Phylogenetic study and the effect of climate change on Duckweeds (Lemnaceae) in South Africa: an ecological niche modelling approach

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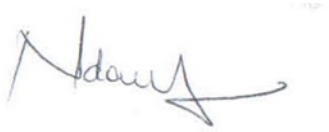

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ABSTRACT

Duckweeds are tiny floating plants that are anthropogenically introduced in many rivers, lakes, and wetlands in South Africa. Because of their miniature habit, they can be very difficult to identify, distinguish with naked eyes and they reproduce rapidly, forming a dense mat that diminishes the potential usage of waterbodies. Therefore, we aim to identify duckweed species using phylogenetic relationships and elucidate how climate change may affect the current and future distribution of duckweed (Lemnaceae) species in South Africa. For DNA molecular technique, we used five plastid regions (*matK, rbcL, rps16, trnK (3')* and *trnK (5')* to reconstruct a complete phylogeny of five duckweed genera with 38 species. 20 samples were collected from different provinces in South Africa. The phylogenetic analysis revealed two distinct clades representing the two subfamilies of Lemnaceae: Lemnoideae and Wolffioideae. Approximately, seven native and 31 invasive alien duckweed species were identified. With regards to results from the species distribution model, current climate models revealed suitable environmental niche for duckweeds species in South Africa, with the following hotspot provinces: KwaZulu-Natal, Eastern cape, Western Cape, Mpumalanga, and Limpopo. However, the future projections have shown that majority of duckweed species will experience great contraction: *Spirodela polyrhiza*, *Lemna aequinoctialis* and *Lemna gibba*. Therefore, this project has confirmed the placement of collected duckweed species within the two subfamilies on the phylogenetic tree and we also predicted the distribution of duckweed species in South Africa. The eastern and southern part of South Africa extending to the northern part has been identified as the most current climatically suitable areas for duckweed species. Furthermore, results differentiated between native and invasive alien duckweed species to South Africa. The study also listed some of the water bodies that may experience range expansion of duckweed species in the future where conservationist may prioritize management of dams.

ABBREVIATIONS

ABI:	Applied Biosystem
ACCTRAN:	Accelerated Transformation
ArcGIS :	Aeronautical Reconnaissance Coverage Geographic Information System
atp:	Adenosine Triphosphate
BSA:	Bovine Serum Albumin
CAES:	College of Agriculture and Environmental Sciences
cpDNA:	Chloroplast Deoxyribonucleic Acid
CSIR:	Commonwealth Scientific and Industrial Research Organization
DELTRAN:	Delayed Transformation
DNA:	Deoxyribonucleic Acid
GBM:	Gradient Boosting Machines
GBIF:	Global Biodiversity Information Facility
GenBank/NCBI:	National Genetic Sequence Data Base
GLM:	Generalized Linear Model
Km:	Kilometer
matK:	Megakaryocyte Associated Tyrosine Kinase
min:	Minute
Min:	Minimum
Max:	Maximum
MP:	Maximum Parsimony
M/sec:	Meter per Second
Multrees:	Multiple equal parsimonious trees
NCBI:	National Center for Biotechnology Information
PCR:	Polymerase Chain Reaction
PRECIS:	Pretoria Computerized information System
rbcL:	Ribulose Biophosphate Carboxylase Large
RF:	Random Forest
rpsl 16:	Ribosomal Proteins
SANBI:	South African National Biodiversity Institute
Sec:	Second
SDMs:	Species Distribution Models
TBP:	Thyroxine-binding Protein

CHAPTER 1

INTRODUCTION

1.1 Background

Native species are those that naturally occur and evolve in a particular ecosystem or geographical area, also they have adapted to the local environmental conditions, climate, and other species present in that ecosystem over time (Lilly Center for lakes and streams 2021).

Invasive alien plants are a nuisance in the ecosystems (Rouget et al. 2004). Invasive alien species are considered as one of the major threats to biodiversity (Mack et al. 2000; Andreu and Vilà 2010). Over the past two centuries, human migration patterns (McNeely et al. 2001) and settlement have introduced flora that were kept apart by natural barriers into new environments (Lee 2001). Invasive alien species are introduced into new areas either intentionally or unintentionally and they are also capable of spreading rapidly without the assistance of people (Henderson 2020).
They are taxa that have been introduced recently and exert substantial negative ecological and socio-economic impact (Mack et al. 2000; Pimentel et al. 2005; Coetzee et al. 2018). The phenomenon of Invasive alien species has sparked worldwide discussions as they have caused ecological crisis by changing plant communities within the area of invasion.

South Africa has been particularly vulnerable to invasive alien plants than any other country in the world (Richardson and Van Wilgen 2004; Trethowan et al. 2011) and it has been long battling with Invasive alien species and management of biological invasions (Richardson and Van Wilgen 2004). Invasive alien plants have costed South Africa an estimated R6.5 billion every year but still they are left unmanaged (De Lange and Van Wilgen 2010). It has been estimated that about 10 million hectares of land in South Africa has been invaded (Nel et al. 2004) to satisfy human needs. Almost close to 9000 invasive alien species are introduced in South Africa and about 750 of them are tree species and almost 8000 shrubby species which are divided into succulent and herbaceous species (Van Wilgen and Scott 2001). This spread beyond the point

of introduction, poses negative ecological and economic challenges to the country (Pimentel et al. 2005).

However, compared to terrestrial plants, aquatic plants are shown to have higher probabilities of becoming invasive alien plants in new environments (Andreu and Vilà 2010). These floating aquatic plants are anthropogenically introduced in many rivers, impoundments, lakes, and wetlands in South Africa (Hill 2003; Martin and Coetzee 2011; Van Wilgen et al. 2020). Once introduced, they reproduce rapidly, form a large mat that diminish the potential usage of waterbodies and reduce the aquatic functioning of the ecosystem (Hill 2003). Invasive alien plants are extremely difficult and expensive to eradicate and therefore require cost effective prevention and management measures methods (Wittenberg and Cock 2001). Therefore, this study, focuses on the Lemnaceae family commonly known as duckweed. Duckweed is commonly found floating on the surface of various waterbodies such as ponds, lakes, marshes, and slow-moving streams (Goopy and Murray 2003).

Duckweed plants belong to Lemnaceae family and is comprised of five genera: *Lemna* L.*, Spirodela* Schleid.*, Landoltia* Les & D.J.Crawford*, Wolffia* Horkel ex Schleid*,* and *Wolffiella* Hegelm (Bog et al. 2019). Leng et al. (1995) stated that duckweed is usually mistaken as algae. Morphologically, duckweed is comprised of small flowering plants that grow exponentially (Bog et al. 2019) and have ability to cover the whole surface of the water body in few weeks (Sengupta et al. 2010). It ranges from 1.5 cm long to less than one millimetre in height (Wang et al. 2011). Duckweed has zero to 21 roots with one to 16 frond veins which are thin or lanceolate in shape (Landolt 1986). *Spirodela*, *Landoltia* and *Lemna* have two meristematic primordia while genera *Wolffia* and *Wolffiella* daughter fronds are formed from the meristematic region in the single reproductive pouch of a mother frond (Klaus et al. 2013*). Spirodela* species have the largest fronds: 20 mm, while those of *Wolffia* are 2 mm or less in diameter and *Lemna* species are mid-size at 6 to 8 mm (Skillicorn et al.1993).

Although duckweed has a cosmopolitan distribution, subtropical and tropical areas are the most concentrated areas (FAO 2000). Most favourable regions of duckweed in Africa are temperate regions which are Southern and tropical side of Africa (Botanical Research Institute 1980).

1.2 Characteristics of duckweed

Duckweed is a prevalent aquatic green plant often encountered in slow-moving or stagnant water bodies such as ponds, lakes, marshes, and sluggish streams. (FAO 2000, Figure 1.1A and Figure 1.1B). They are very tiny green floating plants capable of supplementing feed for livestock (Heuze and Tran 2015). Skilicorn et al. (1993) further mentioned that duckweed's buoyant nature and lack of anchorage to the substrate make it susceptible to being moved by wind and wave action within water bodies (Figure 1.1B). When wind or waves create surface movement, duckweed fronds can be carried towards the banks or shores of lakes, ponds, or other water bodies. Duckweeds consist of flat, ovoid frond and rootlets hairs which functions as a stability organ (Skillicorn et al. 1993) that lack woody tissue (Figure 1.1C). Some individual duckweed species such as *Lemna* and *Spirodela* consist of adventitious roots while the other two genera (*Wolffia* and *Wolffiella*) have no roots (Goopy and Murray 2003; Figure 1.1C). They grow in temperatures that are between 6 and 33 degrees Celsius and sensitive to frost (Van den Berg et al. 2015). When climatic conditions are highly favourable, duckweed is known for its exceptionally fast growth rate, often doubling its biomass in a short period ranging from 16 to 48 hours under optimal conditions (Leng 1999) thus forming a surface mat on top of waters that brings negative impacts on aquatic life (Sullivan and Gublin 2012).

Water bodies rich in decaying organic material provide an abundance of nutrients and trace elements that support the vigorous growth of duckweed populations (Skilicorn et al. 1993). This condition occurs during mid-summer due to favourable temperatures. Duckweed often do not survive a moving water, which means the water speed should be less than 0.3 m/sec for duckweed to survive (Van den Berg et al. 2015). Water current can have a massive impact on growing of duckweed species as they have certain limit of water velocity of 0.1 m/s velocity (Duffield and Edwards 1981) that it can withstand (FAO 2000).

Figure 1.1: Floating duckweed and types of duckweed (Freshwater Habitats 2013). A) Plant collection at Haartebees dam, B) Duckweed blooming at Walter Sisulu Botanical Garden Lake C) Duckweed leaves and fronds

1.3 Species distribution

Duckweed is world widely distributed and most species of duckweed are situated in average climates of tropical and subtropical zones (Iqbal 1999; Oyawoye 2017). They adapt to wide variety of geographic zones but not in waterless deserts and permanently frozen polar regions (Skillicorn et al. 1993). Species of duckweed have wide geographic distribution which indicates a high probability of ample genetic diversity and good potential to improve their agronomic characteristics through selective breeding (Skillicorn et al. 1993). Mtshali et al. (2017) studied the distribution of duckweed in South Africa, and these species are mainly found in the Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Mpumalanga, North-west and Western Cape. Local distribution of duckweed ranged from Helderberg in Western Cape across to Komatipoort extending to Mpumalanga (Mtshali et al. 2017).

1.4 Taxonomic identification of duckweed species

The Lemnaceae family (duckweed) is comprised of 37 species in five genera: *Lemna, Landoltia, Spirodela, Wolffia* and *Wolffiella* (Appenroth et al. 2013; Figure 1.2). Duckweed family (Lemnaceae) is structured into two subfamilies known as, Wolffioideae (members devoid of roots) and Lemnoideae (varying numbers of roots) (Landolt 1986; Figure 1.3). Duckweed genera such as *Spirodela* and *Lemna* are

characterised by a flat and oval leaf-like structure while *Spirodela* has two or more thread-like roots on each frond and *Lemna* has only one thread-like root (FAO 2000). *Wolffiella* and *Wolffia* are thalloid, have no roots and they are smaller compared to *Spirodela* or *Lemna* (FAO 2000).

The reduced morphology of duckweed and the infrequent occurrence of flower, seed formation, and the analogy of many morphological characters limit the number of morphological traits useful for taxonomy (Braglia et al. 2021). It is difficult to differentiate duckweeds due to its simple structure (Figure 1.1). They have a highly reduced morphology which brings difficulties in species recognition (Heuze and Tran 2015).

Therefore, the use of molecular taxonomic tool is the present-day solution to identify different tiny duckweed species (Bog et al. 2019). Braglia et al. (2021), used Tubulin-Based Polymorphism (TBP) Fingerprinting for differentiating duckweed species. TBP is a PCR-Based technique which has been tested for plant genotype differentiation (Braglia et al. 2021). Tubulin-Based Polymorphism Fingerprinting provided distinctive fingerprinting profiles and unravelled the relationships between some species of duckweed (Braglia et al. 2021).

The development of molecular biology techniques, especially those that analyze DNA, has revolutionized our understanding of taxonomy and has provided insights that were not possible through earlier methods such as chemotaxonomy (Bog et al. 2019). Bog et al. (2019), demonstrated that the five genera (*Spirodela, Landoltia, Lemna, Wolffiella* and *Wolffia*) of duckweed are monophyletic taxa. Les et al. (2002) mentioned that previous studies have investigated allozymes (Crawford et al. 1993; 1996; 1997; Crawford and Landolt 1995; Vasseur et al. 1993, Hirahaya and Kadono 1995,) and cpDNA (Beppu in Landolt 1986, Jordan et al. 1996). Les et al. (2002) showed that, there is high divergence at allozyme loci among congeneric species, even when the taxa are difficult to differentiate using morphological and anatomical characters (Crawford et al. 2006). Allozyme also supported the removal of *Wolffiella hyalina* and *Wolffiella repanda* from *Wolffiella* but only by inclusion of *Wolffiella rotunda* (Les et al. 2002). However, enzyme electrophoresis has been valuable in assessing genetic

variation in Lemnaceae species (Crawford et al. 2001).

Although molecular biology techniques have significantly advanced the understanding of taxonomy and systematics, numerous challenges and unanswered questions persist, starting from the precise delimitation of taxa in Lemnaceae, to their inter-relationships (Les et al. 2002). One of the important limitations is that classification of duckweed cannot be clarified with confidence due to lack of information (Bog et al. 2019).

Presently, chloroplast DNA has been used as an important tool in plant systematics and evolutionary studies due to its characteristics (maternal inheritance (in most plants), a relatively low rate of recombination, and a slower rate of evolution) compared to nuclear DNA (Olmstead and Palmer 1994; Jordan et al. 1996). Ding et al. (2017) stated three different kinds of genomes which consist of different evolutionary origins and histories coexist in plant cells which are nuclear, chloroplast and mitochondrial. It is explained that mitochondrial genomes are not the best choice for phylogenetic studies because their rate of rearrangements is fast compared to chloroplast genomes (Palmer and Herbon 1988). Making use of nuclear genomes on phylogenetic studies is restricted due to their complex and infeasibility of enough data but independent genealogical history can be found from chloroplast DNAs (Ding et al. 2017).

1.5 Classification of the Lemnaceae family

Appenroth et al. (2013) explained that Hegelmaier (1868) was the first publication to provide a reasonable classification of Lemnaceae within the taxonomic framework. In his classification, seven species of *Lemna*, two species of *Spirodela* and twelve species of *Wolffia* were identified (Figure 1.2).

Duckweed family was split into two sub-families (Tippery et al. 2015). That resulted into *Wolffia* and *Wolffiella* genera falling under the subfamily Wolffioideae (Figure 1.3). The subfamily Lemnoideae is characterized by *Lemna, Landoltia* and *Spirodela* genera (Figure 1.3). Subfamily Lemnoideae consists of four sections (*Lemna, Alatae, Biformes* and *Uninerves*) while subfamily Wolffioideae consists of six sections which

are *Wolffia, Pseudorrhizae, Pigmentate, Wolffiella, Stipitatae* and *Rotundae* (Tippery et al. 2020).

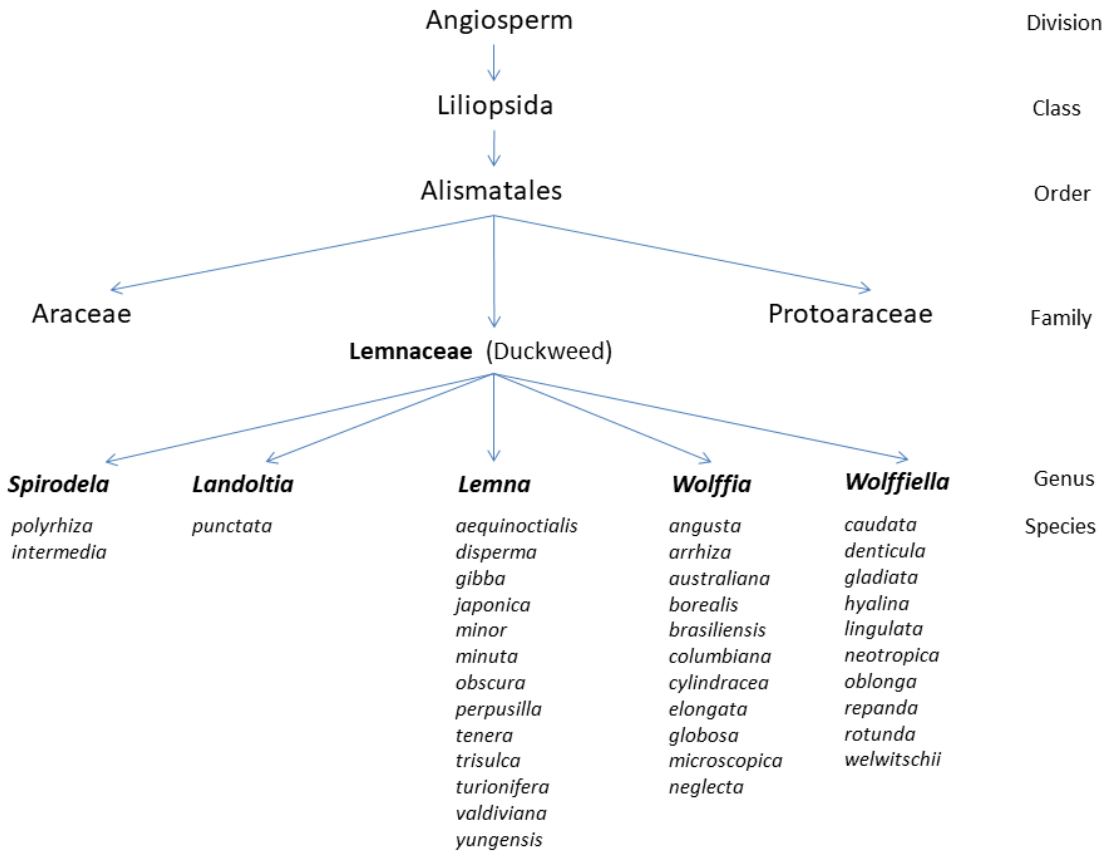

Figure 1.2: Structural classification and origin of the family Lemnaceae (Appenroth et al. 2013).

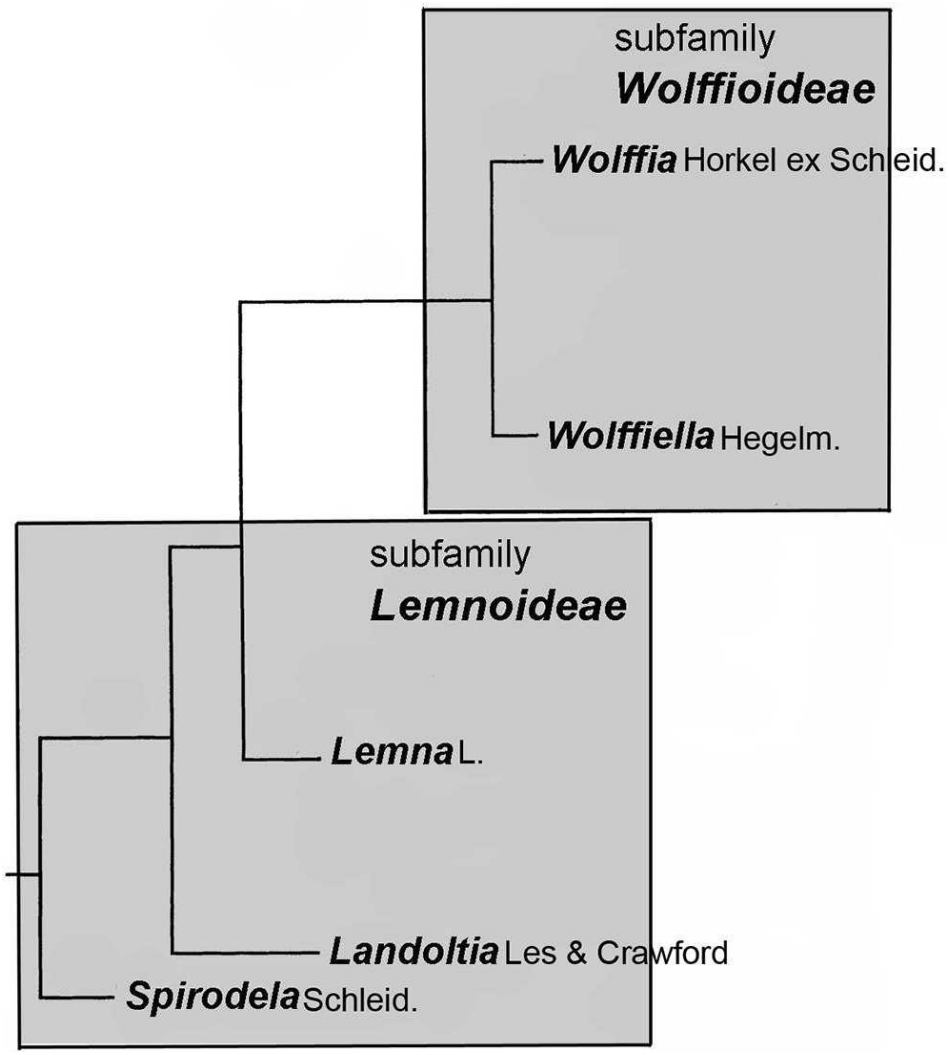

Figure 1.3: Structure of the family Lemnaceae in relation to genera (Tippery et al. 2015)

1.6 Ecological Niche modelling

Ecological niche modelling is a methodology built for species distribution modelling, a tool used to predict the distribution of species across geographic spaces based on the ecological conditions they are known to occupy. They are quantitative models developed by using data of the location of the species and variables that are assumed to influence the distribution (Trethowan et al. 2011; Elith and Franklin 2013). Models help to predict how Invasive alien plants distribution will change in the face of changing climate conditions.

Spatially explicit risk assessment results are provided from these models which in return can help to inform conservation managers with future prevention and control

efforts (Bradley et al. 2010). They can be applied to freshwater, marine, and terrestrial species. SDMs are popular in biodiversity research now due to the availability of geospatial data that has become more widely available.

Other uses for niche models include environmental impact assessments and land planning. For instance, predicting the risk of the species when it is established into a new area and determining appropriate locations for habitat restoration and reintroduction of the species (Elith and Franklin 2013). These models can be calibrated using a combination of both range data and invaded range data, or it can be used individually (Trethowan et al. 2011). Using both the invaded and native range data provides a better indication of the species likelihood to spread (Elith and Franklin 2013; Trethowan et al. 2011). Finding native range data may present a challenge to researchers as it can be a slow and costly process.

The literature may be in a foreign language and therefore hard to interpret the findings. Sampling bias can be a negative influence on ecological niche modelling; the calibration used on the model, based on the data, would not be a true representation of the climatic niche of the species (Wolmarans et al. 2010; Trethowan et al, 2011). The model performance can be assessed by using area under the curve (AUC) which ranges between 0 to 1. Reading of between 0.5 and 0.7 are regarded as poor and therefore considered to be no better than random predictions. Readings between 0.7 and 0.9 are regarded as good and therefore usable (Wolmarans et al. 2010).

1.7 The research problem

Biological invasions have been one of the main causes of biodiversity loss that has driven ecosystem degradation globally (Pyšek and Richardson 2010; Chen 2019). Wetlands are facing significant challenges from invasive alien species due to biological invasion, which can threaten native biodiversity, alter ecosystem processes, and degrade ecosystem services provided by wetlands (With 2002; Zedler and Kercher 2004). Some studies have shown that there is a tight link between species distribution and climate change; such that climate change might either increase or decrease the geographic range of species (Loarie et al. 2008; Pyšek and Richardson 2010; Willis et

al. 2010; Hoveka et al. 2016). Multiple rapid global change factors and anthropogenic effect has greatly aggravated the invasiveness of many plants in the world (Chen 2019). The changing atmosphere has affected invasive alien plants (Hellmann and Byers 2008) in such a way that many introduced invasive alien species change their climatic suitability, fecundities, and phenology (Willis et al. 2010), to adjust and thus becomes difficult to manage (Hellmann and Byers 2008; Chen 2019).

Climate change impact on invasive alien plants have received great attention globally in the past (Invasive species Council Australia 2009). How invasive alien species responds to climatic change has been a complex issue and more information is needed to facilitate the proactive invasive alien species management.

About R6.5 billion is lost every year in South Africa on controlling invasive alien species (De Lange and Van Wilgen 2010). Lemnaceae family commonly known as duckweed has been among the most problematic aquatic plant in South Africa (Freshwater habitats Trust 2013). They are very tiny plants with simplified morphology and difficult to distinguished among them (Les et al. 2002). Although, there are some taxonomic studies of duckweed that have been done in the past (Schleiden 1839; Landolt 1986) but the phylogenetic relationship studies at species level of duckweeds have been sparse. Ivanova (1973) explained a phylogenetic study of duckweed species, but the phylogenetic tree was on intrafamilial relationship with no character based and Borisjuk et al. (2014) used two gene markers to resolve the classification of duckweed species but with a limited number of species.

Duckweed species reproduce asexually, rapidly and increases in biomass (Henderson 2010; Zhao et al. 2014) making it difficult to control its rapid spread. This results in an increase in sedimentation, degraded pastures, crop, and enhanced mosquito breeding (Sainty et al. 1998). However, there is a limited literature on the effects of climate change on the current and future distribution of duckweed (Lemnaceae) species in South Africa. Analysing regional biodiversity in the context of global change is critical, given the rate at which this climate change is occurring. Therefore, this study explored how climate change affects the current and future distribution of the native and invasive alien duckweed (Lemnaceae) species in South Africa as there is a lot of research on invasive alien species and control management in South Africa but little

evidence on distribution prediction of duckweed species. The data gathered in this study will help ecologists and conservationists to pre-empt the distribution of these species and take appropriate actions to protect native biodiversity.

1.8 The aim and objectives of the study

Aim:

The main aim of this study was to identify duckweed species and elucidate how climate change may affect current and future distribution of duckweed (Lemnaceae) species in South Africa.

Objectives of the study:

- To identify duckweed species, phylogenetic relationships within the duckweed species were assessed using molecular DNA sequences from the plastids (*matK, rbcL, rps16, trnK (3')* and *trnK (5').*
- To investigate the effect of climate change on the duckweed plants in South Africa using species distribution models and identify hotspot areas for these duckweed species where management actions can be focused.

CHAPTER 2

MATERIAL AND METHODS

2.1 Plant collection

For plant collection for the study, plant materials were collected from three provinces in South Africa; North-West (Haartebeespoort Dam), Gauteng (Walter Sisulu Botanical Garden and Florida Dam) and Mpumalanga (Bankenveld Golf Club Emalahleni) as duckweeds are prevalent in these areas.

After collection, duckweeds were stored in a brown paper bag to drain off excess water. Duckweed plants were then stored in a cool room with their paper bags and the plant materials were pressed on a plant press and labelled accordingly. They were then placed at the University of South Africa herbarium. The location of samples collection was recorded. All the safety collection precautions were followed during collection.

2.2 Taxon sampling, DNA Extraction and PCR amplification

2.2.1 Taxon sampling

Molecular datasets of 33 taxa were undertaken resulting in 165 samples from previously published sequence data (Les et al. 2002, Table 2.1). The samples were retrieved from GenBank/NCBI. Additional 20 taxa resulting in 100 samples were collected from the field (i.e. North-West, Gauteng and Mpumalanga). Some collected duckweed plants were contaminated amongst the 20 plants collected while some were duplicates, and this resulted in 5 taxa and 50 samples. A molecular sequence data of five plastid region (*matK*, *rbc*L, *rpl*16, *trn*K (3') and *trn*K (5')) was generated. Sampling included five recognized genera (*Wolffiella*, *Wolffia*, *Lemma*, *Spirodela* and *Landoltia*) from the Lemnaceae family, with 39 currently accepted taxa that results in 195 samples from the two subfamilies (Lemnoideae and Wolffioideae).

Table 2.1: List of accession and voucher numbers. The species with asterick * represent voucher numbers collected in North-West, Gauteng, and Mpumalanga. Accession numbers were retrieved from Genbank (Les et al. 2002).

Species	**Genbank accession number**				
	matK	*rpL16*	*rbcL*	*trnK intron (3')*	*trnK intron (5')*
***Wolffiella rotunda* Landolt**	AY034200	AY034277	AY034238	AY034355	AY034316
***Wolffiella repanda* (Hegelm.)**	AY034201	AY034278	AY034239	AY034356	AY034317
***Wolffiella hyalina* (Delile) Monod**	AY034202	AY034279	AY034240	AY034357	AY034318
***Wolffiella denticulata* (Hegelm.) Hegelm.**	AY034209	AY034286	AY034247	AY034364	AY034325
***Wolffiella neotropica* Landolt**	AY034208	AY034285	AY034246	AY034363	AY034324
***Wolffiella welwitschii* (Hegelm.) Monod**	AY034207	AY034284	AY034245	AY034362	AY034323
***Wolffiella oblonga* (Phil.) Hegelm.**	AY034204	AY034281	AY034242	AY034359	AY034320
***Wolffiella lingulata* (Hegelm.) Hegelm.**	AY034203	AY034280	AY034241	AY034358	AY034319
***Wolffiella gladiata* (Hegelm.) Hegelm.**	AY034205	AY034282	AY034243	AYO34360	AY034321
***Wolffiella caudata* Landolt**	AY034206	AY034283	AY034244	AY034361	AY034322

***Wolffia brasiliensis* Wedd.**	AY034210	AY034287	AY034248	AY034365	AY034326
***Wolffia borealis* (Engelm.) Landolt**	AY034212	AY034289	AY034250	AY034367	AY034328
***Wolffia microscopica* (Griff.) Kurz**	AY034211	AY034288	AY034249	AY034366	AY034327
***Wolffia australiana* (Benth.) Hartog & Plas**	AY034213	AY034290	AY034251	AY034368	AY034329
***Wolffia elongata* Landolt**	AY034220	AY034297	AY034258	AY034375	AY34336
***Wolffia columbiana* H.Karst.**	AY034217	AY034294	AY034255	AY034372	AY034333
***Wolffia cylindracea* Hegelm.**	AY034218	AY034295	AY034256	AY034373	AY034334
***Wolffia arrhiza* (L.) Horkel ex Wimm.**	AY034216	AY034293	AY034254	AY034371	AY034332
***Wolffia globosa* (Roxb.) Hartog & Plas**	AY034219	AY034296	AY034257	AY034374	AY034335
***Wolffia neglecta* Landolt**	AY034214	AY034291	AY034252	AY034369	AY034330
***Wolffia angusta* Landolt**	AY034215	AY034292	AY034253	AY034370	AY034331
***Lemna yungensis* Landolt**	AY034188	AY034265	AY034226	AY034343	AY034304
***Lemna valdiviana* Phil.**	AY034187	AY034264	AY034225	AY034342	AY034303
***Lemna minuta* Kunth**	AY034186	AY034263	AY034224	AY034341	AY034302
***Lemna tenera* Kurz**	AY034189	AY034266	AY034227	AY034344	AY034305
***Lemna perpusilla* Torr.**	AY034191	AY034268	AY034229	AY034346	AY034307
***Lemna aequinoctialis* Welw**	AY034190	AY034267	AY034228	AY034345	AY034306

***Lemna trisulca* L.**	AY034199	AY034276	AY034237	AY0343541	AY034315
***Lemna japonica* Landolt**	UNA4*	UNA4*	UNA4*	UNA4*	UNA4*
***Lemna turionifera* Landolt**	AY034192	AY034269	AY034230	AY034347	AY034308
***Lemna obscura* (Austin) Daubs**	AY034194	AY034271	AY034232	AY034349	AY034310
***Lemna ecuadorensis* Landolt**	AY034193	AY034270	AY034231	AY034348	AY034309
***Lemna minor* L.**	UNA8*	UNA8*	UNA8*	UNA8*	UNA8*
***Lemna gibba* L.**	UNA20*	UNA20*	UNA20*	UNA20*	UNA20*
***Lemna disperma* Hegelm.**	UNA10*	UNA10*	UNA10*	UNA10*	UNA10*
***Spirodela polyrhiza* (L.) Schleid.**	AY034184	AY034261	AY034222	AY034339	AY034300
***Spirodela intermedia* (G.Mey.) C.H.Thomps.**	AY034183	AY034260	U68092	AY034338	AY034299
***Landoltia punctata* (G.Mey.) C.H.Thomps.**	UNA2*	UNA2*	UNA2*	UNA2*	UNA2*

2.2.2 DNA extraction and PCR amplification

Genomic DNA was extracted from 0.1g to 0.15g fresh plant materials using DNA extraction kit (i.e., Zymo Research, USA). The DNA extraction was done following the manufacturer protocol. Purification of samples was done using Zymo research kit.

All PCRs was performed using ReadyMix Master (Advanced Biotechnologies, Epson, Surrey, UK). Bovine serum albumin (3.2% BSA) was added to both plastid reactions. This additive serves as stabilizer for enzymes, reduces problems with secondary structure, and improves annealing (Palumbi 1996). PCR amplifications were performed using 9800 Fast Thermal Cycler. Program protocol for the amplification of *matK* and *rbcL* regions were as follows: denaturation 95° C for 1min 15 sec, annealing 55° C for 2 min, extension 72° C for 2 min 15 sec and final extension was 72° C for 5 min with 30 cycles. Program protocol for the amplification of *rpl*16 and *trn*K's regions were as follows: denaturation 95° C for 45 sec, annealing 52° C for 45 sec, extension 72° C for 45 sec, final extension 72° C for 5 min with 35 cycles. The primers used for PCR reactions are listed in Table 2.2. Verification of PCR products were done by electrophoresis in 1% agarose gels stained with ethidium bromide. The PCR products were sent to the Inqaba laboratory Biotechnology for cycle sequencing.

Table 2.2: Details of primers used for PCR amplification.

Locus	**Primer**	**Reference**
Rpl16		
F71	5'-GCTATGCTTAGTGTGTGACTCGTTG-3'	Jordan et al. 1996
R622	5'- CCAACCCAATGAATCATTAGGATT-3'	Posno et al.1986
matK		
390F	5'-CGATCTATTCATTCAATATTTC-3'	Les et al. 1999
1326R	5'-TCTAGCACACGAAAGTCGAAGT-3'	Les et al. 1999
trnK		
F (3914F)	5'-ATG TGG GTT GCT AAC TCA ATG G-3'	Les et al. 1999
2R	5' AAC TAG TCG GAT GGA GTA G-3'	Les et al. 1999
rbcL		
1F	5′-ATG TCA CCA CAA ACA GAA ACT AAA GC-3'	Les et al.1993
724R	5'TCGCATGTACCTGCAGTAGC-3'	Les et al.1993

2.3 Data analyses

Complementary strands were assembled, and edited using Sequencer 3.1 (Gene Codes, Ann Arbor, Michigan, USA). *matK, rbcL, rpl16, trnK (3')* and *trnK (5')* sequences were aligned manually in PAUP* (version 4.0b.10; Swofford 2003). All the sequences were aligned using the program MUSCLE.

2.4 Phylogenetic analyses

Maximum parsimony (MP) was performed for both separated and combined analysis. Incongruence length difference test and visual comparisons were employed to detect incongruences among the data sets. Aligned data matrix had a combined 4504 characters and thirty-nine taxa. The tree searches were analysed using Heuristic search which was performed with PAUP version.4.0b10 program (Swofford 2003) with 1000 replications of random taxon addition, holding 10 trees at each step during stepwise addition with tree bisection reconnection (TBR) branch swapping algorithm and saving multiple equal parsimonious trees (MulTrees). Data matrices were analyzed with uninformative characters excluded.

Internal support was accessed with one thousand bootstrap replicates using simple stepwise addition, but only holding one tree per replicate. Only groups of greater than 50% frequency were reported. DELTRAN (Delayed transformation) characteristic optimization was used instead of (ACCTRAN) acceleration of transformation due to reported errors with the version of PAUP version.4.0b10.

Bootstrap analysis was used to estimate the support for each clade (Felsenstein, 1985) using TBR swapping with Fitch weights and retaining one tree per replicate. Bootstrap support was categorized as high (85–100%), moderate (75–84%) and low (50–74%).

Bayesian Inference (BI) analysis was done using MrBayes version 3.2.7 (Ronquist and Huelsenbeck 2003) using four parallel Markov Chain Monte Carlo (MCMC) that runs for ten million generations. Prior to Bayesian analysis, a model test was run to determine which evolutionary model best suits the data sets. All five regions had the same model test. The resulting trees were plotted against their likelihoods. The point

where the likelihoods converged on a maximum value was determined. All tree file outputs were burnt using tree annotator v. 2.7.4 and the trees were visualized on FigTree, a graphical viewer of phylogenetic trees. Since there was confusion of duckweed species being invasive alien or native, the species were then verified using POWO (2017).

2.5 Species occurrence data

To obtain the species occurrence data, the distribution data for duckweed species were sourced from the Botanical Research and Herbarium Management System (BRAHMS). However, duplicate records were removed from the data. Data obtained from this platform is up to date. This study included 38 duckweed species which 31 are invasive alien and seven are native species. Point data were cleaned to remove records with indefinite localities. Species with less records were removed.

2.6 Climate data

Climate data was collected from 19 raster-based bioclimatic parameters (Table 3.2) for both current and future climate scenarios. Thereafter, spatially downscaled estimates of future climate for the year 2080 were obtained from the WorldClim database (Philips et al. 2006 (www.worldclim.org)) at a spatial resolution of 2.5 arcminutes using the Canadian Earth System Model (CanESM5), Model for Interdisciplinary Research on Climate (MIROC6) and Earth Consortium System Model (EC-Earth3-Veg). After, environmental variables were interpolated onto ArcGIS grids to ensure that all spatial data have the same geographic bounds and cell size as the study region. Spatially downscaled estimates of future climate for the year 2080 was obtained using the most up to date climate change projections.

2.7 Species distribution modelling

Predictive models for current and future distribution of duckweed species were generated from MaxEnt version 3.4.4. Even if there is an issue of spatial autocorrelation in most species' distribution models, methods on how to correct or test for correlation between climatic variables are still not standardized (Lennon 2000;

Dormann 2007). Nevertheless, spatial autocorrelation was tested in all environmental variables to address the issue of multicollinearity. Analysis excluded variables that showed correlation strength above this range. Jackknife statistics was used to evaluate the relative contribution of each of the 19 predictor variables to the models using the area under the curve (AUC) score (Pearson et al. 2007). An AUC value of 0.5–0.7 indicates poor performance, 0.7–0.9 indicates acceptable performance, and AUC 0.9 indicates high performance (Peterson et al. 2011). “Do jackknife to measure variable important” was checked on MaxEnt to run a jackknife test which helps to identify important variables when running a model. The results of the jackknife appear in the “bradypus.html” files in three bar charts. Comparing the three jackknife plots can be very informative on selecting the suitable environmental variables (Philips and Dudik 2008). All models were ran again using only the best predictor variables, assigning 75% of the occurrence data for model training and the remaining 25% for model testing. About 15 subsampling replicates were run for each model to measure the variability in the model performance, and the default iteration parameter was adjusted to 5 000, which is sufficiently large to ensure model convergence. The 10th percentile training presence threshold to generate prediction probability maps was employed (Phillips and Dudik 2008). Model outputs followed a logistic distribution, with values ranging from 0 (indicating areas that are climatically unsuitable) to 1 (indicating areas that are climatically suitable) for species persistence.

2.8 Determination of habitat suitability

Conversion of MaxEnt output projection (current and predicted future climate) parameters from ASCII to Raster float was performed using the ArcGIS software (ESRI ArcGIS version 10.8). Spatial Analyst tools in ArcGIS software was used to calculate changes in geographical ranges of each species between current and future climate (Hoveka et al. 2016). The differences in projected shifts in climatic extent was calculated (estimated as the number of pixels gained or lost) such that species with an increased probability of occurrence under future climate projections were assigned a positive value (i.e., range expansion), whereas species with a decreased probability of occurrence under future projections were assigned a negative value using Zonal Statistics extension (i.e., range contraction).

CHAPTER 3

RESULTS

3.1 Phylogeny

The combined DNA matrix was made of five plastids regions (*rbcL* + *matK* + *rpl16* + *trnk (3')* + *trnK (5'))* which resulted in combined 4504 number of characters, 3262 constant characters and 809 parsimony informative characters (Table 3.1). Among the plastid regions*, trnK (5')* showed the lowest number of parsimony informative characters (69), *rpl16* (104), *rbcL* (116), *trnK (3')* 165 and *matK* recorded the highest parsimony information characters (355) (Table 3.1). Number of parsimony uninformative characters for *matK* (169) was higher when compared to *trnK (3')* (95), *rpL16* (72), *rbcL* (55), and *trnK (5')* (42).

Using this DNA matrix dataset, the Bayesian phylogenetic tree constructed yielded a strong bootstrap support (91 BP) for the family Lemnaceae. The phylogenetic results revealed two distinct major clades with strong bootstrap support for the subfamily Lemnoideae (100 bootstrap support) and subfamily Wolffioideae (100 bootstrap support) (Figure 3.1). The phylogenetic tree of this study recovered duckweed subfamilies and their sections. Subfamily Lemnoideae consists of four sections (*Lemna, Alatae, Biformes*, and *Uninerves*). Then, subfamily Wolffioideae consists of six sections which are *Wolffia, Pseudorrhizae, Pigmentate, Wolffiella, Stipitatae*, and *Rotundae* that were strongly supported (Figure 3.1). Within the subfamily Wolffiodeae, the clade of the genus *Wolffiella* had three sections (*Rotundae, Stipitatae* and *Wolffiella*) that was highly supported (100BP). While *Wolffia* and *Pseudorrhizae* sections have 99 bootstrap supports. Section *Lemna, Uninerves, Biformes* and *Alatae* are also well supported with a bootstrap support of 100. All the sections within the subfamilies were found to be monophyletic.

Spirodela polyrhiza and *Spirodela intermedia* indicated a strong support of 100 BP (Figure 3.1). *Spirodela polyrhiza, Spirodela intermedia* and *Landoltia punctata* were assigned as outgroups based on Les et al. (2002). There has been an argument regarding Lemnaceae species being invasive alien or native to South Africa. Therefore, the results of this study confirmed that there are seven native Lemnaceae

species within the phylogenetic tree (Figure 3.1). Within the two subfamilies, the subfamily Lemoideae had *Lemna aequinoctialis*, *Lemna minor*, *Lemna gibba* and *Spirodela polyrhiza* as native species. Subfamily Wolffioideae included, *Wolffiella welwitschii*, *Wolffiella denticulata*, *Wolffia arrhiza* and *Wolffia globosa* as native to South Africa. The phylogenetic tree was dominated by 31 invasive alien species indicated in red colour while native species are indicated in green colour (Figure 3.1).

Table 3.1: The summary of DNA matrix and Maximum Parsimony (MP) statistics for the aligned, analysed and number of informative for each gene regions used.

	matK	***rpl16***	***rbcL***	***trnK(3')***	***trnK(5')***	**Combined Plastids**
No. of taxa	38	38	38	38	38	38
No. of included characters	1554	512	1348	810	280	4504
No. of constant characters	1030	336	1177	550	169	3262
No. of parsimony informative sites	355	104	116	165	69	809
No. of trees (Fitch)	880	327	319	466	205	2233
No. of parsimony uninformative sites	169	72	55	95	42	433

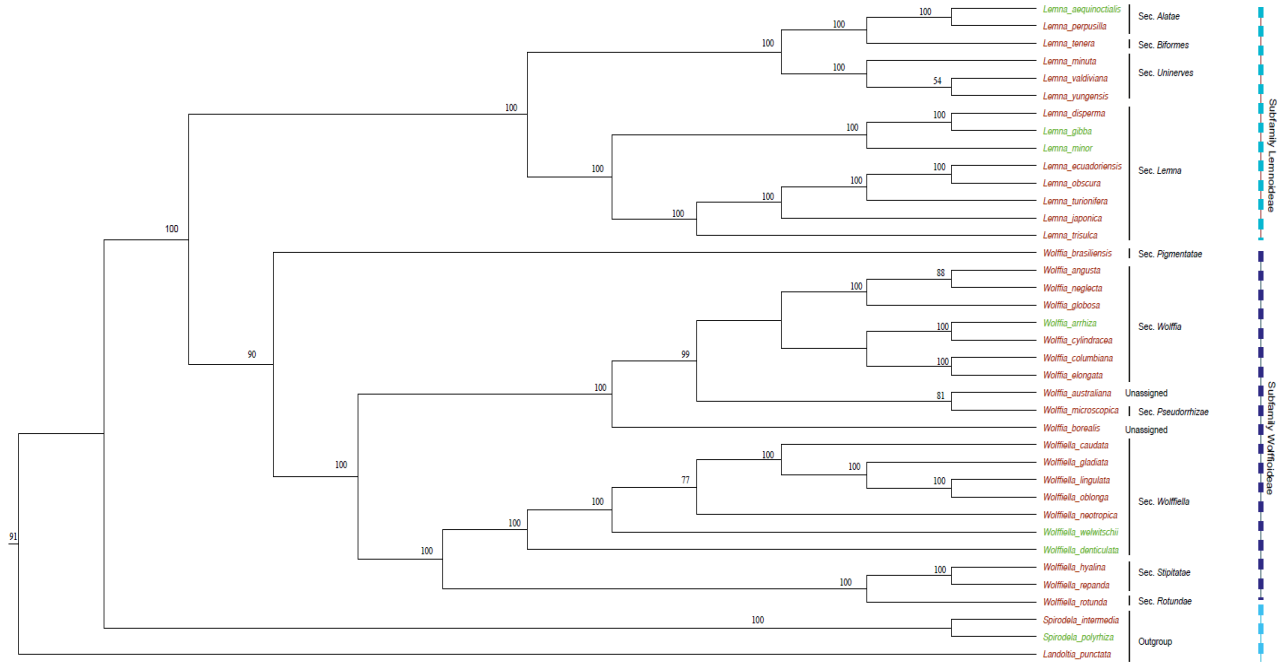

Figure 3.1: Bayesian phylogenetic tree of duckweed species based *on matK, rpl16, rbcL,trnK (3')* and *trnK (5')* regions. Bootstrap values are shown above the branches. Green colour indicates native species and red colour indicates invasive alien species. Light blue represents subfamily Lemnoideae and navy blue represents subfamily Wolffioideae

3.2 Climate change

For ecological niche modelling, 19 climatic variables (Table 3.2) were used for current and future scenarios. Values of minimum temperature, maximum temperature and precipitation were processed for three General Circulation Models. Four shared Socio-Economic Pathways (SSPs): 126, 245, 370 and 585 were used to run the models.

Table 3.2: 19 Bioclimatic variables used when running current and future models.

Bioclimatic variable code	Meaning
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (×100)
BIO4	Temperature Seasonality (standard deviation ×100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

An expected change in species richness maps across South Africa for current and future climate was obtained (Figure 3.2–3.8).

Current richness map showed abundance of duckweed species in the coastal provinces such as KwaZulu-Natal, Eastern Cape, and some parts of Western-Cape (Figure 3.2). Future climate suitability maps obtained from stacking individual duckweed species distribution showed coastal provinces as more suitable areas for duckweed species (KwaZulu-Natal, Eastern Cape, and Western Cape (Figure 3.3–3.5)). Mapping the difference between current and future climate scenarios showed

contraction of duckweed species in coastal provinces such as KwaZulu-Natal, Eastern Cape, and Western Cape (Figures 3.6B and 3.7D). Nevertheless, predictions have indicated that most duckweed species will experience contraction in the future. Fundamentally, we predicted further spread of duckweed species into several areas such as North-West, Gauteng, Mpumalanga, and Free State (Figure 3.6C; Figure 3.6D). The provided mapping has shown that North-West, Gauteng, Mpumalanga and Free State provinces might experience range expansion of duckweed species (Figure 3.6C; Figure 3.6D).

We obtained averagely consistent results across emission scenarios and GCMs. Current and future models showed some of the dams in different provinces that may be infested with duckweed species in the future (Table 3.3). For example, Free State, Gauteng, Limpopo, Mpumalanga, and North-West provinces showed range expansion of duckweed species (Table 3.3).

The results predicted range expansion of *Lemna aequinoctialis* (Figure 3.9A, Table 3.4) and contraction of six duckweed species (*Lemna gibba, Lemna minor, Spirodela polyrhiza, Landoltia punctata, Wolffia arrhiza* and *Wolffiella denticulata*) in the future (Figure 3.10; Figure 3.11; Table 3.4).

EC-Earth3-Veg and MIROC6 Global Circulation Models suggested that over 90% of duckweed species in our analyses may experience a decrease in climatic suitability (Figure 3.10; Figure 3.11; Table 3.4). The models showed great contraction of *Lemna aequinoctialis*, *Lemna gibba* and *Spirodela polyrhiza* in future projections (Figure 3.10A; Figure 3.10B; Table 3.4). However, *Spirodela polyrhiza* has decreased in climatic suitability in the future (Figure 3.9B; Figure 3.11D; Table 3.4).

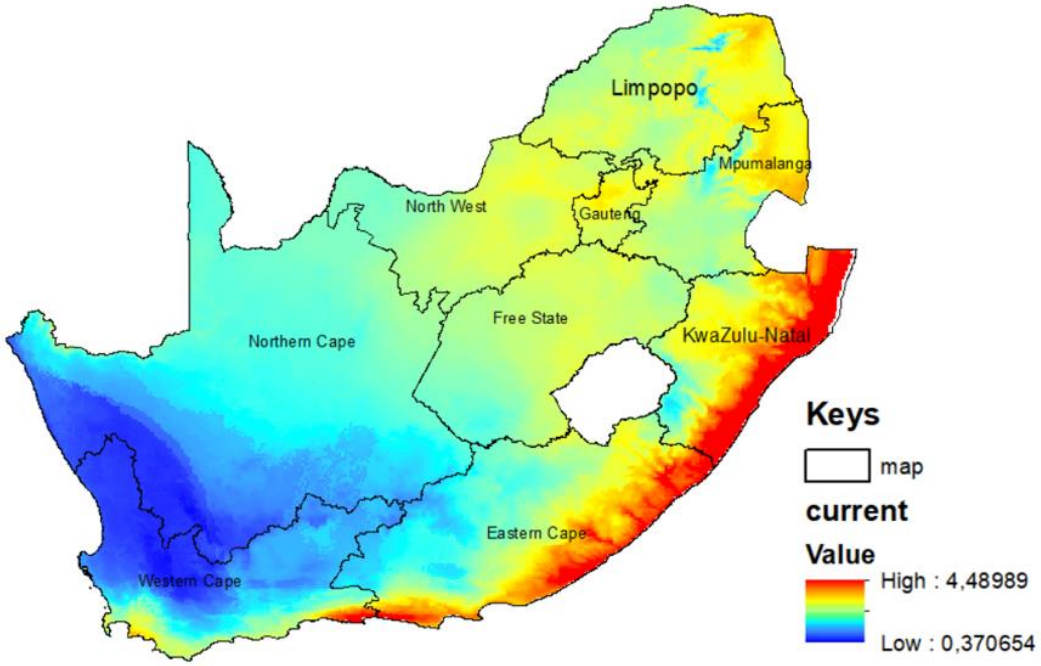

Figure 3.2: Current climate suitability map obtained from stacking individual duckweed species distributions. Red colours indicate most suitable areas and blue colours indicate least suitable areas.

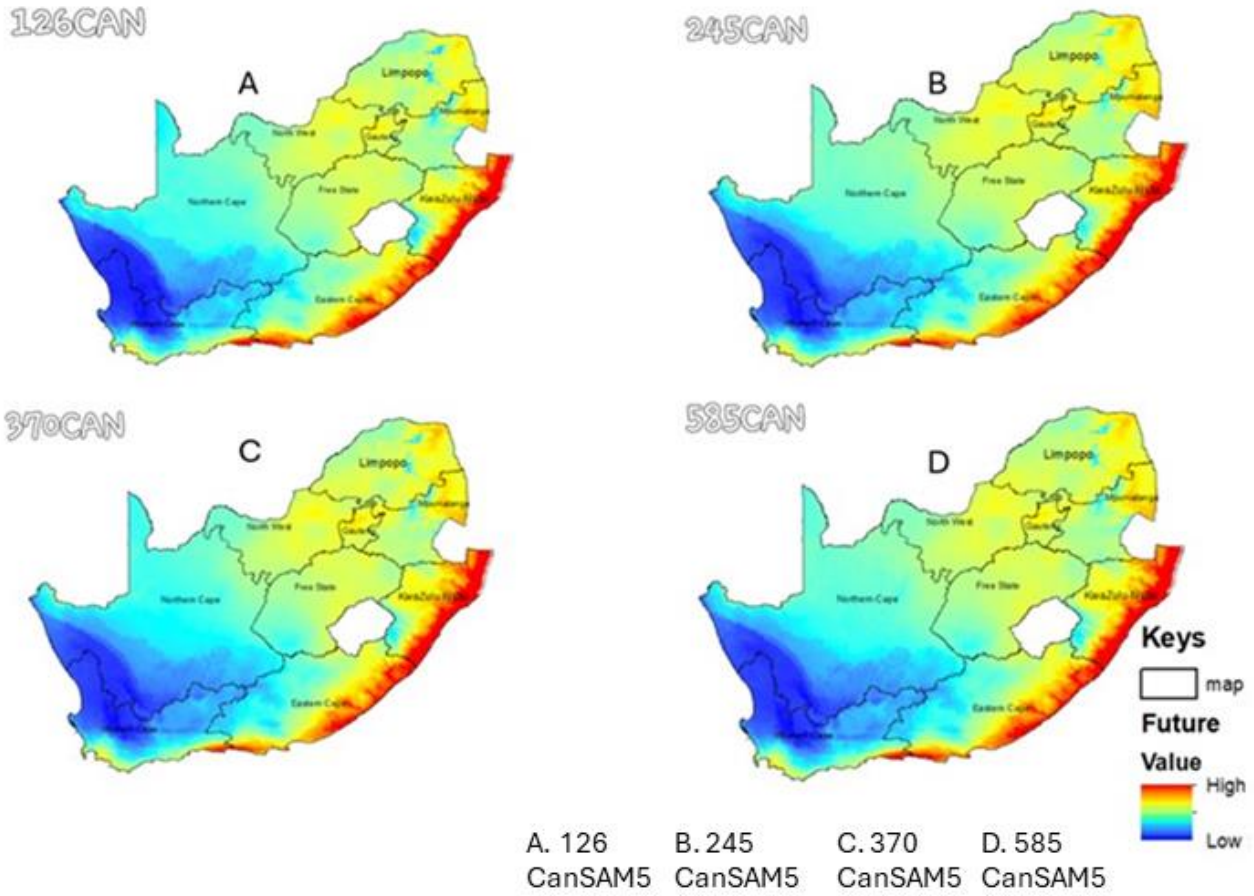

Figure 3.3: Future climate suitability map obtained from stacking individual duckweed species distributions run with CanSAM5 GCM

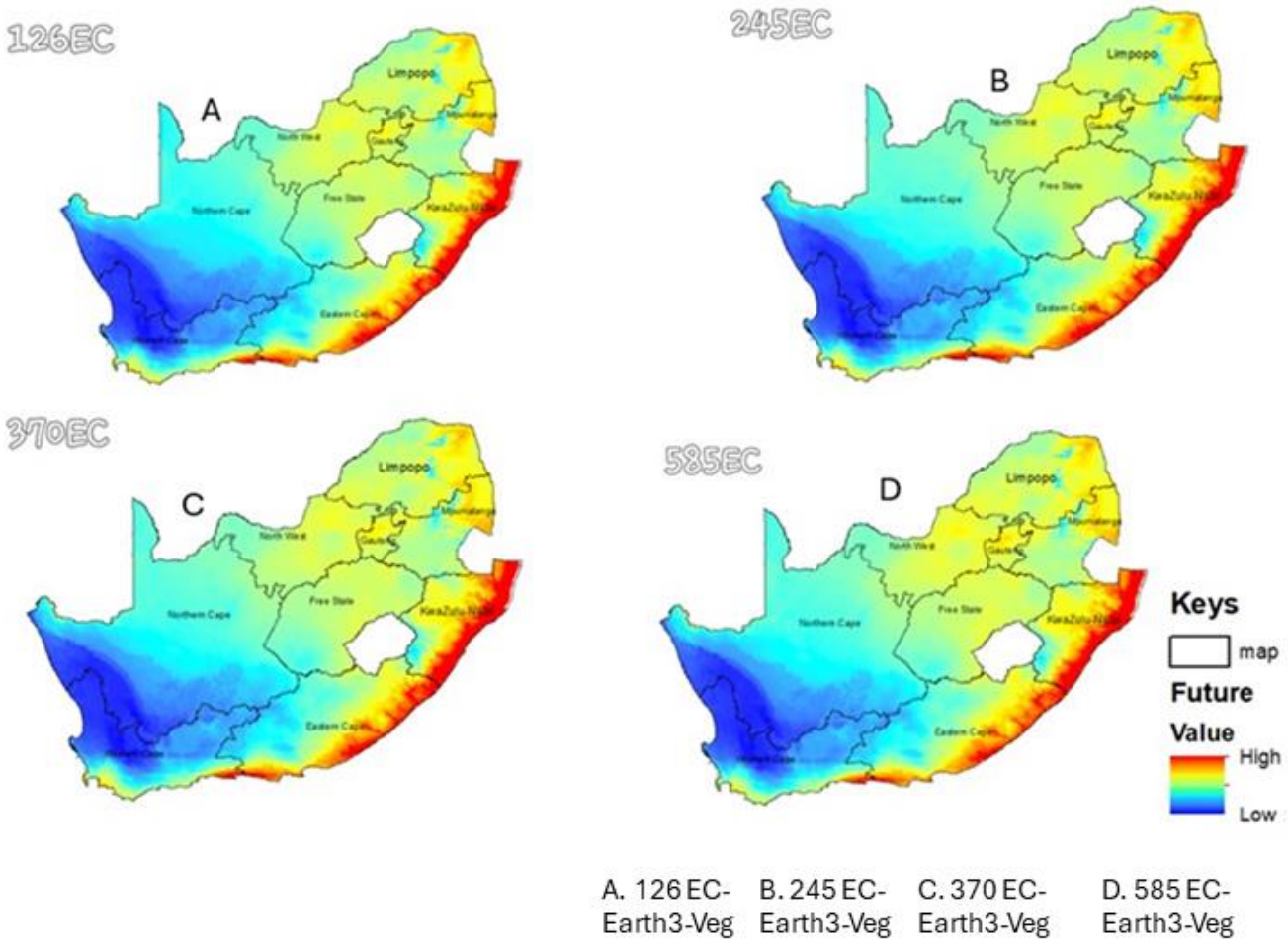

Figure 3.4: Future climate suitability map obtained from stacking individual duckweed species distributions run with EC-Earth3-Veg GCM

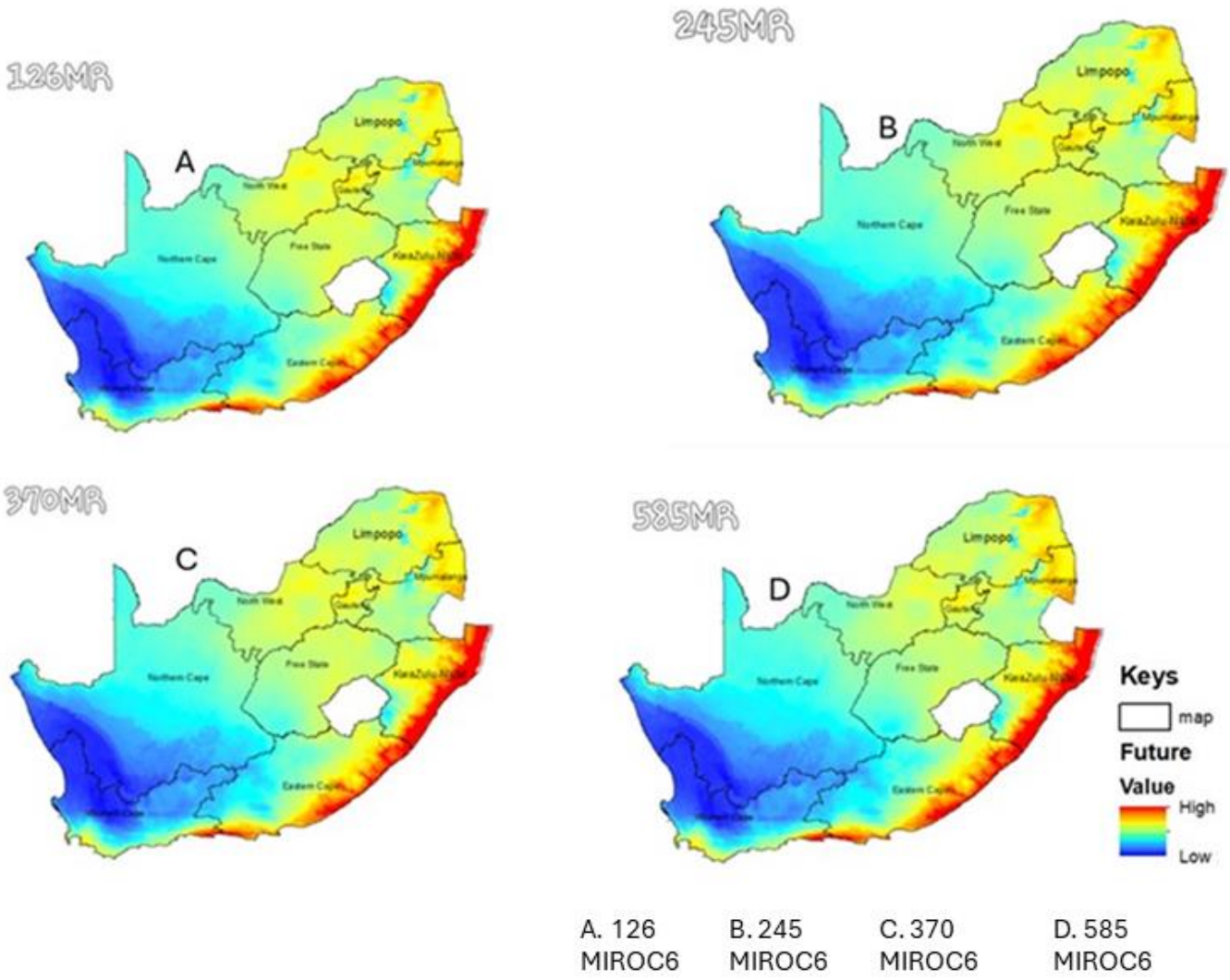

Figure 3.5: Future climate suitability map obtained from stacking individual duckweed species distributions run with MIROC6 GCM.

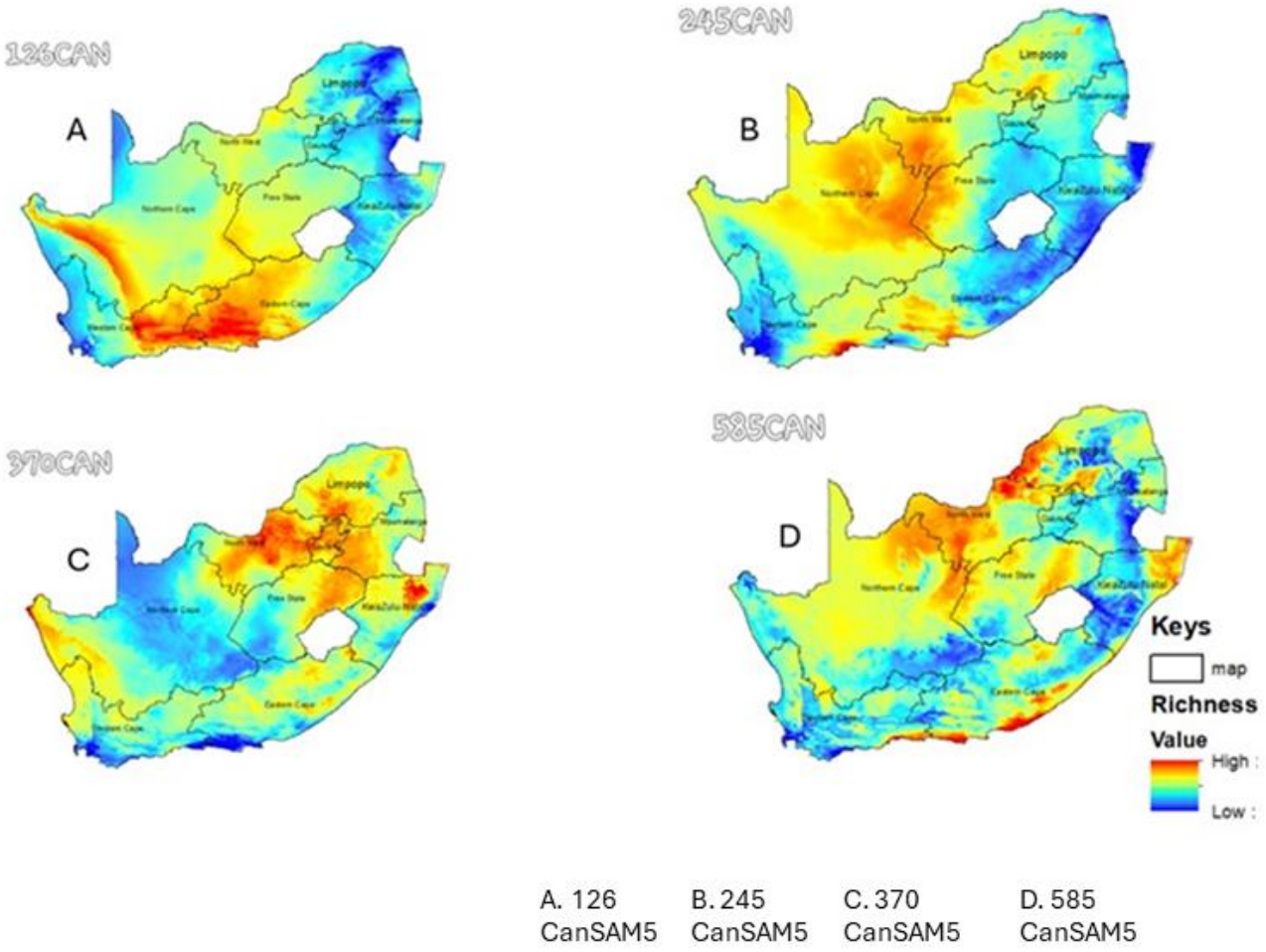

Figure 3.6: Difference (Future - Current) suitability map contoured from stacking individual duckweed species distributions ran with CanSAM5 GCM.

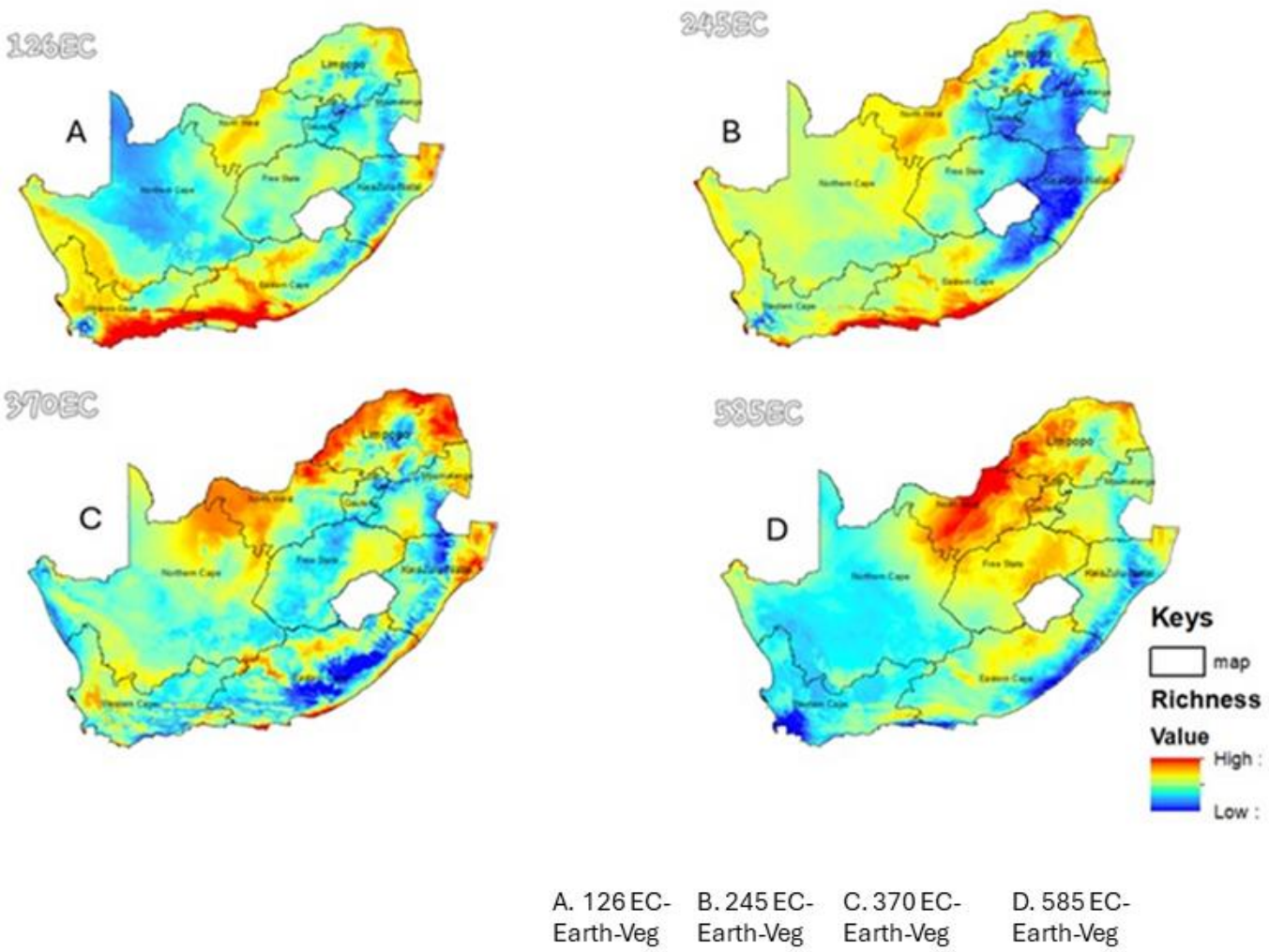

Figure 3.7: Difference (Future - Current) suitability map contoured from stacking individual duckweed species distributions ran with EC-Earth-Veg GCM

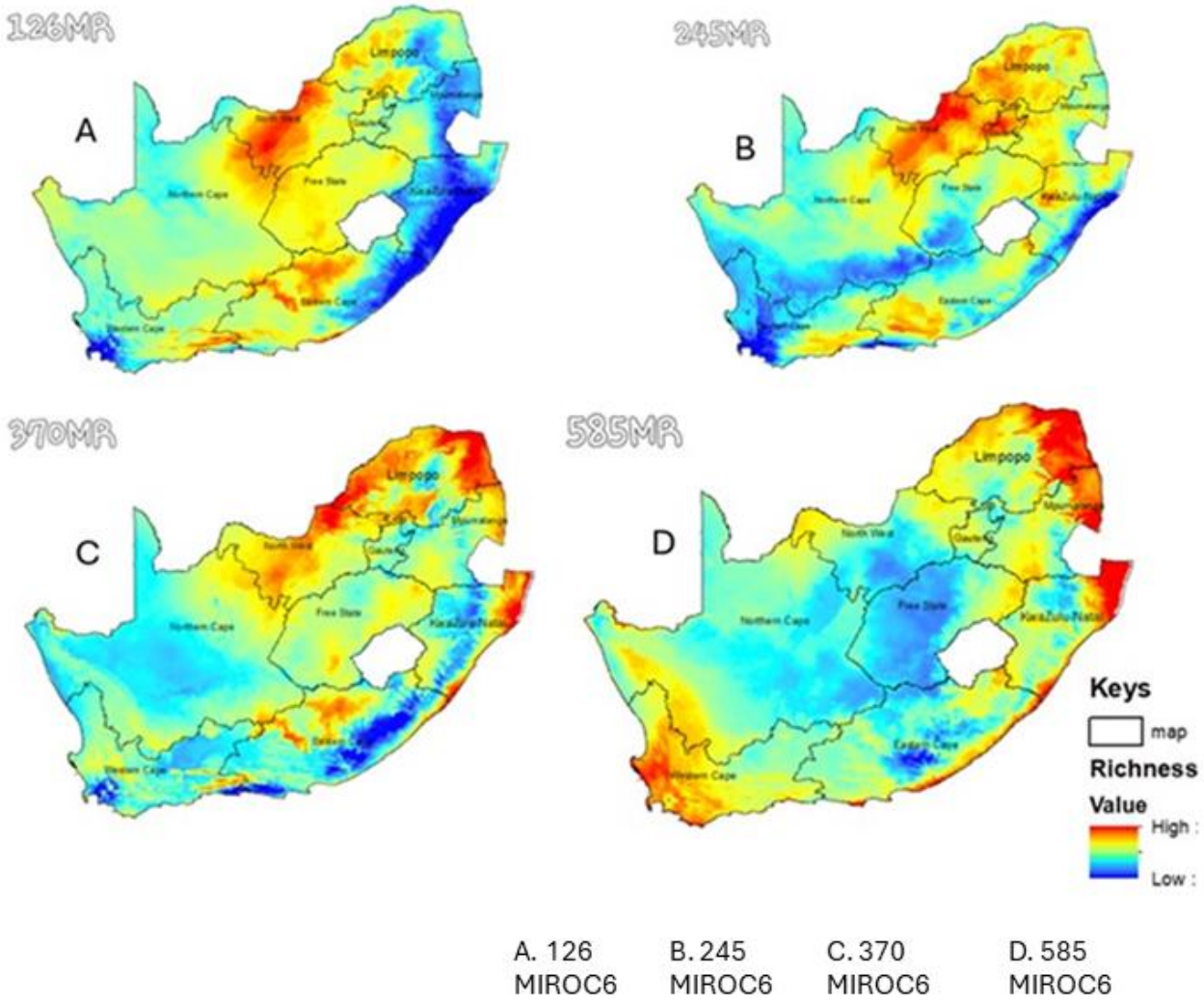

Figure 3.8: Difference (Future - Current) suitability map contoured from stacking individual duckweed species distributions ran with MIROC6 GCM.

Table 3.3: Dams that are most likely to be infested by duckweed species as per future models.

Provinces	Name of Dams
1. Free State	a. Vaal (Border of Gauteng and Free State) b. Saliba
2. Gauteng	a. Bon Accord b. Roodeplaat c. Rietvlei d. Evaporation e. Modderfontein Number 1, 2, 3 and 4 f. Darrenwood g. Emmarentia h. Westdene i. Cinderella j. Monument k. Eeufees l. Princess m. Fleurhof n. New Canada o. Orlando p. Premiermyn
3. Limpopo	a. Donkerpoort b. Matukwala c. Phugwane d. Mooigesig c. Shangoni d. Hoenderkop e. Hudson Ntsanwisi f. KaMakhaveni g. Mintomeni

4. Mpumalanga	a. Kruger b. Robertson c. Graham d. Athlone e. Pienaars f. Doringpoort g. Middelburg h. Witbank i. Clewer
5. North-West	a. Taaibosspruit b. Madikwane c. Lehujwane d. Kromellenboog e. Marico-Bosveld f. Molatedi g. Linley's poort h. Swartruggens i. Klein-maricopoort j. Klerkskraal k. Boskop l. Hartbeespoort

Table 3.4: Differences between three future GCMs values and current values.

	Specie name	Current value	GCM CanSAM5 (Future)				GCM EC-Earth3-Veg (Future)				GCM MIROC (Future)			
1	*Lemna aequinoctialis*	21667;838	**126**	**245**	**370**	**585**	**126**	**245**	**370**	**585**	**126**	**245**	**370**	**585**
			22354;3488	507;410239	-1035;622621	323;39367	-3123;522236	1470;16164	-373;32325	139;347222	-1578;740326	-429;039766	-2100;124435	899;509072
	Future - Current		686;5108	-21160;42776	-22703;46062	-21344;44433	-24791;36024	-20197;67636	-22041;16125	-21528;49078	-23246;57833	-22096;87777	-23767;96244	-20768;32893
2	*Lemna gibba*	21144;65574	**126**	**245**	**370**	**585**	**126**	**245**	**370**	**585**	**126**	**245**	**370**	**585**
			1323;274367	-578;037711	479;997429	1438;116922	1373;07544	469;931977	2999;515449	-956;786568	1717;129964	1379;325035	1954;780764	1023;289882
	Future - Current		-19821;38137	-21722;69345	-20664;65831	-19706;53881	-19771;5803	-20674;72376	-18145;14029	-22101;4423	-19427;52577	-19765;3307	-19189;87497	-20121;36585
3	*Lemna minor*	13063;86944	**126**	**245**	**370**	**585**	**126**	**245**	**370**	**585**	**126**	**245**	**370**	**585**
			464;324311	218;970923	470;571219	190;175858	662;375961	75;334425	174;109867	68;150806	966;893549	747;259722	419;806855	-635;103383
	Future - Current		-12599;54512	-12844;89851	-12593;29822	-12873;69358	-12401;49347	-12988;53501	-12889;75957	-12995;71863	-12096;97589	-12316;60971	-12644;06258	-13698;97282
4	*Spirodela*	23967;468	**126**	**245**	**370**	**585**	**126**	**245**	**370**	**585**	**126**	**245**	**370**	**585**

	polyrhiza		157;833609	5084;125099	1962;139554	56;986312	254;194963	-1563;235722	-1602;610894	1677;208349	3437;65307	3323;680441	-13;124961	-4829;929811
	Future - Current		-23809;63439	-18883;3429	-22005;32845	-23910;48169	-23713;27304	-25530;70372	-25570;0789	-22290;25965	-20529;81493	-20643;78756	-23980;59296	-28797;39781
5	*Landolia punctata*	9751;648887	**126**	**245**	**370**	**585**	**126**	**245**	**370**	**585**	**126**	**245**	**370**	**585**
			-578;423085	-894;019483	67;078966	163;396595	-9;291339	-910;400719	-429;193573	150;530582	-926;334078	810;796767	-225;375389	193;160698
	Future - Current		-10330;07197	-10645;66837	-9684;569921	-9588;252292	-9760;940226	-10662;04961	-10180;84246	-9601;118305	-10677;98297	-8940;852217	-9977;024276	-9558;488189
6	*Wolffia arrhiza*	14203;23229	**126**	**245**	**370**	**585**	**126**	**245**	**370**	**585**	**126**	**245**	**370**	**585**
			1536;822247	761;149579	-309;203048	544;241301	-55;104487	-482;042935	10;028864	61;482501	1174;944704	828;793204	1060;769624	156;327118
	Future - Current		-12666;41005	-13442;08272	-14512;43534	-13658;99099	-14258;33678	-14685;27523	-14193;20343	-14141;74979	-13028;28759	-13374;43909	-13142;46267	-14046;90518
7	*Wolffiella denticulata*	2766;822461	**126**	**245**	**370**	**585**	**126**	**245**	**370**	**585**	**126**	**245**	**370**	**585**
			220;229946	507;955117	37;016438	192;075017	291;768662	483;069028	641;396419	-245;116012	-278;863593	42;609197	65;94601	229;091469
	Future - Current		-2546;592515	-2258;867344	-2729;806023	-2574;747444	-2475;053799	-2283;753433	-2125;426042	-3011;938473	-3045;686054	-2724;213264	-2700;876451	-2537;730992

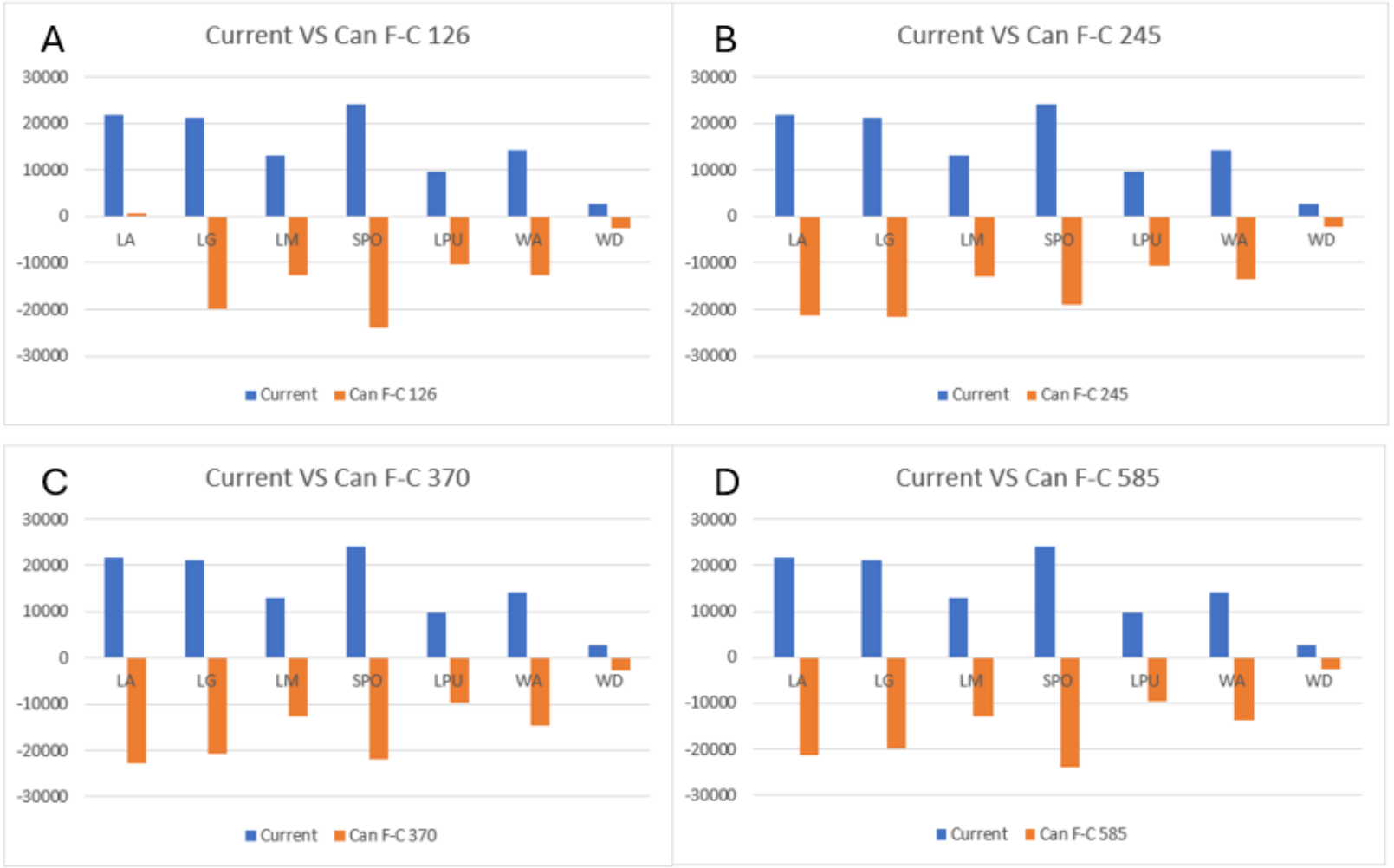

Figure 3.9: Percent change in habitat suitability from species distribution models contoured to current climate and CanSAM5 GCM

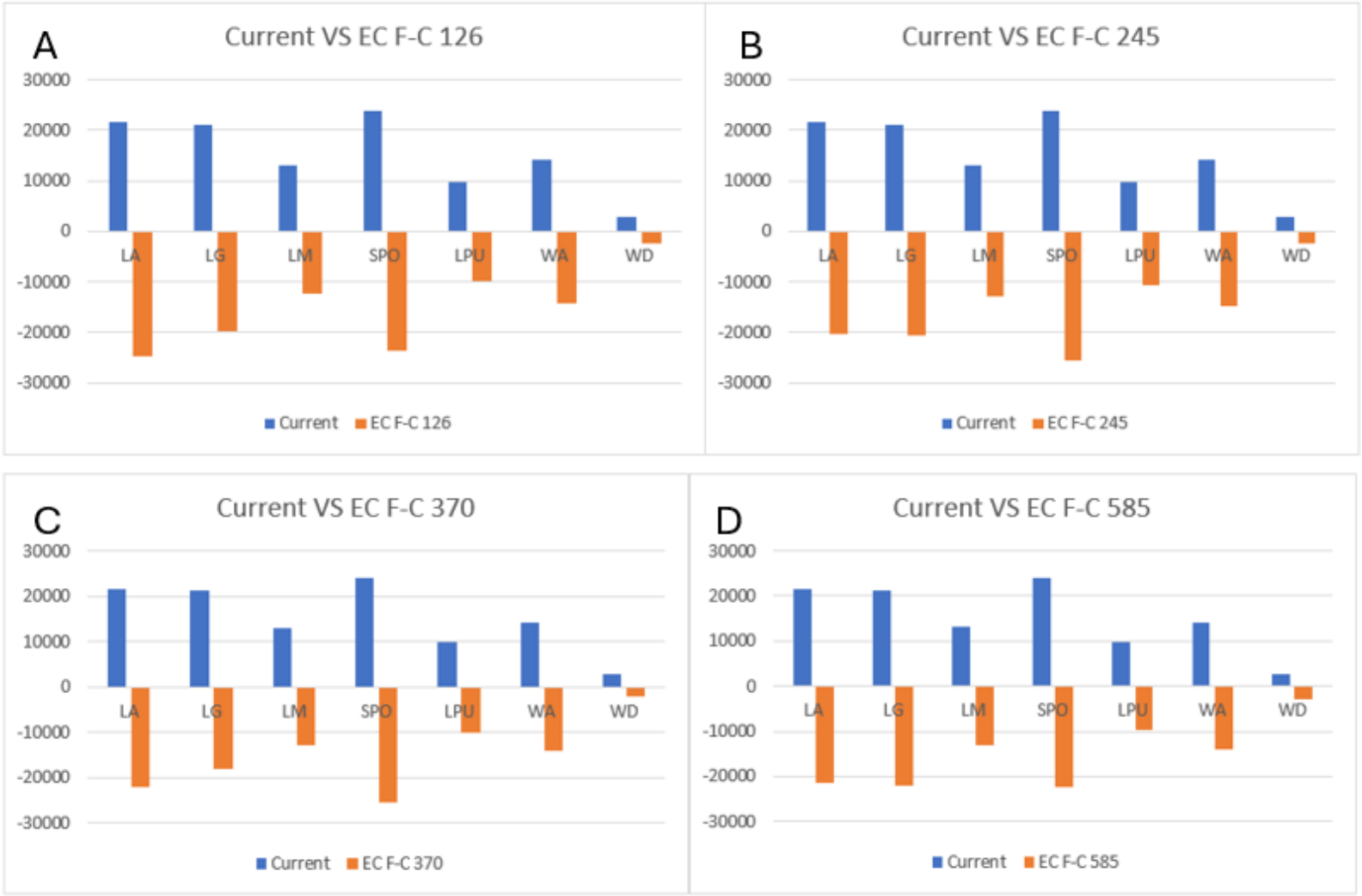

Figure 3.10: Percent change in habitat suitability from species distribution models contoured to current climate and EC-Earth3-Veg GCM

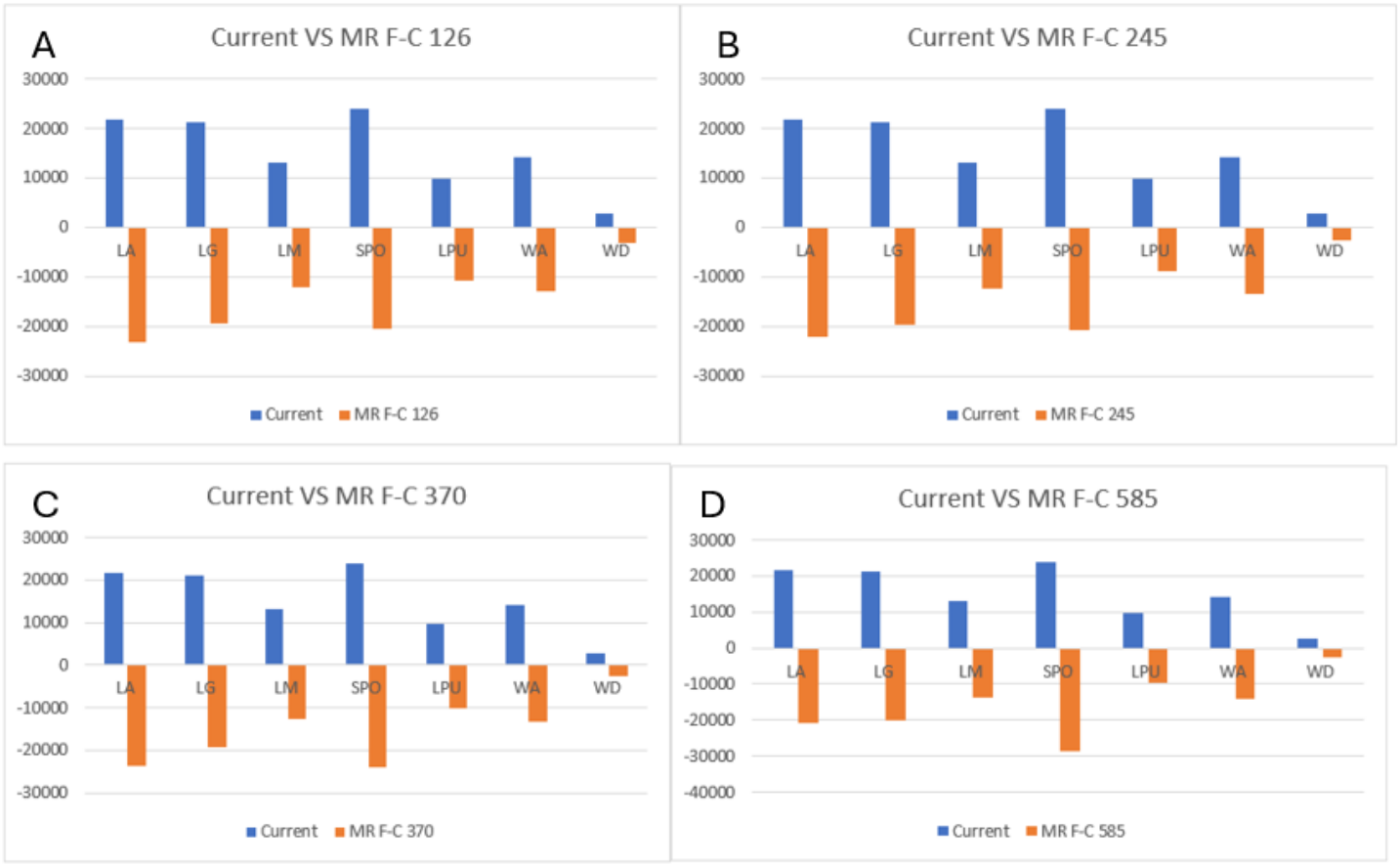

Figure 3.11: Percent change in habitat suitability from species distribution models contoured to current climate and MIROC6 GCM

CHAPTER 4

DISCUSSION

4.1 Phylogeny

When there is a high degree of reduction in structural complexity among closely related species of duckweeds, distinguishing between them becomes even more challenging (Braglia et al. 2021). Without distinct morphological differences to rely on, taxonomists may need to resort to other methods for species identification, such as molecular techniques like DNA sequencing (Bog et al. 2019). While some earlier studies may have used a limited number of genes for duckweed identification (Borisjuk et al. 2014), this study efforts have embraced larger-scale genomic approaches to overcome these limitations and provide a more robust understanding of interspecific relationships within the group.

The first part of this research focused on the identification and reconstruction of a robust phylogeny of duckweed species using five plastids' gene regions as they have reduced morphology. With the inclusion of the collected species from different locations in South Africa, the overall topology, and the node support of the phylogeny of the Lemnaceae family was found to be like previous studies (Les et al. 2002; Blog et al. 2019; Tippery et al. 2015; Tippery and Les 2020) with two distinct subfamilies (Wolffioideae and Lemnoideae). The species within Wolffioideae subfamily were regarded as species with devoid roots and Lemnoideae as species with varying number of roots (Blog et al. 2019).

The findings of the research have indicated *Landoltia punctata* as a separate genus (Les and Crawford 1999). The genera *Spirodela* and *Landoltia* were found distinct from other duckweed species. *Wolffiella* consistently had strong support in phylogenetic analyses for being monophyletic genus (Tippery and Les 2020). *Wolffia* and *Lemna* were also confirmed to be monophyly genera. Sections Alatae, Biformes, Lemna, and Uninerves were recognized to be phylogenetically distinct (Tippery and Les 2020).

Botanical Research Institute Pretoria (1980) elucidated that nine duckweed species were native to South Africa. ARC-Plant Protection Research Institute (2010) also indicated that some species of *Lemna, Spirodela* and *Wolffia* are invasive alien to

South Africa. Mtshali et al. (2017) indicated *Lemna minor* as invasive alien species to South Africa. *Lemna gibba* was also listed as invasive alien to South Africa (Cholo and Foden 2006).However, the results of this study confirmed that *Lemna minor* and *Lemna gibba* are native to South Africa (Figure 3.1). The results of the study also illustrated that out of 38 duckweed species, 31 duckweed species are invasive alien and seven being native to South Africa (POWO 2017, Figure 3.1). This study elucidated a clear difference between two distinct major clades of the Lemnaceae family (Subfamilies Lemnoideae and Wolffioideae). After this study, there are still several knowledge gaps left to be filled, some are:

- Resolution of species complexes: Some duckweed species show high levels of morphological similarity, which is a challenge to differentiate between closely related taxa. Resolving species complexes and accurately delineating species boundaries within these groups remains a major challenge.

- Hybridization: Duckweeds are known to hybridize readily, leading to complex patterns of genetic exchange and introgression. Understanding the extent of hybridization between different species and its impact on phylogenetic relationships and species boundaries is crucial for reconstructing accurate phylogenies (Braglia et al. 2021).

4.2 Climate Change

Climate change has been considered as one of the main factors that is influencing the spread of aquatic invasive alien plants (Willis et al. 2010). Evidence from previous studies suggests that humankind activities have influence on climate change (IPCC. 2014). Dispersal ability is influencing the distribution and spread of duckweed species in South Africa (Václavík and Meentemeyer 2009). The issue of aquarium trade seems to be getting more serious as it contributes to introduction of duckweed species into new areas (Azan et al. 2015). Surveillance of this activity should be prioritized as it is one of the several factors that is promoting spread of duckweed species across the country. Therefore, based on the results obtained from this study, climate change does influence the distribution of duckweed in South Africa.

Species Distribution Models (SDMs) and General Circulation Models (GCMs) were used to survey the possible shift of duckweed species from one area to another due to climate change. Ecological niche modelling of seven duckweed species was performed to obtain current and future projections. Jackknife AUC (Area Under Curve) graphs were used to determine important climatic variables for all the species. Most of the models ran, showed a sum of negative value which may favour the contraction of duckweed species in South Africa (Figure 3.10). With projected climate change, Mpumalanga and Limpopo may be vulnerable to expansion of duckweed species in the future (Figure 3.8D). *Lemna aequinoctialis* seems to be the favourable duckweed species that might spread or expand in the future (Figure 3.9A). Mpumalanga and Limpopo are in a high elevation area which might influence many species to move into these areas (Loarie et al. 2009, Bellard et al. 2013). KwaZulu-Natal and Mpumalanga show range contraction of duckweed species (Figure 3.7B).

Species distribution modelling is an important tool for climate change exploration on species invasion but there are challenges in relation to this tool. It may happen that generated SDMs for some species may not yet have had enough time to reach equilibrium with their environments (Václavík and Meentemeyer 2011). Furthermore, some of the duckweed species might not have occupied all the available suitable climate areas yet. Duckweed species may continue to expand in their geographic distribution irrespective of the unchanged area of suitability (García-Valdés et al. 2013).

Water-specific variables such as flow rate, pH and others were not taken into consideration due to unavailability of these variables. The reason why there is this limitation on this study is because South African water bodies variables are not yet available which makes it complex to include them in running the models (Coetzee et al. 2009). The impact of these variables is not yet known. However, the provided ecological niche models provided in this study are still important and useful for management of duckweed species as they also identify areas that may be in danger of being invaded currently and in future by these species.

Even though there are limitations in these studies, results of this study still match the earlier studies on native and invasive alien species contraction in South Africa (Bezeng et al. 2017; Adedoja et al 2024). For example, a study on predicted climate-induced

mismatch between Proteaceae species and their avian pollinators, (Adedoja et al. 2024) showed that the range of these species will likely contract in the future. Also, Bezeng et al. (2017) have indicated that climate change may reduce the spread of invasive alien species and they further suggested that climate change may also promote the development of new invasive alien species threat in South Africa. These previous studies (Bezeng et al. 2017; Adedoja et al. 2024) and our study have illustrated this trend of range contraction with projected climate change that seems to be a general trend for native and invasive alien species distribution in South Africa.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The reduced morphology of duckweed has made it difficult to identify these species with naked eyes. This study used molecular techniques to identify and distinguish duckweed species. The use of five gene markers resulted in good and clear phylogenetic results. Species collected fell into two respective clades which confirmed two sub-families of duckweed. Duckweed sections (*Alatae, Biformes, Lemna*, and *Uninerves*) were also proved to be phylogenetically distinct. It also listed *Lemna minor* and *Lemna gibba* as native species to South Africa. Phylogenetic tree topology was supported by various findings of previous studies of duckweed subfamilies (Wolffioideae and Lemnoideae). Furthermore, the results illustrated that duckweed is characterized by subfamilies Lemnoideae and Wolffioideae. The phylogenetic tree showed a clear relationship among species of Lemnaceae family. Resolution of species complexes and Hybridization of duckweed species is still a problem that should be further addressed and researched about as it affects phylogenetic results. Relationship between Lemnaceae family species was justified and explained clearly.

Duckweed distribution can be influenced by several factors but one of the most important factors is climate change. Models ran in this study gave an insight on how duckweed may be distributed in the future. The output models showed that there will be range contraction of duckweed species in KwaZulu-Natal and Eastern Cape. However, there are areas that could still be suitable or favourable for duckweed species such as North-West, Gauteng, Limpopo, and some parts of Mpumalanga. Therefore, the eastern and southern part of South Africa extending to the northern part has been identified as the most current climatically suitable areas for duckweed species. Coastal areas are shown to be prone to duckweed species invasion currently because of human disturbances and high nutrient availability. Looking at the future models provided, the mentioned provinces should be prioritized when controlling duckweed. This study also provided some of the water bodies that should be prioritized when controlling duckweed species in South Africa.

5.2 Recommendations

Even though the findings of this research confirmed and showed a clear duckweed phylogenetic tree, there is still a missing information about duckweed genera that are not yet assigned to their sections. This research could not assign some of the duckweed genera into their sections which is something that can be investigated in the future.

Although the results from this study will help conservationists to know where to start management of duckweed, there is still a gap about the missing geospatial data which affect the climate change results. Some of the dams and rivers could not be listed in this study as they did not have names from the geospatial data.

CHAPTER 6

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UNISA university of south africa

UNISA-CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 06/09/2021

Dear Mr Ndou

Decision: Ethics Approval from 06/09/2021 to 31/08/2024

NHREC Registration # : REC-170616-051
REC Reference # : 2021/CAES_HREC/130
Name : Mr UH Ndou
Student #: 12961612

Researcher(s): Mr UH Ndou
Ndou.uh@gmail.com; 076-396-7887

Supervisor (s): Dr L Mankga
mankglt@unisa.ac.za; 011-471-3642

Working title of research:

The effect of climate change on Lemnaceae family (duckweeds) in South Africa: an ecological niche modelling approach

Qualification: MSc Agriculture

Thank you for the application for research ethics clearance by the Unisa-CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is granted for three years, **subject to further clarification and submission of yearly progress reports. Failure to submit the progress report will lead to withdrawal of the ethics clearance until the report has been submitted.**

The researcher is cautioned to adhere to the Unisa protocols for research during Covid-19.

Due date for progress report: 31 August 2022

Please note the points below for further action:

1. How was the sample size determined? Is it not too small for a genetic diversity study?
2. Where will the sequencing be done?
3. Please submit the CVs of the researcher and the supervisor.

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4. Please provide a brief description of each of the statistical methods (GLM, RF, GNM and Ensemble Forecast niche modelling approach) Which R software package will be used?

*The **low risk application** was **reviewed** by the UNISA-CAES Health Research Ethics Committee on 02 September 2021 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

The proposed research may now commence with the provisions that:

1. The researcher will ensure that the research project adheres to the relevant guidelines set out in the Unisa Covid-19 position statement on research ethics attached.
2. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
3. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the Committee.
4. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
5. Any changes that can affect the study-related risks for the research participants, particularly in terms of assurances made with regards to the protection of participants' privacy and the confidentiality of the data, should be reported to the Committee in writing, accompanied by a progress report.
6. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.
7. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data require additional ethics clearance.
8. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

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Note:
*The reference number **2021/CAES_HREC/130** should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

Yours sincerely,

Prof MA Antwi
Chair of UNISA-CAES Health REC
E-mail: antwima@unisa.ac.za
Tel: (011) 670-9391

Prof SR Magano
Executive Dean : CAES
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