *B. nemorale* and *B. caeruleogula* are distantly related. These two species have similar habitat preferences high up in the forest canopies. The high PE of the Entumeni forests could be attributed to the isolation caused by the difference in habitat types. The forest dwellers of this area were isolated from the species that prefer shorter trees and plants closer to the ground, thereby promoting
speciation and high PE. Recent radiations in the area could have also lead to the high PE (Tolley et al. 2008).

*Helichrysum* distribution

Everlasting daisies are widespread in southern Africa and were found to occur in all the countries in the study region. Botswana had the fewest recorded occurrences of *Helichrysum* and this could be due to insufficient collections or records of the genus, or it may be that the aridity and nutrient poor soils of the country cannot support many species (Nicholson & Farrar 1994). The genus is widespread in the rest of the region with a concentration in the Western and Eastern Cape provinces, as well as in the Great Escarpment which runs along the eastern side of the region. The most likely explanation for this pattern is the origin of the genus; it is believed that it originated in the Cape and then dispersed to the afro-montane regions of east southern Africa, mainly the Drakensberg, where it diversified considerably (Galley et al. 2007; Galbany-Casals et al. 2009). After this diversification, it possibly migrated northwards into tropical Africa as is the case in other plant genera (Linder 2003).

Species metrics

The Drakensberg escarpment contains the highest number of *Helichrysum* species with many being endemic to the area. This is not surprising due to the diversification of the genus that is believed to have occurred after dispersal from the Cape (Galley et al. 2007). The diversification can be attributed to the wide variety of ecological niches offered by the DAC (Travers et al. 2014). The central and northern Drakensberg regions are the most diverse – due to the variety of ecological niches here. The escarpment slopes are north-east facing and experience considerably less cold than the southern region with its south-east facing slopes (Hilliard & Burtt 1987). The more northerly regions of the KZN Drakensberg escarpment have pockets of afro-montane forests on south facing slopes and in river gorges where the trees are protected and able to grow (Carbutt & Edwards 2006). The mix of afro-montane and riverine forests and also grasslands in this topographically variable landscape provide a wide range of ecological niches that have allowed *Helichrysum* to diversify (Carbutt & Edwards 2003).
The Sekhukhuneland area mainly characterised by grassland with patches of forest also has high species richness and endemism. The heterogeneous topography of the region offers an explanation for the high species richness (Van Wyk & Smith 2001a). Due to tectonic forces and magma upliftment, the area has a complex topography (Thomas et al. 1993). It consists of flat to undulating valleys as well as rocky ridges and mountains (Siebert et al. 2002). This heterogeneity acts in the same way as in the Drakensberg escarpment to provide a variety of ecological niches that Helichrysum species were able to colonise and in which they have diversified. The Wolkberg – an area that is climatically similar to the Sekhukhuneland, is also an area of high species richness. This area is very mountainous with steep slopes but also has some gorges that form forest pockets (Van Wyk & Smith 2001a). This topographic heterogeneity has allowed Helichrysum to diversify thereby leading to high species richness in the area.

The Western Cape surprisingly has lower Helichrysum species richness than would be expected especially considering that this is where the genus originated. This could be due to a failure by Helichrysum to diversify as extensively in the Cape as in the Drakensberg region – possibly due to competition with other genera (Linder 2003). Helichrysum originated in the Western Cape (Galbany-Casals et al. 2014) and is a component of the fynbos biome. This biome occurs in the Western Cape and extends slightly into the Eastern Cape where the vegetation is characterised by fynbos and renosterveld (Linder & Hardy 2004; Daru et al. 2015). The fynbos is very rich in species and many are endemic to the areas with this type of vegetation (Linder & Hardy 2004).

Interestingly, the Gauteng and Northwest provinces have notable species richness values. Even though these provinces are being developed there are still some remaining patches of natural vegetation. There are three main types of vegetation in these provinces, sourish mixed bushveld, sour bushveld, and the bankenveld which shares floristic affinities with the Wolkberg (Van Wyk & Smith 2001a; Grobler 2006). Helichrysum species could be surviving in these natural patches of vegetation (Grobler et al. 2002).

Phylogenetic metrics

PD was also highest in the KZN Drakensberg, Sekhukhuneland, as well as in the Wolkberg. These areas encompass species with long branches on the phylogenetic tree revealing distantly related clades. Another explanation for the high PD is that there is an
assemblage of species in each region that is not the result of a single migration into the region, but several migrations, resulting in diversification in a number of different clades leading to high PD. These areas of high PD contain species that diversified a long time ago (Faith 1992) or where there has been competitive exclusion of relatives (Schmidt-Lebuhn et al. 2015). Another likely explanation for the high PD could be that these areas are refugial, i.e., they contain relict species, however from the CANAPE analysis it can be seen that these areas do not have a concentration of palaeo-endemics but rather mixed endemics.

The areas with low PD such as Botswana and Namibia imply that they are centres of recent local radiations thereby resulting in the low diversity in evolutionary history (Faith 1992). These areas contain species that are closely related and reflect a lack of diversification in the clade. The species found in Namibia are most likely relicts since quite a number of palaeo-endemics are revealed by the CANAPE analysis. There are also a lot of mixed endemics in Namibia and Botswana showing that even though there might be relicts causing low PD, there are also neo-endemics that have failed to diversity. This lack of diversification of the genus could be due to the conditions in these areas that seem to limit SR as well as further diversification.

There is low Helichrysum PE in most of the study region with the highest PE being in the Drakensberg region followed by the Wolkberg. Therefore these are areas where the units of PD have restricted ranges (Rosauer et al. 2009). The DAC is an area of high endemism and this is not a surprising result. The area is home to many endemic species (Carbutt & Edwards 2006). The rest of the region has low PE showing that there are no PD units restricted to it. The gorges, forest pockets and heterogeneous topography of these areas likely caused isolation of some species thereby resulting in high PE.

Comparison of hotspots

The results for both Bradypodion and Helichrysum showed remarkable overlap between traditional and phylogenetic biodiversity metrics. The phylogenetic metrics however revealed additional hotspots that were not revealed using traditional biodiversity metrics. There are many cases where this same pattern has been observed e.g. SR and PD were found to highly correlate in Australian endemic conifer species (Lee & Mishler 2014) and Laity et al. (2015) also found that phylogenetic metrics were correlated with traditional metrics with additional information provided by the phylogenetic metrics in a variety of Australian taxa.
This congruence is expected in taxa where evolutionary diversification has been relatively constant over time and in taxa with phylogenies that poorly reflect the geography of the taxa (Rodrigues et al. 2005a; Laity et al. 2015).

There are, however, a number of other cases where traditional species metrics and phylogenetic metrics do not overlap e.g. in southern African tree species where there is no congruence between areas of high PD and SR (Devictor et al. 2012; Daru et al. 2015) also reveal an incongruence between phylogenetic and traditional metrics in birds and butterflies. This incongruence could infer differences in the biogeographical and evolutionary histories of the species or reveal differences in what shapes the biodiversity of the species. There is contention when it comes to conclusions on the congruence of phylogenetic and traditional metrics because the congruence is dependent on the genus of interest, its biogeography as well as evolutionary history (Tucker & Cadotte 2013; Daru et al. 2015).

**Categorical Analysis of Neo- and Palaeo-Endemism analysis**

Only a few cells were significantly high in endemism for *Bradypodion* species. *Bradypodion* is a small genus with relatively few records of the species’ localities, which could be the reason there were only a few significant cells. The CANAPE analysis (Mishler et al. 2014) revealed only one hotspot of neo-endemism in the Drakensberg region. Glaciation events in the area could explain the rise of neo-endemics (Grab 2002; Grab 2009). These events caused periods of extreme cold thereby wiping out some organisms and creating niches for neo-endemics to occupy. This region is important for conservation as it harbours platforms of speciation. Vicariance also aided in the diversification of the genus through the isolation of species due to the gorges and gullies with islands of forest patches and this could be the reason behind the neo-endemism hotspot (Tolley et al. 2011).

The remaining significant cells are hotspots of mixed endemism, i.e. they contain both neo- and palaeo-endemics. Mixed endemism hotspots are usually areas where there are refugia and a cradle for speciation in one place. These areas possibly came about due to vicariance of species which led to allopatric speciation in the fragmented areas as well as extinction filtering that allowed some relict species to survive there (Balmford 1996; Jordan et al. 2016). Pluvial periods prior to the Miocene and during the Pliocene would have caused high levels of erosion (Jacobs 2004), thereby causing isolation of rocky inselbergs which then became refugia during the Neogene through aridification (Demenocal 2004; Jacobs 2004).
Some of the species restricted to these refugia would have diversified (Bowie et al. 2006; Tolley et al. 2011), thereby creating a hotspot of both neo- and palaeo-endemism.

There were many more significant endemic grid cells for *Helichrysum* as compared to *Bradydodion*. Four hotspots of neo-endemism were revealed by the CANAPE analysis with most of them in South Africa. The endemism hotspots mostly occur in mountainous areas where there are higher chances of allopatric speciation occurring (Lomolino et al. 2010). Areas of recent allopatric speciation would then form hotspots of neo-endemism.

Hotspots of palaeo-endemism were mainly located in southern Namibia – an area of high species richness for the Asteraceae (Maggs et al. 1998). The region also features many specialist species that can only be found there due to the aridity of the land (Maggs et al. 1998). The south of Namibia is mainly desert land but a portion of it is encompassed in the succulent Karoo where the vegetation ranges from succulent shrubs, few grasses and scarce tall shrubs and trees. Namibia can be considered an island of great aridity amidst a sea of more dynamic, less arid habitats (Kingdon 1990). This could potentially explain the reason behind the high number of palaeo-endemic hotspots in the region.

There are a lot of mixed endemism hotspots in the study region showing that a wide variety of species are supported, both relict and newly diversified species. Interestingly, areas of super endemism for *Helichrysum* were revealed to be scattered across the study region. These are areas that have high WE and PE and a possible explanation for this is that there are both rare, short phylogenetic branches and rare, long phylogenetic branches which occupy these grid cells. The vegetation in these areas might have changed over time and there are likely to be relict endemics as well as new endemics that recently dispersed.

The northern Drakensberg, Sekhukhuneland, Wolkberg and parts of the Western Cape are all areas of *Helichrysum* super endemism (i.e. with a concentration of many neo- and palaeo-endemics). The Sekhukhuneland and Wolkberg have undergone periods of volcanic activity and magma upliftment which could have led to the rise of new endemics in the newly formed environment (Thomas et al. 1993). Patches of vegetation that survived the upliftment and volcanic activity likely contain relict species leading to an area of super endemism. This super endemism could be due to the climatic history of these areas. Relatively stable climatic histories give rise to palaeo-endemic hotspots (Jordan et al. 2016). For example, in Australia,
Mishler et al. (2014) found that the scattered areas of palaeo-endemism were located in regions where the climate has been stable for long periods of time.

There is very low overlap between the hotspots of neo- and palaeo- endemism for the two genera. Bradypodion is very data deficient and the analysis of endemism shows scarce evidence of important areas. Protection of the important areas of endemism still needs to be prioritised for the different genera but there is no overlap.

The information from the CANAPE analysis can be utilised in conservation assessments. Areas of neo-endemism (harbouring unique, new taxa) need to be conserved in light of the current global change. Such areas of neo-endemism will be essential because they are centres of recent speciation (González-Orozco et al. 2015; González-Orozco et al. 2016). This means that the Drakensberg, a hotspot of neo-endemism, needs to be prioritised for conservation. This prioritisation is not dependent on the taxa contained within the area, but due to the area being a cradle for speciation (González-Orozco et al. 2016). Conservation of the speciation process is very important with the current rates of species decline and conserving hotspots of neo-endemism such as the Drakensberg will ensure the preservation of this potential.

Areas of super endemism such as the northern Drakensberg, Sekhukhuneland, Wolkberg and southern Namibia are even more important in conservation. Such areas contain irreplaceable evolutionary information, but also act as cradles of speciation and adaptation (González-Orozco et al. 2015; Laffan et al. 2016). Conservation of areas of palaeo-endemism is essential as it ensures that refugia are conserved which harbour irreplaceable relict species in danger of extinction.

Analysis of neo- and palaeo-endemism is very important for conservation decision making as it facilitates conservation of evolutionary and ecological processes. To help focus conservation efforts, the CANAPE method uses extensive datasets and computer simulations to find locations where new species have evolved and where older species have taken refuge (González-Orozco et al. 2016). The method is also informative about the major centres of genetic diversity. There is therefore a need for the conservation field to adopt the CANAPE method of identifying endemism hotspots as it not only highlights important areas for conservation, but also identifies the possible underlying processes leading to endemism.
Since conservation efforts have to prioritise ‘important’ areas, CANAPE allows for the identification of these areas and maximising of resources. This method of endemism analysis can be a valuable tool for conservation scientists as it also aids in the identification of sections of the tree of life that should be protected.

Comparisons of areas of endemism (PD-dissimilarity)

The PD-dissimilarity analysis gives insight into relationships among the significant areas of endemism based on shared evolutionary history. Bradypodion did not show any cohesive geographic clustering of significant areas of endemism. This can be attributed to the small size of the genus plus its limited distribution (or the small number of known localities for the genus).

In contrast, the everlasting daisy genus, Helichrysum, showed geographic clustering of the areas of significant phylogenetic endemism to some extent. The north-eastern and southern parts of the study region were found to be closely related on the phylogenetic tree. This could be due to the diversification of species from the Western Cape to the Drakensberg and northern areas, which reportedly happened a number of times (Linder 2003). This means that the Drakensberg and Western Cape clades would be closely related since they originated in the same place. The significant grid cells in Namibia cluster together on the phylogenetic tree and it is likely that the species here originated from the same ancestor resulting in the clustering.

The rest of the study region shows no clear clustering of significant grid cells. One would expect a well-established genus like Helichrysum to show more patterns of clustering than currently observed, however, the phylogenetic tree used for this analysis was not fully resolved (nor was the sampling complete), and so some evolutionary information was missing.

Conservation implications

Analysis of the level of protection of important areas in southern Africa reveals an interesting pattern, SR and WE hotspots for Bradypodion are adequately protected in southern Africa, as are the PD and PE hotspots. This is a very good finding as it shows that conservation efforts are adequately protecting the diversity and endemism of Bradypodion.
The *Bradypodion* hotspot of neo-endemism in the DAC is protected within the Ukhahlamba Drakensberg Transfrontier Park, while the mixed endemism hotspots are scattered across a range of nature reserves in the forest areas of KwaZulu-Natal and Eastern Cape.

There are, however, a few hotspots of mixed endemism in the Eastern Cape just south of Willowmore and in KwaZulu-Natal in the Nongoma area that are not contained within protected areas and attention should be paid to these areas to ensure no loss of endemic *Bradypodion* species occurs. It is important to conserve *Bradypodion* species due to the level of vulnerability of some of the species. For example, one species is listed as Critically Endangered, *B. taeniabronchum*, one as Endangered, *B. setaroi* and two as Near Threatened, *B. thamnobates* and *B. nemorale* on the International IUCN Red List (Hilton-Taylor 2000; Tolley *et al.* 2004a).

Similar to *Bradypodion*, the PD and SR hotspots for *Helichrysum* are well protected in various nature reserves and national parks. In contrast, some of the *Helichrysum* WE hotspots in KwaZulu-Natal do not fall within a protected area. Loss of these endemic species would be irreversible as they are only found in those restricted localities. The PD hotspots are fairly well protected in southern Africa since they are contained in various protected areas across the region. The species occurring in hotspots that do not fall into any protected areas need to be assessed for vulnerability to extinction and plans need to be drawn up on how they can be incorporated into future conservation areas.

The significantly high endemic *Helichrysum* hotspots inferred by the CANAPE analysis (Mishler *et al.* 2014) are mostly inadequately protected. Only one neo-endemism hotspot is protected in Namibia in the Okamatipati Communal Conservancy. This is concerning because areas of neo-endemism are invaluable and irreplaceable areas of speciation important for the diversification of future lineages. The super endemic hotspots also need to be monitored due to their importance in being both refugia for relict species and cradles of speciation for new species. *Helichrysum* species found in the four super endemism hotspots that are not protected in southern Namibia should be included into conservation plans to avoid loss of rich evolutionary historic areas.

In contrast to the lack of protection for neo-endemism in *Helichrysum*, palaeo-endemism hotspots are the most protected of all the *Helichrysum* endemism hotspots. This is good because the irreplaceable, valuable evolutionary history of these areas is being
conserved. This rich evolutionary history is important for predicting future patterns in vegetation as well as climatic patterns. The mixed endemism hotspots are however, poorly protected and together with the super endemism hotspots they need to be included in future conservation hotspots and special attention should be paid to them. Special attention needs to be paid to southern and central Namibia, as well as scattered areas of Eastern Cape and Northern Cape where *Helichrysum* species in hotspots are inadequately protected.

**Conclusions and recommendations**

The main findings from this study were that:

Hotspots inferred by phylogenetic metrics for both genera studied here were largely congruent with those inferred by traditional species metrics. Therefore, for *Helichrysum* and *Bradypodion*, traditional metrics can be used as surrogates of the phylogenetic metrics. However, phylogenetic metrics do reveal additional information that species metrics alone cannot due to their inclusion of the evolutionary history of the taxa studied. It is advisable for all biodiversity assessments to include the use of phylogenetic metrics to ensure adequate information is captured for effective conservation decision making. It can also be concluded that the southern African region has a number of neo-, palaeo and mixed endemism hotspots for both *Helichrysum* and *Bradypodion*. The main areas of endemic importance are the Drakensberg region, the Eastern Cape, Wolkberg, Maputaland diversity hotspot, Pondoland centre of endemism, Sekhukhuneland, and various areas of southern and northern Namibia.

The small genus size caused a lack of geographic clustering for *Bradypodion* using the PD-dissimilarity analysis. It is advised that care be taken when using a small genus to assess biodiversity of an area as they might mislead the analyses. Choosing biodiversity hotspots using a small genus presents a challenge since there is scant evidence concerning the evolutionary processes at play. It is important that the data (genus size and extent of distribution) utilised in such an analysis contain enough data for appropriate inferences to be made. *Helichrysum* showed clustering to a certain extent but the lack of full resolution of the phylogeny used caused less than expected clustering patterns. The level of resolution of the phylogeny used should always be considered when interpreting results for a biodiversity assessment so as to avoid bias.

It can also be concluded that conservation of both plants and animals (as represented by *Bradypodion* and *Helichrysum*) in southern Africa needs more attention as there are many
endemism hotspots that are not at all or only partially protected. These occur mainly in southern and central Namibia as well as some scattered areas in KwaZulu-Natal, Eastern and Northern Cape. Protection of these areas of endemism will ensure less extinction of the important and evolutionary rich taxa, some of which provide valuable ecosystem services as well as medicinal (and other) resources for humans (e.g. *H. odoratissimum*). The use of a small genus like *Bradypodion* versus a large genus like *Helichrysum* reveals some interesting results. Smaller genera do not fully explain biodiversity patterns at the same level as large genera. Large genera reveal more information and work better for most biodiversity analyses. Genera for such analyses need to be selected carefully.

The results of this study provide critical information that can guide conservation planning because they locate biodiversity centres in terms of evolutionary history and potential refugia. No taxon can be managed in isolation and co-occurrence is important in conservation and phylogenetic metrics that can be utilised across multiple genera should continue to be developed to allow for effective assessments that maximise conservation for various taxa. However, it should be noted that the hotspots identified via this approach do not consider complementarity, an aspect that aims to maximise representation of different species (Williams *et al.* 1996). The methodology utilised does not assess complementarity of the hotspots, it only indicates areas of endemic and diversity importance. There is a possibility that the areas of high SR contain the same set of taxa and do not represent the true diversity of the area. Including an analysis of the complementarity of the identified hotspots would be beneficial for conservation.

Understanding the history of an area can help to predict responses to future environmental changes and thus inform climate adaptation strategies (Pennington *et al.* 2004; Böhm *et al.* 2013). The information revealed by this study could be used to further assess the response of taxa to future environmental change in the region. This is especially important in *Bradypodion* where species are listed as Critically Endangered (*B. taeniabronchum*), Endangered (*B. setaroi*) and Near Threatened (*B. thamnobates* and *B. nemorale*) on the International IUCN Red List (Hilton-Taylor 2000; Tolley *et al.* 2004a). Understanding southern African climate and geomorphological history is also important for *Helichrysum* since many species are local endemics that need to be protected due to vulnerability to extinction. Examples of such species are *H. albertense* and *H. album* which are restricted to the Western Cape and the high Drakensberg in KwaZulu-Natal respectively.
Limitations

The study should include more taxon groups for more effective testing of the metrics across multiple taxa to enable better conservation decisions for a wide variety of taxa since no species occurs alone in an area. Including more phylogenetic and traditional metrics could also aid in their comparison. Ecological and evolutionary patterns may differ at different spatial scales, thus, it is important to re-analyse the data accordingly. A fine scale assessment of the level of protection of the important grid cells should also be performed. Sometimes, the fact that a grid cell is protected is not enough because there might be other fine scale factors that affect the taxa in the protected areas (Freitag & Van Jaarsveld 1998).

The use of point locality data for this study could have potentially underestimated the occurrence of the two genera. Point locality data is usually affected by geographic sampling bias (Parnell et al. 2003). Sampling is mostly conducted in areas easily accessible to researchers as well as close to areas of human habitation (Ponder et al. 2001; Funk et al. 2002). Reddy & Dávalos (2003) found that sampling in sub-Saharan Africa is skewed towards rivers and places of human residence while Costa et al. (2009) found that plant collections were mainly conducted close to roads and on mountain sides. This bias in sampling under-represents the occurrence of a species. In this study, point locality data were utilised and there is likely to be some sampling bias that affected the results. An improvement to this study would include an offset of the potential bias presented by the use of point locality data using specie range maps, GIS technology and potentially distribution models (Ponder et al. 2001).

Another shortcoming of the study is that the branch lengths of the phylogenetic trees utilised were not time calibrated. Time calibrated branch lengths provide time-based information for divergence events in the phylogenetic tree. Other advantages of time calibrated phylogenies are: they provide information on processes such as speciation, provide more information for future phylogenetic comparative studies and promote simpler phylogenetic inference (Forest 2009). In this study, the branch lengths were not time calibrated and this means some phylogenetic information could have been left out. An improvement to this study would include phylogenetic analyses using time calibrated phylogenies.
In conclusion, phylogenetic metrics reveal additional valuable information that should be included in every biodiversity assessment. Species metrics are congruent with phylogenetic ones in some genera but they should still be used in conjunction with phylogenetic metrics so as to gain additional information. Many hotspots of the two genera studied here are not protected and this indicates a need for similar assessments to be conducted using other taxa. Various areas of endemic importance were revealed and this information can be utilised in conservation assessments and plans. Future conservation plans for the areas of neo- and palaeo-endemism identified in southern Africa need to be prioritised. Preserving the cradles for speciation and refugia might just be the solution to the current global species loss.
References


UNEP-WCMC. 2012. Data standards for the world database on protected areas. Cambridge, UK.


Appendix A

List of *Bradypodion* species and an outgroup included in molecular phylogenetic analyses, with available voucher information and localities provided. Eight undescribed *Bradypodion* are included in the current study and indicated in the table with an asterisk*

<table>
<thead>
<tr>
<th>Species</th>
<th>Voucher information</th>
<th>Location</th>
<th>Sample number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Kinyongia tavetana</em></td>
<td></td>
<td>Mount Kilimanjaro</td>
<td>Tolley CT207</td>
</tr>
<tr>
<td><em>Bradypodion damaranum</em> (Boulenger, 1887)</td>
<td></td>
<td>Witelsbos</td>
<td>Tolley BS01</td>
</tr>
<tr>
<td><em>Bradypodion pumilum</em> (Gmelin, 1789)</td>
<td>PEM-R5729</td>
<td>Franschhoek</td>
<td>Tolley CT098</td>
</tr>
<tr>
<td><em>Bradypodion occidentale</em> (Hewitt, 1935)</td>
<td>PEM-R5716</td>
<td>Namaqualand</td>
<td>Tolley CT94</td>
</tr>
<tr>
<td><em>Bradypodion ventrale</em> (Gray, 1845)</td>
<td>PEM-R5704</td>
<td>Port Elizabeth</td>
<td>Tolley CT073</td>
</tr>
<tr>
<td><em>Bradypodion gutturale</em> (Smith, 1849)</td>
<td>PEM-R5685</td>
<td>George</td>
<td>Tolley CT007</td>
</tr>
<tr>
<td><em>Bradypodion taeniabronchum</em> (Smith, 1831)</td>
<td>PEM-R5697</td>
<td>Van Stadensberg</td>
<td>Tolley CT076</td>
</tr>
<tr>
<td><em>Bradypodion caffer</em> (Boettger, 1889)</td>
<td>PEM-R5692</td>
<td>Port St Johns</td>
<td>Tolley CT009</td>
</tr>
<tr>
<td><em>Bradypodion nemorale</em> (Raw, 1978)</td>
<td></td>
<td>Zululand</td>
<td>Tolley KTH484</td>
</tr>
<tr>
<td><em>Bradypodion melanocephalum</em> (Gray, 1865)</td>
<td>PEM-R5696</td>
<td>Durban</td>
<td>Tolley CT072</td>
</tr>
<tr>
<td><em>Bradypodion transvaalense</em> (Fitzsimons, 1930)</td>
<td>PEM-R5719</td>
<td>Haenertsburg</td>
<td>Tolley CT026</td>
</tr>
<tr>
<td><em>Bradypodion dracomontanum</em> (Raw, 1976)</td>
<td></td>
<td>Drakensberg region</td>
<td>Tolley CPBD01</td>
</tr>
<tr>
<td><em>Bradypodion setaroi</em> (Raw, 1976)</td>
<td></td>
<td>St Lucia</td>
<td>Tolley DA061</td>
</tr>
<tr>
<td><em>Bradypodion thamnobates</em> (Raw, 1976)</td>
<td>PEM-R5721</td>
<td>Nottingham Road</td>
<td>Tolley CT018</td>
</tr>
<tr>
<td><em>Bradypodion atromontanum</em> (Tolley &amp; Tilbury, 2006)</td>
<td>PEM-R5721</td>
<td>Swartberg Pass</td>
<td>Tolley KT016</td>
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<tr>
<td>Bradypodion caeruleogula (Raw &amp; Brothers, 2008)</td>
<td>KwaZulu-Natal</td>
<td>Tolley CT068</td>
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<tr>
<td>-----------------------------------------------</td>
<td>---------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>Bradypodion kentanicum (Hewitt, 1935)</td>
<td>Kentani</td>
<td>Tolley KTH459</td>
<td></td>
</tr>
<tr>
<td>Bradypodion ngomeense (Tilbury &amp; Tolley, 2009)</td>
<td>PEM-R5690 Ngome Forest</td>
<td>Tolley CT065</td>
<td></td>
</tr>
<tr>
<td>Bradypodion sp. Baviaanskloof*</td>
<td>Baviaanskloof</td>
<td>Tolley KT029</td>
<td></td>
</tr>
<tr>
<td>Bradypodion sp. emerald*</td>
<td>KwaZulu-Natal</td>
<td>Tolley KTH434</td>
<td></td>
</tr>
<tr>
<td>Bradypodion sp. Groendal*</td>
<td>PEM-R5860 Nelson Mandela Bay</td>
<td>Tolley KTH166</td>
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</tr>
<tr>
<td>Bradypodion sp. Knysna*</td>
<td>PEM-R5725 Knysna</td>
<td>Tolley CT006</td>
<td></td>
</tr>
<tr>
<td>Bradypodion sp. nebula*</td>
<td>Pietermaritzburg</td>
<td>Tolley KTH447</td>
<td></td>
</tr>
<tr>
<td>Bradypodion sp. Tsitsikamma*</td>
<td>Tsitsikamma</td>
<td>Tolley KTH098</td>
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<tr>
<td>Bradypodion damar_witelsbos*</td>
<td>Witelsbos</td>
<td>Tolley KTH131</td>
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<tr>
<td>Bradypodion taenia_vanstadensberg*</td>
<td>PEM-R5863 Van Stadensberg</td>
<td>Tolley KTH108</td>
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</tr>
</tbody>
</table>
## Appendix B

List of *Helichrysum* species included in molecular (ITS and ETS) phylogenetic analysis, with voucher information. Herbarium acronyms follow (Holmgren & Holmgren 1998). Placeholder species (indicated with asterisk*) included in phylogenetic analysis, but not in southern Africa were excluded from the Biodiverse analyses.

<table>
<thead>
<tr>
<th>Species</th>
<th>Voucher information</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Filago pyramidata</em></td>
<td>Galbany et al. s.n. (BCN6124)</td>
<td>Spain, Balearic Islands, Ibiza, Sta. AgnPs</td>
</tr>
<tr>
<td><em>H acutatum</em></td>
<td>Koekemoer 3400 (PRE)</td>
<td>RSA, MP, along Weltevreden Rd., between Makobulaan and Buffelskloof, S of Lydenburg</td>
</tr>
<tr>
<td><em>H adenocarpum</em></td>
<td>Cron &amp; Goodman 1281 (J)</td>
<td>RSA, KZN, Injisuthi</td>
</tr>
<tr>
<td><em>H albilanatum</em></td>
<td>Romo et al. 14603 (BC)</td>
<td>RSA, MP,</td>
</tr>
<tr>
<td><em>H albobrunneum</em></td>
<td>Cron &amp; Cingo 1122 (J)</td>
<td>Lesotho, near Black Mountain Pass</td>
</tr>
<tr>
<td><em>H allioides</em></td>
<td>Bester 7360 (PRE)</td>
<td>RSA, EC, Wodehouse District</td>
</tr>
<tr>
<td><em>H alsiniioides</em></td>
<td>AndrJs-Sánchez et al. SA756 (NBG, BOL, SALA)</td>
<td>RSA, WC, Holrivier</td>
</tr>
<tr>
<td><em>H anomalum</em></td>
<td>Romo et al. 14463 (BC)</td>
<td>RSA, EC</td>
</tr>
<tr>
<td><em>H appendiculatum</em></td>
<td>Cron 1091 (J)</td>
<td>RSA, KZN, Monk's Cowl Reserve</td>
</tr>
<tr>
<td><em>H argyrolepis</em></td>
<td>Koekemoer 3599 (PRE)</td>
<td>RSA, LIM, Lekgalameets Nature Reserve</td>
</tr>
<tr>
<td><em>H argyrophyllum</em></td>
<td>Koekemoer 3498 (PRE)</td>
<td>RSA, MP, S oF Buffelskloof Nature Reserve</td>
</tr>
<tr>
<td><em>H argyrosphaerum</em></td>
<td>Koekemoer 3532 (PRE)</td>
<td>RSA, FS, along the R42 between Sasolburg and Parys</td>
</tr>
<tr>
<td><em>H asperum</em></td>
<td>Bergh 1077 (NBG)</td>
<td>RSA, EC, between Nieu-Bethesda and Aliwal North</td>
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<tr>
<td><em>H aureofolium</em></td>
<td>Jardine 1906 (NBG)</td>
<td>RSA, WC, Knolfontein, Swartruggens, 60 km NE of Ceres</td>
</tr>
<tr>
<td><em>H aureolum</em></td>
<td>Koekemoer 3494 (PRE)</td>
<td>RSA, MP, Buffelskloof Nature Reserve</td>
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<tr>
<td>Species</td>
<td>Collector</td>
<td>Location</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------------------------</td>
<td>--------------------------------------------------------------------------</td>
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<td><em>H. aureonitens</em></td>
<td>Koekemoer 3693</td>
<td>Angola, Huila Province, N of Humpata</td>
</tr>
<tr>
<td><em>H. aureum</em></td>
<td>Romo et al. 14414 (BC)</td>
<td>RSA, EC</td>
</tr>
<tr>
<td><em>H. aureum</em> var. <em>monocephalum</em></td>
<td>Koekemoer 3387 (PRE)</td>
<td>RSA, MP, Long Tom Pass</td>
</tr>
<tr>
<td><em>H. aureum</em> var. <em>serotinum</em></td>
<td>Cron &amp; Goodman 1098 (J)</td>
<td>RSA, KZN, Monk's Cowl Reserve</td>
</tr>
<tr>
<td><em>H. auriceps</em></td>
<td>Romo et al. 14371 (BC)</td>
<td>RSA, KZN</td>
</tr>
<tr>
<td><em>H. bellidiastrum</em></td>
<td>Koekemoer 3545 (PRE)</td>
<td>RSA, EC, Naude's Nek</td>
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<tr>
<td><em>H. bellum</em></td>
<td>Cron &amp; Cingo 1123 (J)</td>
<td>Lesotho, near Black Mountain Pass</td>
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<tr>
<td><em>H. brownei</em> Kenya*</td>
<td>Galbany &amp; Arrabal s.n. (BC 867843)</td>
<td>Kenya, Mount Kenya</td>
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<tr>
<td><em>H. caespititium</em></td>
<td>Koekemoer M3531 (PRE)</td>
<td>RSA, FS, between Sasolburg and Parys</td>
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<tr>
<td><em>H. caespitosum</em></td>
<td>Cron 1151 (J)</td>
<td>RSA, Gauteng, Melville Koppies</td>
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<tr>
<td><em>H. callicomum</em></td>
<td>Koekemoer 3497 (PRE)</td>
<td>RSA, MP, Buffelskloof Nature Reserve</td>
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<tr>
<td><em>H. calocephalum</em></td>
<td>Cron 1156 (J)</td>
<td>RSA, MP, Barberton Mountainlands</td>
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<td><em>H. candolleanum</em></td>
<td>Burrows 8560 (BNRH)</td>
<td>Mozambique, Gaza Province</td>
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<tr>
<td><em>H. cephaloideum</em></td>
<td>Romo et al. 14578 (BC)</td>
<td>RSA, MP</td>
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<tr>
<td><em>H. cerastioides</em></td>
<td>Burrows 8508 (BNRH)</td>
<td>RSA, MP</td>
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<tr>
<td><em>H. chionosphaerum</em></td>
<td>Koekemoer 3392 (PRE)</td>
<td>RSA, MP, Long Tom Pass, Mauchsberg</td>
</tr>
<tr>
<td><em>H. chrysargyrum</em></td>
<td>McMurtry 8871 (BNRH)</td>
<td>RSA, MP, NE of Nelspruit, Uitkyk loop road</td>
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<tr>
<td><em>H. cochleariforme</em></td>
<td>Bergh 2279 (NBG, BOL, SALA)</td>
<td>RSA, WC, Velddrit, Roche Pan Nature Reserve</td>
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<tr>
<td><em>H. confertifolium</em></td>
<td>Romo et al. 14599 (BC)</td>
<td>RSA, MP</td>
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<tr>
<td><em>H. cooperi</em></td>
<td>Cron &amp; Goodman 1284 (J)</td>
<td>RSA, KZN, Injisuthi</td>
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<td><em>H. cordifolium</em></td>
<td>Bayer et al. MAD-04003 (CANB)</td>
<td>Madagascar, Antananarivo Province</td>
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<td><em>H. crispum</em></td>
<td>Romo et al. 14532 (BC)</td>
<td>RSA, WC</td>
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<td>H cymosum subsp. calvum</td>
<td>Koekemoer 3535 (PRE)</td>
<td>RSA, EC, 10 km S of Tiffendal on road to Rhodes</td>
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<tr>
<td>H dasyanthum</td>
<td>BCN 6107</td>
<td>Ex J. Bot. Mar i Murtra Blanes</td>
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<tr>
<td>H dasycephalum</td>
<td>Koekemoer 3446 (PRE)</td>
<td>RSA, EC, Between Rhodes and Naude's Nek</td>
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<td>H dasymallum</td>
<td>Cron &amp; Goodman 1152 (J)</td>
<td>RSA, MP, Kurisa Moya</td>
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<td>H difficile</td>
<td>Romo et al. 14588B (BC)</td>
<td>RSA, MP</td>
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<tr>
<td>H diffusum</td>
<td>Abbott 8922 (PRU)</td>
<td>RSA, KZN, Umtamvuna Nature Reserve, The Terracees</td>
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<td>H drakensbergense</td>
<td>Cron &amp; Goodman 1258 (J)</td>
<td>RSA, KZN, Sani Pass</td>
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<td>H dregeanum</td>
<td>Bester 1605 (PRU)</td>
<td>RSA, EC, Maclear, Farm: Wide Valley</td>
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<td>H dunense</td>
<td>AndrJs-Sánchez et al. SA780(NBG, BOL, SALA)</td>
<td>RSA, WC, Lambert's Bay</td>
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<td>H ecklonis</td>
<td>Koekemoer 3538 (PRE)</td>
<td>RSA, EC, 8 km from Tiffendal on road to Rhodes</td>
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<td>H elegantissimum</td>
<td>Romo et al. 14435 (BC)</td>
<td>RSA, EC</td>
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<tr>
<td>H epapposum</td>
<td>Koekemoer 3504 (PRE)</td>
<td>RSA, MP, Sterkspruit Nature Reserve</td>
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<td>H ephelos</td>
<td>Koekemoer 3491 (PRE)</td>
<td>RSA, MP, S of Lydenburg, Makobulaan</td>
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<td>H erubescens</td>
<td>Hall 380 (NBG)</td>
<td>Namibia, Kaokoveld, Orupembe</td>
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<tr>
<td>H evansii</td>
<td>Cron &amp; Goodman 1298 (J)</td>
<td>RSA, KZN, Mont Aux Sources</td>
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<td>H excisum</td>
<td>Koekemoer 3433 (PRE)</td>
<td>RSA, WC,</td>
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<tr>
<td>H felinum</td>
<td>Koekemoer 3737 (PRE)</td>
<td>RSA, WC, Franschhoek Pass</td>
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<td>H flammeiceps Malawi*</td>
<td>Koekemoer 1854 (BC)</td>
<td>Malawi, Nyika National Park</td>
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<td>H flanaganii</td>
<td>Cron &amp; Cingo 1113 (J)</td>
<td>Lesotho, near Tshelanyane River</td>
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<td>H foetidum</td>
<td>Cron &amp; Goodman 1139 (J)</td>
<td>RSA, WC, Swartberg Pass</td>
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<tr>
<td>H galpinii</td>
<td>Koekemoer 3408 (PRE)</td>
<td>RSA, MP, Steenkampsberg, between Lydenburg and Roossenekal</td>
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<td>Species</td>
<td>Collector</td>
<td>Location</td>
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<tr>
<td><em>H. gariepinum</em> var. gariepinum</td>
<td>Koekemoer</td>
<td>Namibia, 88 km S of Helmeringhausen on road to Aus</td>
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<tr>
<td><em>H. gariepinum</em> var. roseo</td>
<td>Koekemoer</td>
<td>Namibia, dry river bed between Noerdoewer and Rosh Pinah</td>
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<tr>
<td><em>H. gerberifolium</em></td>
<td>Koekemoer</td>
<td>RSA, MP, along Weltevreden Rd., between Makobulaan and Buffelskloof, S of Lydenburg</td>
</tr>
<tr>
<td><em>H. glaciale</em></td>
<td>Bester</td>
<td>RSA, EC, SW of Maclear, Farm: Aurora Peak</td>
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<tr>
<td><em>H. globosum</em> Kenya*</td>
<td>Galbany &amp; Arrabal s.n. (BC 867838)</td>
<td>Kenya, Mount Kenya</td>
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<tr>
<td><em>H. glomeratum</em></td>
<td>Cron &amp; Goodman</td>
<td>RSA, KZN, Injisuthi</td>
</tr>
<tr>
<td><em>H. grandibracteatum</em></td>
<td>Bester</td>
<td>RSA, KZN</td>
</tr>
<tr>
<td><em>H. griseolananatum</em></td>
<td>Koekemoer</td>
<td>RSA, EC, Between Rhodes and Naude's Nek</td>
</tr>
<tr>
<td><em>H. gymnocomum</em></td>
<td>Cron &amp; Goodman</td>
<td>RSA, KZN, Sani Pass</td>
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<tr>
<td><em>H. hamulosum</em></td>
<td>Koekemoer</td>
<td>RSA, WC, between Montagu and Matroosberg</td>
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<tr>
<td><em>H. hebelepis</em></td>
<td>Koekemoer</td>
<td>RSA, NC, Namakwa National Park</td>
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<tr>
<td><em>H. herbaceum</em></td>
<td>Cron &amp; Goodman</td>
<td>RSA, KZN, Monk's Cowl Reserve</td>
</tr>
<tr>
<td><em>H. herniarioides</em></td>
<td>Koekemoer</td>
<td>Namibia, SW of Kakamas on farm DroNgrond</td>
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<tr>
<td><em>H. heterolasium</em></td>
<td>Cron &amp; Goodman</td>
<td>RSA, KZN, Sani Pass</td>
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<tr>
<td><em>H. homilochrysum</em></td>
<td>Burrows</td>
<td>RSA, MP</td>
</tr>
<tr>
<td><em>H. hypoleucum</em></td>
<td>Koekemoer</td>
<td>RSA, FS, Retiefklip at the foor of Kerkenberg</td>
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<tr>
<td><em>H. incarnatum</em></td>
<td>Bergh</td>
<td>RSA, WC, top of Daskop Pass</td>
</tr>
<tr>
<td><em>H. indicum</em></td>
<td>Romo et al. 14547 (BC)</td>
<td>RSA, WC</td>
</tr>
<tr>
<td><em>H. infaustum</em></td>
<td>Cron &amp; Goodman</td>
<td>RSA, KZN, Monk's Cowl Reserve</td>
</tr>
<tr>
<td><em>H. inornatum</em></td>
<td>Bester</td>
<td>RSA, EC, Maclear, Farm Cervantes, N of Ugie</td>
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<tr>
<td>Species</td>
<td>Collectors</td>
<td>Locality</td>
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<tr>
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<tr>
<td><em>H. interjacens</em></td>
<td>Koekemoer 2181 (PRE)</td>
<td>RSA, MP, Buffelskloof Nature Reserve</td>
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<tr>
<td><em>H. isolepis</em></td>
<td>Romo et al. 14477 (BC)</td>
<td>RSA, EC</td>
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<tr>
<td><em>H. italicum</em></td>
<td>Redžić et al. s.n. (BCN 20756)</td>
<td>Bosnia Herzegovina, Herzegovina</td>
</tr>
<tr>
<td><em>H. jubilatum</em></td>
<td>AndrJs-Sánchez et al. SA740 (NBG, BOL, SALA)</td>
<td>RSA, NC, Lekersing</td>
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<tr>
<td><em>H. kilimanjari</em></td>
<td>Galbany &amp; Arrabal s.n. (BC 867836)</td>
<td>Kenya, Mount Kenya</td>
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<tr>
<td><em>H. kraussii</em></td>
<td>Koekemoer 3674 (PRE)</td>
<td>Angola, Huila Province, Huila</td>
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<tr>
<td><em>H. krebsianum</em></td>
<td>Cron &amp; Goodman 1261 (J)</td>
<td>RSA, KZN, Sani Pass</td>
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<tr>
<td><em>H. krookii</em></td>
<td>Koekemoer 3556 (PRE)</td>
<td>Lesotho, Sehlabathebe National Park</td>
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<tr>
<td><em>H. lambertianum</em></td>
<td>Romo et al. 14556 (BC)</td>
<td>RSA, WC</td>
</tr>
<tr>
<td><em>H. leontonyx</em></td>
<td>Koekemoer 3009 (PRE)</td>
<td>RSA, NC, Namakwa National Park</td>
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<tr>
<td><em>H. lepidissimum</em></td>
<td>Koekemoer 3495 (PRE)</td>
<td>RSA, MP, Weltevreden Rd., S of Buffelskloof Nature Reserve</td>
</tr>
<tr>
<td><em>H. lesliei</em></td>
<td>Koekemoer 3493 (PRE)</td>
<td>RSA, MP, Kemps Height on Weltevreden Rd., S of Buffelskloof Nature Reserve</td>
</tr>
<tr>
<td><em>H. lineare</em></td>
<td>Koekemoer 3544 (PRE)</td>
<td>RSA, EC, Rhodes</td>
</tr>
<tr>
<td><em>H. lineatum</em></td>
<td>Koekemoer 2895 (PRE)</td>
<td>Lesotho, between Oxbow and Mahlasela Pass</td>
</tr>
<tr>
<td><em>H. lingulatum</em></td>
<td>Bester 990 (PRU)</td>
<td>RSA, EC, Maclear, Farm Rutherford</td>
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<tr>
<td><em>H. litorale</em></td>
<td>Koekemoer 3476 (PRE)</td>
<td>RSA, WC, Cape of Good Hope</td>
</tr>
<tr>
<td><em>H. lucilioides</em></td>
<td>Koekemoer 3637 (PRE)</td>
<td>RSA, WC, along the Groot Graffwater turn-off from the N7</td>
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<tr>
<td><em>H. marginatum</em></td>
<td>Koekemoer 3546 (PRE)</td>
<td>RSA, EC, Naude's Nek Pass</td>
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<tr>
<td><em>H. mariepscopicum</em></td>
<td>Koekemoer 3489 (PRE)</td>
<td>RSA, MP, Mariepskop</td>
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<tr>
<td><em>H. marmoralepis</em></td>
<td>Boucher 6768 (NBG)</td>
<td>RSA, WC, Vanrhynsdorp</td>
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<tr>
<td><em>H. melanacme</em></td>
<td>Romo et al. 14334 (BC)</td>
<td>RSA, FS</td>
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<tr>
<td><em>H. miconiifolium</em></td>
<td>Cron &amp; Goodman 1086 (J)</td>
<td>RSA, KZN, Cathedral Peak, Mike's Pass</td>
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<tr>
<td><strong>H micropoides</strong></td>
<td>AndrJs-Sánchez et al. SA752(NBG, BOL, SALA)</td>
<td>RSA, WC, Vanrhynsdorp</td>
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<tr>
<td><strong>H milfordiae</strong></td>
<td>Cron &amp; Goodman 1247 (J)</td>
<td>RSA, KZN, Sani Pass (summit)</td>
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<td><strong>H milleri</strong></td>
<td>Koekemoer 3861 (PRE)</td>
<td>RSA, MP</td>
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<tr>
<td><strong>H mimetes</strong></td>
<td>Romo et al. 14610 (BC)</td>
<td>RSA, MP</td>
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<tr>
<td><strong>H mixtum var. mixtum</strong></td>
<td>Romo et al. 14374 (BC)</td>
<td>RSA, KZN</td>
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<tr>
<td><strong>H mixtum var. grandiceps</strong></td>
<td>Cron et al. 1187 (J)</td>
<td>RSA, MP, Barberton Mountainlands</td>
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<tr>
<td><strong>H mollifolium</strong></td>
<td>Cron &amp; Goodman 1108 (J)</td>
<td>RSA, KZN, Cathedral Peak, near Catchment 8</td>
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<tr>
<td><strong>H montanum</strong></td>
<td>Koekemoer 4320 (PRE)</td>
<td>RSA, KZN/EC</td>
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<tr>
<td><strong>H monticola</strong></td>
<td>Cron &amp; Goodman 1343 (J)</td>
<td>RSA, FS, Platberg near Harrismith</td>
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<tr>
<td><strong>H mundtii</strong></td>
<td>Romo et al. 14368 (BC)</td>
<td>RSA, KZN</td>
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<tr>
<td><strong>H mutabile</strong></td>
<td>Koekemoer 3603 (PRE)</td>
<td>RSA, LIM, Lekgalameetse Nature Reserve</td>
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<td><strong>H nanum</strong></td>
<td>Cron &amp; Goodman 1288 (J)</td>
<td>RSA, KZN, Injisuthi</td>
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<td><strong>H natalitium</strong></td>
<td>Burrows 8431 (BNRH)</td>
<td>RSA, KZN, near Coleford turnoff on road from Kokstad to Underberg</td>
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<tr>
<td><strong>H nicolai</strong> Cape Verde*</td>
<td>Kilian et al. (BC)</td>
<td>Cape Verde, São Nicolau, Alto das Cabaças</td>
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<tr>
<td><strong>H nimbicola</strong></td>
<td>Cron &amp; Goodman 1299 (J)</td>
<td>RSA, KZN, Mont Aux Sources</td>
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<tr>
<td><strong>H niveum</strong></td>
<td>Koekemoer 3425 (PRE)</td>
<td>RSA, WC, Stilbaai</td>
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<tr>
<td><strong>H nudifolium var. nudifolium</strong></td>
<td>Koekemoer 3548 (PRE)</td>
<td>RSA, EC, Pitseng Pass road, between Rhodes adn Mount Fletcher</td>
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<tr>
<td><strong>H nudifolium var. pilosellum</strong></td>
<td>Cron &amp; Goodman 1199 (J)</td>
<td>RSA, MP, Barberton Mountainlands</td>
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<tr>
<td><strong>H obductum</strong></td>
<td>Romo et al. 14573 (BC)</td>
<td>RSA, MP</td>
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<tr>
<td><strong>H obtusum</strong></td>
<td>Koekemoer 3517 (PRE)</td>
<td>Namibia, between Noordoewer and Rosh Pinah</td>
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<tr>
<td><em>H. odoratissimum</em></td>
<td>Cron &amp; Goodman 1286 (J)</td>
<td>RSA, KZN, Injisuthi</td>
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<tr>
<td><em>H. opacum</em></td>
<td>Romo et al. 14593 (BC)</td>
<td>RSA, MP</td>
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<tr>
<td><em>H. oreophilum</em></td>
<td>Koekemoer 3389 (PRE)</td>
<td>RSA, MP, Long Tom Pass</td>
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<td><em>H. paleatum</em></td>
<td>Cron &amp; Goodman 1259 (J)</td>
<td>RSA, KZN, Giant’s Cup trail</td>
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<td><em>H. pallidum</em></td>
<td>Koekemoer 3353 (PRE)</td>
<td>RSA, NC, Tankwa Karoo National Park</td>
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<tr>
<td><em>H. panduratum</em></td>
<td>Heyman 74 (PRU)</td>
<td>RSA, KZN, Dalton Midlands, Grayton</td>
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<td><em>H. pandurifolium</em></td>
<td>Koekemoer 3413 (PRE)</td>
<td>RSA, MP, ca, 85 km from Machadoendorp on road to Badplaas</td>
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<tr>
<td><em>H. pannosum</em></td>
<td>Abbott 9353 (PRU)</td>
<td>RSA, KZN, Ken Gaze's Farm</td>
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<td><em>H. paronychioides</em></td>
<td>Bester s.n. (PRE764790)</td>
<td>RSA, NW, Klerkdorp</td>
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<td><em>H. patulum</em></td>
<td>Koekemoer 3395 (PRE)</td>
<td>RSA, MP, Three Falls Estate, S of Lydenburg</td>
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<td><em>H. petiolare</em></td>
<td>Cron &amp; Goodman 1142 (J)</td>
<td>RSA, WC, Swartberg Pass</td>
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<td><em>H. plantago</em></td>
<td>Bayer et al. MAD-04062 (CANB)</td>
<td>Madagascar, Antananarivo Province, Mt. Ibity</td>
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<td><em>H. platycephalum</em></td>
<td>Bayer et al. MAD-04022 (CANB)</td>
<td>Madagascar, Fianarantsoa Province</td>
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<td><em>H. platypterum</em></td>
<td>Koekemoer 2186 (PRE)</td>
<td>RSA, MP, Buffelskloof Nature Reserve</td>
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<td><em>H. polycladum</em></td>
<td>Romo et al. 14598 (BC)</td>
<td>RSA, MP</td>
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<td><em>H. populifolium</em></td>
<td>BCN 8218</td>
<td>RSA, ex Silverhill Seeds</td>
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<tr>
<td><em>H. praecurrens</em></td>
<td>Cron &amp; Cingo 1130 (J)</td>
<td>Lesotho, near Black Mountain Pass</td>
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<tr>
<td><em>H. psilolepis</em></td>
<td>Romo et al. 14461 (BC)</td>
<td>RSA, EC</td>
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<td><em>H. pumilio</em></td>
<td>Desmet et al. 3711 (NBG)</td>
<td>RSA, NC, Bushmanland</td>
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<td><em>H. reflexum</em></td>
<td>Romo et al. 14571 (BC)</td>
<td>RSA, MP</td>
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<tr>
<td><em>H. retortoides</em></td>
<td>Cron &amp; Goodman 1297 (J)</td>
<td>RSA, KZN, Mont Aux Sources</td>
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<tr>
<td><em>H. retortum</em></td>
<td>BCN 6112</td>
<td>ex Silverhill Seeds</td>
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<td>H revolutum</td>
<td>Koekemoer 3319 (PRE)</td>
<td>RSA, NC, Namakwa National Park</td>
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<td>H roseo niveum</td>
<td>Bergh 1469 (NBG)</td>
<td>Namibia, Kuiseb River bed</td>
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<td>H rosum var. arcuatum</td>
<td>Romo et al. 14494 (BC)</td>
<td>RSA, EC</td>
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<td>H rosum var. rosum</td>
<td>Romo et al. 14462 (BC)</td>
<td>RSA, EC</td>
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<tr>
<td>H rugulosum</td>
<td>Romo et al. 14331 (BC)</td>
<td>RSA, FS</td>
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<tr>
<td>H rutilans</td>
<td>Koekemoer 3403 (PRE)</td>
<td>RSA, MP, 24 km from Lydenburg on Road to Roossenekal</td>
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<td>H schimperi Tanzania*</td>
<td>Galbany &amp; Arrabal s.n. (BC 867821)</td>
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<td>H sessile</td>
<td>Glennon 51 (J)</td>
<td>RSA, KZN, Sani Pass</td>
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<td>H sessilioides</td>
<td>Koekemoer 3547 (PRE)</td>
<td>RSA, EC, Naude's Nek Pass</td>
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<tr>
<td>H setosum</td>
<td>Cron 1149 (J)</td>
<td>RSA, Gauteng, Melville Koppies</td>
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<td>McMurtry 11424 (BNRH)</td>
<td>Mozambique, Licuati Sand Forest</td>
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<td>Koekemoer 3443 (PRE)</td>
<td>RSA, KZN, Matatiele</td>
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<td>AndrJs-Sánchez et al. SA755 (NBG)</td>
<td>RSA, NC, Holrivier</td>
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<td>Bester 7433 (PRE)</td>
<td>RSA, EC, Elliot District</td>
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<td>H splendidum</td>
<td>Koekemoer 3445 (PRE)</td>
<td>RSA, EC, Between Rhodes and Naude's Nek</td>
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<td>Venter 11713 (PRU)</td>
<td>RSA, LIM, Wolkberg Wilderness, Farm Wolkberg</td>
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<td>H stellatum</td>
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<td>RSA, NC, between Soutfontein and Nareip, We of Garies</td>
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<td>Bester 7659 (PRE)</td>
<td>RSA, MP, Witbank District, Goedgevonden Coal Mine, Ca. 10 km S of Ogies</td>
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<td>Cron &amp; Goodman 1239 (J)</td>
<td>RSA, KZN, Sani Pass</td>
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<td>H subglomeratum</td>
<td>Cron &amp; Goodman 1248 (J)</td>
<td>RSA, KZN, Sani Pass (summit)</td>
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<td>Koekemoer 3398 (PRE)</td>
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<td>H summomontanum</td>
<td>Koekemoer 3505 (PRE)</td>
<td>RSA, MP, Sterkspruit Nature Reserve</td>
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<td>(BNRH)</td>
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<td>Heyman</td>
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<td><em>H. wilmsii</em></td>
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<td><em>H. witbergense</em></td>
<td>Cingo &amp; Cron</td>
<td>08 (J)</td>
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<td><em>H. xylocladum</em> Madagascar*</td>
<td>Bayer et al.</td>
<td>MAD-04073 (CANB)</td>
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<td><em>H. zeyheri</em></td>
<td>Romo et al.</td>
<td>14542 (BC)</td>
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<td><em>H. zwartbergense</em></td>
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<td>3474 (PRE)</td>
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</tbody>
</table>
Appendix C

How to use Biodiverse


**Note: Biodiverse works better when the PC decimal symbol setting is set to a point.

For species analyses

1. Follow the official manual to find out how to open Biodiverse.
2. Makes sure the data file matches the example file that is included in the Biodiverse folder of files.
3. When it is now open, click on the + sign to the immediate right of the ‘Basedata’ tab to add the data.
4. Choose the .csv file you wish to input as your Basedata and press Next.
5. Leave everything as is on this page and press Next.
6. Set ‘num’ to ignore from the dropdown menu and ‘genus’ and ‘species’ as labels from the dropdown menu. Latitude and longitude should be set to group from the dropdown menu.
7. For group cell size for both latitude and longitude insert 0.25 and insert 0.25 for cell origin.*
8. Make sure to choose ‘is lat’ on the row for latitude and ‘is lon’ for the longitude one under ‘data in degrees’.
9. Press OK to go to the next step.
10. A coordinate is represented as Y (latitude), X (longitude), however, the default setting in Biodiverse is X (longitude), Y (latitude). Therefore, swap longitude and latitude in this step. To swap: select longitude and choose ‘Up’ from the tabs in the middle then press ‘OK’.
11. After the data is imported, double click on the Basedata in the left to view the data.
12. To run the actual analyses select ‘Analyses’ from the top menu and choose ‘Spatial’
13. For species richness: expand the ‘List and Counts’ tab using the + sign and select ‘Richness’ from the list of metrics then press ‘Go!’.
14. For endemism: expand the ‘Endemism’ tab and choose the desired endemism metric and press ‘Go!’ for the analysis to run.
15. Display results after an analysis and select ‘Export’ from the left menu on the results page. Export any desired file format.

*Choose 0.25 only if your data is recorded per QDS (Quarter Degree Square). A QDS measures 25 x 25 km.

For phylogenetic analyses

1. Trim the tree to ensure the taxa in the data matches the taxa phylogeny.
2. Add a tree by selecting the + to the right of the ‘Tree’ tab.
3. Select ‘Yes’ or ‘No’ depending on whether your tree is in tabular format or not.
4. Select the tree file to be imported into Biodiverse.
5. Select the ‘Yes’ to remap the tree labels.
6. Choose the remap file prepared as a .txt file and press ‘Ok’ (follow the steps in the official manual to find out how to create this file).
7. Leave everything as is in the next step and select ‘Next’
8. Set row 0 to ‘ignore’ from the dropdown menu, ‘Input_element’ for row 1 and ‘Remapped_element’ for row 2 and press ‘OK’ and ‘OK’ again.
9. For the actual analyses select ‘Analyses’ from the top menu and choose the ‘Spatial’ option.
10. Choose the desired metric from the list and press ‘Go!’ for the analysis to run.

**Results can be exported from Biodiverse in many formats that can be read using any desired software.

For CANAPE analysis

Please consult the following resources for information on how to run a CANAPE analysis. Please note that a complete CANAPE analysis cannot be fully conducted in Biodiverse alone; to complete the analysis, R will need to be utilised.


Appendix D

Figure D1. The most resolved Bayesian phylogeny of the everlasting daisy genus, *Helichrysum* (Asteraceae, Gnaphalieae). The phylogeny has been split into three parts to allow for easier representation, the three parts (from left to right) are in the order in which they would appear if viewed as one upright image.