Modelling current and future distributions of *Warburgia* species at continental (Africa) and local (South Africa) scales

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Declaration

I, Samista Kim Rooplal, declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand. It has not been submitted before for any other degree or examination at other University.

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Abstract

Warburgia is a genus of trees and shrubs that is greatly valued in Africa for its use in traditional medicine. The genus contains four species, one of which has two subspecies: *Warburgia elongata, W. salutaris, W. stuhlmannii, W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis*. Individuals are harvested primarily for their bark, which contains pharmacological compounds that are used to treat various ailments. Due to the high demand, species within the genus are overharvested throughout their range and have consequently become threatened by extinction. *Warburgia salutaris*, the pepper-bark tree, is the only species of the genus that naturally occurs in South Africa. Like its congeners, *W. salutaris* is heavily exploited for its bark and has been listed as IUCN Endangered in South Africa. This dissertation, therefore, assesses the distributions of *Warburgia* species in eastern and southern Africa to identify new, potentially suitable areas to increase population numbers to aid in the conservation of the genus.

The aim of the first part of the study was to assess the eastern and southern African distributions of *Warburgia* species. Species distribution models (SDMs) were created for the four *Warburgia* species and two subspecies, and the geographic distributions and key environmental predictors were identified for each taxon. Environmental niche analyses were also performed to understand whether the two subspecies of *W. ugandensis* should be considered as a single species in accordance with the ecological species concept. The second part of the study aimed to assess how the current South African distribution of *W. salutaris* will be affected by future climate change. An SDM was produced to assess the current distribution of *W. salutaris* in South Africa and identify its key predictor climate variables. The SDM was then extrapolated into the future (2070) using two climate change scenarios, RCP 4.5 and RCP 8.5, which are greenhouse gas emission scenarios that predict future climates under a probable and extreme scenario, respectively. Ecological niche analyses were also used to assess the degree to which *W. salutaris*' climatic niche will change in response to the two climate change scenarios.

Results showed that the distributions of *Warburgia* species are restricted and primarily influenced by climatic variables that likely impact their seeds' and seedlings' sensitivity to water stress and desiccation. Ecological niche modelling results show that the climatic niches of *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* are identical and should therefore be considered as a single species according to the ecological species

concept. The South African distribution of *W. salutaris* is fragmented and restricted. The species' current and future distribution is influenced by its sensitivity to frost and the fact that it produces seeds that are susceptible to drying out. SDMs predicted that the distribution of *W. salutaris* will shrink in eastern Limpopo and in parts of Mpumalanga, but increase in eastern KwaZulu-Natal by 2070 under both climate change scenarios. Overall, this species was predicted to contract from the Indian Ocean coastal belt and grassland biomes and expand into the savanna biome.

This study has expanded our knowledge of the distributions and environmental drivers of *Warburgia* species. It was found that in general, *Warburgia* species have restricted ranges that are likely governed by their sensitivity to desiccation at the seed and seedling stages. Climate change is anticipated to negatively impact the populations of many plant species, especially those in Sub-Saharan Africa. While the environmental niche of *W. salutaris* will remain stable, its geographical distribution was predicted to expand further in the savanna biome along the eastern coast of South Africa in response to climate change. Results from this study support pursuing different conservation techniques, including propagating *Warburgia* populations around the Great Lakes of Africa and Mt. Kenya and in western Limpopo and eastern KwaZulu-Natal for *W. salutaris* only. This study therefore emphasizes the importance of using SDMs as a baseline to inform effective conservation efforts for important medicinal plant species.

Keywords: climate change, conservation, niche analyses, overharvesting, species distribution modelling, traditional medicine

Structure of dissertation

This dissertation is composed of four chapters. Chapter 1 is an introductory chapter, which outlines the distribution, uses and importance of the five *Warburgia* species. It provides and explains the conservation statuses of all *Warburgia* species and details species distribution modelling. Finally, the chapter contains the research problem, aim and objectives of this research study. Chapters 2 and 3 are structured as manuscripts; and therefore, contain some repetition.

Chapter 2 highlights the application of species distribution modelling to present the current distributions of all *Warburgia* species in eastern and southern Africa. This chapter also identifies the most important variables driving the distributions of the *Warburgia* species. Finally, the chapter makes taxonomic inferences regarding whether the two subspecies of *W. ugandensis* should be sunk into a single species.

Chapter 3 assesses the current and future South African distributions of *W. salutaris* to examine how the distribution will be affected by two climate change scenarios. The chapter also identifies the most important variables that drive the current and future distributions of *W. salutaris.* Lastly, the chapter estimates range size change and examines environmental niche indices between the current and future distributions. Chapter 4 synthesises Chapters 2 and 3 and provides conclusions and recommendations for the conservation of the ethnobotanically-important *Warburgia* species.

Glossary of abbreviations

AUC/AUROC	Area Under Curve/Area Under Receiver Operating Curve
ENM	Environmental Niche Model
GBIF	Global Biodiversity Information Facility
GIS	Geographic Information System
HSM	Habitat Suitability Model
IPCC	Intergovernmental Panel on Climate Change
IUCN	International Union for Conservation of Nature
KNP	Kruger National Park
masl	Metres above sea level
PCA-env	Principal Component Analysis
RCP	Representative Concentration Pathway
SAEON	South African Environmental Observation Network
SANBI	South African National Biodiversity Institute
SANParks	South African National Parks
SDM	Species Distribution Model
TSS	True Skill Statistic

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1. Chapter 1: Literature review and Rationale

1.1. Literature review

1.1.1. Biogeography of plants

Biogeography integrates biological and geographical sciences and is primarily focused on the biologically inhabited section of the biosphere (Briggs, 1995; Tivy, 2018). Essentially, biogeography is the study of the distributions of biota on Earth and the processes and implications thereof that have led to their current distributions. The field seeks to understand why taxa live where they do. To achieve this, biogeography is at the intersection of a multitude of disciplines, including climatology, genetics and botany (Briggs, 1995; Cox et al., 2016; Tivy, 2018).

The geographically centred aspect of biogeography examines the spatial variation of two key aspects (Tivy, 2018). Firstly, it studies the interrelationship between the biotic (living) and abiotic (non-living) elements of Earth's environment, (i.e., the biosphere). Secondly, it explores the often-debated relationship between the biosphere and humans (Cox et al., 2016; Huggett, 2004; Tivy, 2018). Ecologists have only recently recognised humans as a vital component of Earth's environment, as they now acknowledge that they are the ecologically dominant organism on the planet. Plants are generally better studied than animals in biogeography (Spellerberg, 1999; Tivy, 2018), likely because they constitute the majority of Earth's biomass. Additionally, plants are more influenced by the abiotic factors of their habitats than animals, partly due to their limited mobility. Consequently, plants serve as a more comprehensive indicator of the physical, biological and anthropogenic characteristics of the areas they inhabit (Tivy, 2018).

Like all organisms, plants require specific habitats for their survival (Chase, 2011; Godsoe *et al*., 2017). The biogeography of plants is driven by an assortment of living (e.g., competition and predation) and non-living (e.g., climate and soil ecology) factors, called an ecological niche (Chase, 2011; Spellerberg, 1999). Ecological niches can be differentiated into two main types: fundamental niche and realised niche. A fundamental niche is the collection of environmental conditions and resources that a species potentially uses in the absence of other species that allows it to persist (Chase, 2011; Godsoe *et al*., 2017; Hutchinson, 1957), whereas a realised niche is range of environmental conditions that an organism occupies

when resources and environmental conditions are considered in conjunction with biotic factors, such as competition, predation and diseases (Chase, 2011; Godsoe *et al*., 2017). Fundamental niches can be further subdivided into various types of niches, such as habitat, thermal or climatic niches. Godsoe *et al*. (2017) highlights that when there are no biotic interactions, and there is minimal dispersal (like in many plants), a species' habitat should closely align with its fundamental niche, given that exposure to environments beyond this may lead to extinction. This gives rise to a generative field in ecology – the ecological niche theory (Chase, 2011; Vandermeer, 1972).

Ecological niche theory is a requirement-based concept that connects the fitness (survival and reproduction) of a species to a set of environmental variables (Bardou *et al*., 2021; Hirzel and Le Lay, 2008). These variables, however, can be affected by anthropogenic activity and climate change, which may result in the decoupling of a species and its ecological niche. In the Anthropocene, humans possess the ability to exploit the biosphere at levels higher than any other life form and drive climatic changes faster than ever before. Consequently, climate change has affected the abundance and distribution of many plant species worldwide (Bardou *et al*., 2021). García-Valdés *et al*. (2015) highlighted this by investigating the relationships between 23 species' distributions and climate change and land use change in Spain. They found that climate change threatened the future geographic occurrence of 15 of their 23 study species. Furthermore, when coupled with land use change, climate change threatened the fitness of all of their study species. García-Valdés *et al*. (2015), along with others (e.g., Hirzel and Le Lay, 2008; Melo-Merino *et al*., 2020; Peterson and Soberón, 2012; Sharifian *et al*., 2022), highlight the importance of studying species' distributions, which can be achieved using species distribution models (SDMs).

1.1.2. Species distribution modelling and ecological niche modelling

The ability to quantify why and how biota are distributed in space is a central element of biogeographical research and is an application of ecological niche theory (Hirzel and Le Lay, 2008). Species distribution models (SDM) are used to assess how the distribution of a biological organism is defined by various environmental factors and can determine the environmental niche of the organism (Čengić *et al*., 2020; Miller, 2010). The technique and associated methodologies have become extremely popular in recent years, with hundreds of papers being published annually (Peterson and Soberón, 2012). Guisan and Zimmermann

(2000) highlight that the core of predictive species modelling relies on the quantification of species-environment relationships. These relationships are explained by the interplay of climate and environmental variables and how they affect floral and faunal patterns around the world (Guisan and Zimmermann, 2000). SDMs extract the environmental variables associated with the occurrence of the selected species from the occurrence data and generate an environmental niche for the species. The generated environmental niche is then projected into geographic space based on what is known about the environmental characteristics of the occurrence pixels and the remaining pixels in different geographic locations (Guisan and Thuiller, 2005; Miller, 2010; Pecchi *et al*., 2019). The created environmental niche can then be used to compare specific environmental variables between species to identify differences between their environmental niches, examine environmental change and its consequences, and to predict whether a species occurs within an unsampled area and if a species can exist in specific areas in the future (Elith and Leathwick, 2009; Guisan and Zimmermann, 2000; Miller, 2010).

The generalized nature of SDMs have enabled other uses, including mapping communities or assemblages and improving faunal and floral atlases (Guisan and Zimmermann, 2000), measuring species richness, determining potential of invasive species, and identifying vectors of disease. SDMs are also used for conservation purposes, particularly identifying suitable areas for species introduction or re-introduction and comparing current species distributions to predicted distributions (Miller, 2010; Pecchi *et al*., 2019). Additionally, SDMs can be used in protected area planning and establishing corridors that will allow for species movement. For comparative studies, SDMs are used to interpolate areas of similar environmental conditions to predict species' distributions, compare the predictions to known distributions, and then extrapolate environmental variables into future periods and compare those extrapolations to present environmental variables (Elith and Leathwick, 2009; Miller, 2010). Developing predictions of species distributions as a function of future environmental variables and anthropogenic activity is useful when prioritizing conservation activities (Guisan and Thuiller, 2005).

There is a wide array of methods to choose from when modelling species distributions, with over two dozen statistical and machine learning approaches introduced in the last two decades (Carlson, 2020). With the advances in statistics and geographical information systems (GIS), the use of SDMs has swiftly increased in the field of ecology, including

biogeography, habitat management, climate change science and conservation biology (Brown, 2014; Srivastava *et al*., 2019; Guisan and Zimmermann, 2000). Ecological research makes use of regression analysis models, like generalised linear models (GLM) and generalised additive models (GAM). These methods are preferred over others as they can account for the complexity of ecological data and allow for non-linear variances in data (Guisan *et al*., 2002). Other methods include Bayesian models, boosted regressions and ensemble methods.

Most SDMs require both presence and absence data; therefore, artificial absences, or pseudo-absences, are created when true absence data is unavailable (Barbet-Massin *et al*., 2012). Absence and pseudo-absence data are used in SDMs to compare the environmental conditions of occurrence points versus non-occurrence points, which give a better understanding of where the selected species does and does not occur (Barbet-Massin *et al*., 2012). It is important to consider the nature of the pseudo-absence points selected, as this choice may have residual effects on the SDM produced.

The basic steps of species distribution modelling are as follows: 1) collecting and cleaning species occurrence data and environmental predictor variables, 2) selecting an appropriate modelling algorithm, 3) fitting, evaluating and improving the model and 4) mapping geographic distributions with environmental predictors (Elith and Leathwick, 2009). Elith and Leathwick (2009) explain that the strength and realism of a model is dependent on the choice of predictor variables (e.g., abiotic and biotic variables) and the model method, scale, and the degree of extrapolation. In situations where SDMs are used to test a species' response to future climate conditions, Global Climate Models (GCM) are recommended for suitable predictor variables. Uncertainties in the model come about due to the contrasts between alternate models, characteristics of species and different future climate scenarios (Beaumont *et al*., 2008). Selecting the appropriate method is instrumental when modelling species distributions (Beaumont *et al*., 2008; Guisan and Thuiller, 2005). In addition to modelling future scenarios, techniques used in SDMs can be employed to model ecological niches. When considering ecological niche theory, an extension or synonym of SDMs can be used, termed ecological niche models (ENMs). These models use the fundamental niche of a species to map its potential distribution, rather than its actual geographic distribution (Melo-Merino *et al*., 2020). ENMs can be used to infer ecological niche theory concepts and is based on the ecological niche concept (Sillero, 2011).

1.1.3. Ecological species concept

The ecological species concept highlights how species are differentiated based on their habitat and environmental requirements (Mayr, 1947). Every species has a unique ecological niche, comprising biotic and abiotic factors (Slagsvold and Wiebe, 2007; Wiens *et al*., 2010). An extension of ecological theory suggests that no two species can have identical niches. Slagsvold and Wiebe (2007) explain that closely related species usually have separate ecological niches to promote coexistence by limiting competition. Characterising the ecological niches of taxa and determining the environmental requirements for taxa to persist has great significance in conservation (Papuga *et al*., 2018). Patterns of species occurrence are influenced by their specific environmental needs and preferences. Papuga *et al*. (2018) emphasises the need for ecological niche identification in plants – a sessile life form – as changes in their environments may compromise their survival and persistence. Characterising ecological niches is especially beneficial in distinguishing closely related species from each other (Eisenring *et al*., 2016). One example is two sister species of the genus *Roscoea* (Zingiberaceae, *R. humeana* Balf. f & W. W. Sm and *R. cautleoides* Gagnep.) occurring in the Hengduan-Mountain region, China. A study by Zhao *et al*. (2016) examined how Quaternary climate change promoted the speciation of these sister species using ecological niche modelling. They found that the sister species' ecological niches were significantly different and that Quaternary climate change fragmented populations of the original species, which likely facilitated the speciation of the two sister species (Zhao *et al*., 2016). Modelling species distributions and ecological niches can aid in informing decisions for conservation and management.

1.1.4. Conservation and traditional medicine in Africa

The International Union for Conservation of Nature (IUCN) is a global organisation dedicated to the conservation of nature and promotes the sustainable use of natural resources (Vié *et al*., 2009). It aims to engage in the current extinction crisis by collating and updating essential information on the state of wild species. The IUCN is commonly known for its Red List of Threatened Species, which is a freely available system created to assess and characterise the global risk of extinction for the earth's biota (www.iucnredlist.org). The list provides valuable data that can answer various questions about Earth's biodiversity, including

its overall state, changes over time, the factors driving biodiversity loss, and how, where and when to take conservation action (Mace *et al*., 2008; Vié *et al*., 2009).

The system categorises plant and animal species into eight categories based on five criteria regarding geographic range and population trend, size and structure (Table S1.1). These criteria are further subdivided to gain a complete understanding of a species' state. The first criterion (criterion A) is a reduction in population size, which is the rate of population decline of a species over a specific number of generations (usually three or 10). The rate of decline is measured as a percentage and can be estimated from the past, future, or both (Mace *et al*., 2008). Criterion B examines the extent of a species' geographic range in the form of either its extent of occurrence or its area of occupancy. A species qualifies as threatened under Criterion B when it meets at least two of the following conditions: severely fragmented population or limited number of locations, continuing decline in the population or habitat quality, or extreme fluctuations in the population or range (Mace *et al*., 2008).

Criterion C focuses on the numerical size of the population and its decline over time. This criterion assesses whether a species is vulnerable, endangered or critically endangered due to a small and continually declining population. The penultimate criterion, Criterion D, qualifies species with a very small or restricted population to qualify as threatened. This criterion does not need supporting evidence regarding whether there has been or will be a decline in population, since small populations are generally susceptible to extinction. The last Red List criterion, Criterion E, allows assessors to use quantitative analyses to evaluate the risk of extinction. These analyses are compared to specific extinction-risk thresholds of each of the Red List categories to determine a species' status (Mace *et al*., 2008). The Red List criteria, along with the sub criteria, allow ecologists and conservationists to gain insight into the conservation status of various species. This classification system is central in identifying and monitoring high priority species and prioritising conservation efforts, especially for those species that provide human benefits, such as medicinal species.

Before the introduction of allopathic medicine, the dominant medicinal system in Africa was traditional medicine (Mander *et al*., 2007). Although allopathic medicine has become more readily available, many people, especially those in developing areas, still rely on the traditional medicine market (Mander *et al*., 2007; Senkoro, 2021, Van Wyk and Wink, 2018; Williams *et al*., 2013). For reference, 60 to 79% of some east African populations still rely on traditional medicine as their primary source of healthcare (Senkoro, 2021). The high demand

and degree of collection of plant material from natural populations have affected the sustainability of using wild plants as a medical product. McMullin *et al.* (2014) and Williams *et al*. (2000) report that traditional medicine material is now distributed by and between the informal and formal sectors of African countries, and no longer restricted to traditional healers. The growing number of traders of traditional medicine has raised concern about its sustainability. Consequently, many traditional medicinal species require urgent conservation intervention due to their declining numbers. An example of an important application of the IUCN Red List was conducted by Williams *et al*. (2013), where they found that 10% of South Africa's flora is used for traditional medicine. After categorising the taxa into conservation categories, they found that 82 of 2062 examined traditional medicine species were threatened with extinction in South Africa. They also found that the majority of taxa within the categories that are declining, near threatened and vulnerable are traded in traditional medicine markets, highlighting that traded plants are at a higher risk of extinction than non-traded plants. These findings emphasize the need to prioritise conservation efforts for these valuable and highly sought-after medicinal plants, such as the African genus *Warburgia* (pepper-bark tree).

1.1.5. Genus of concern

Warburgia is a genus of trees and shrubs that is known for its ethnomedicinal uses (Maroyi, 2014; Naidoo and Lamb, 2006; Senkoro, 2021). The genus is part of the Canellaceae family (White Cinnamon Family, Burrows *et al*., 2018) which contains four species and two subspecies: *Warburgia elongata* Verdc., *W. salutaris* (Bertol. F.) Chiov., *W. stuhlmannii* Engl. and *W. ugandensis* Sprague, with subspecies *W. ugandensis* subsp. *longifolia* Verdc. and *W. ugandensis* Sprague subsp. *ugandensis*. It is the only Canellaceae genus that occurs in mainland Africa (Maroyi, 2013; Naidoo and Lamb, 2006). The genus is endemic to Africa (Burrows *et al*., 2018; Leonard and Viljoen, 2015; Naidoo and Lamb, 2006) and is spread primarily across eastern and southern Africa, with small range extensions into the Democratic Republic of Congo (DRC, Figure 1.1).

The species within the genus are highly sought-after for their medicinal properties and this has resulted in a high demand for these trees and shrubs. This has led to an increase in the harvesting of wild populations of *Warburgia* (McMullin *et al*., 2014). In addition, the trade of *Warburgia* now occurs internationally between African countries, which has further increased the demand for plant material and has resulted in poaching, unsustainable use and

insufficient management of wild populations. South Africa, for example, is an international exporter of *Warburgia* (and other plant species) to other African countries, such as Mozambique, Malawi and Zimbabwe (Williams *et al*., 2000). As a result, *W. salutaris* has been listed as a threatened and over-exploited species since the 1990s. The unsustainable use of *Warburgia* species is worsened by the medicinal treatments that primarily depend on harvesting the roots and barks of the trees and shrubs, which is the most damaging form of harvesting (McMullin *et al*., 2014; Senkoro, 2021).

Members of the genus are all evergreens and grow to an approximate height of 10 to 27 m. The species in the genus are well-known for their dark and pungent bark, which, along with the leaves and roots, is used for medicinal purposes (Felhaber and Mayeng, 1997; Leonard and Viljoen, 2015). Species of *Warburgia* contain high levels of drimane and colorotane sesquiterpenoids, which are plant-produced compounds that act as anti-feedants. These compounds are responsible for many medicinal purposes, such as antibacterial, anti-inflammatory, antimalarial properties (Leonard and Viljoen, 2015).

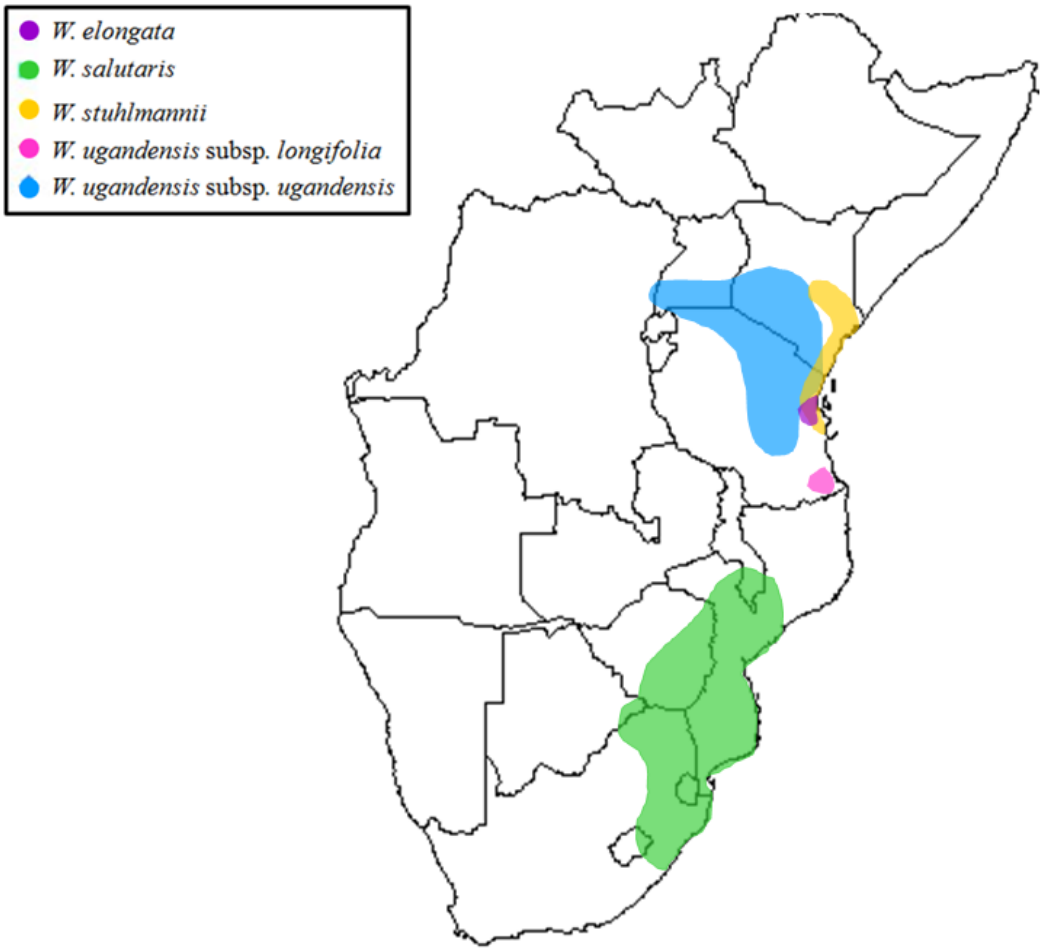

Figure 1.1. General distributions of *Warburgia elongata*, *W. salutaris*, *W. stuhlmannii*, *W. ugandensis*, *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* in eastern and southern Africa**.** Created using locality data obtained from GBIF, iNaturalist and SAEON.

All *Warburgia* species are used for similar traditional purposes, primarily traditional medicine. Other human uses include using the species for firewood, charcoal, tools, timber, perfume, fire breaks, and soil conservation in the form of mulch (Leonard and Viljoen, 2015; Maroyi, 2014; Senkoro, 2021; Van Wyk and Wink, 2018). In addition to offering a wide range of medicinal uses for humans, *Warburgia* species provide advantages for animals due to their pharmacological properties (Leonard and Viljoen, 2015; Maroyi, 2014). Animals, such as elephants and blue monkeys, also use *Warburgia* for self-medication and travel out of their usual feeding ranges to find and consume the plant (Leonard and Viljoen, 2015). Unfortunately, due to these extensive traditional uses, there has been unsustainable harvesting of all members of the genus.

Although the species of the genus share many similarities, they can be differentiated primarily by their distributions and morphological traits (summarised in Tables S1.2 – S1.4). *Warburgia elongata* is a shrub endemic to Tanzania and grows in the undergrowth of lowlands and coastal riverines as well as in groundwater or swamp forests (Lovett *et al*., 2006; Maroyi, 2013). It is a small, evergreen shrub and has a restricted range and a restricted number of mature individuals, which has deemed it a critically endangered species (CR B2ab) on the IUCN Red List. The species is distinguished from the others by its elongated, ellipsoid fruits (Leonard and Viljoen, 2015; Lovett *et al*., 2006; Verdcourt, 1954).

Warburgia stuhlmannii is endemic to the dry lowland forests and woodlands of Tanzania and Kenya (Lovett *et al*., 2006; Maroyi, 2014), and is commonly known as "*karambusi*" or "*mkaa*" in Swahili (Leonard and Viljoen, 2015). The species has small flowers and spherical fruit (Lovett *et al*., 2006) that are the smallest fruits relative to congeners, with an approximate diameter of only 1.5cm (Leonard and Viljoen, 2015). *W. stuhlmannii* has a decreasing population and is ranked as vulnerable (VU B1ab + 2ab, IUCN, 2023).

Warburgia ugandensis, commonly referred to as the "Uganda greenheart" (Leonard and Viljoen, 2015) comprises two subspecies: *W. ugandensis* subsp. *longifolia*, which is endemic to southern Tanzania, and *W. ugandensis* subsp. *ugandensis*, which is relatively widespread across East Africa, including Kenya, Uganda and Ethiopia (Maroyi, 2014). The species has relatively large flowers with fruit that measure approximately 5cm in diameter (Leonard and Viljoen, 2015). *Warburgia ugandensis* exhibits a mixed mating system, which is made up predominantly of outcrossing rather than selfing (Senkoro *et al*., 2020). *Warburgia ugandensis* subsp. *longifolia* has yellow bark when young and black-grey bark when mature and the bark splits into irregular patches. On the other hand, *W. ugandensis* subsp. *ugandensis* has dark brown to black bark that splits into rectangular patches. For both subspecies, the leaves are simple and alternate, and the fruit turn purple when ripe and grow up to 5cm in diameter (Lovett *et al*., 2006). *Warburgia ugandensis* subsp. *longifolia* is found in lowland forest habitats in southern Tanzania (Leonard and Viljoen, 2015; Lovett *et al*., 2006), while *W. ugandensis* subsp. *ugandensis* occurs in groundwater forests and dry montane forests across Uganda, Tanzania, Kenya and Ethiopia (Leonard and Viljoen, 2015; Lovett *et al*., 2006). *W. ugandensis* subsp. *longifolia* is listed as a critically endangered and possibly extinct species (CR B2ab) and *W. ugandensis* subsp. *ugandensis* has not been reviewed, likely due to misidentification of the subspecies, which has been observed from herbaria records (IUCN,

2023; Maroyi, 2013). Due to the many morphological similarities that the two subspecies of *W. ugandensis* share, as well as their overlapping distributions, the taxonomy of *W. ugandensis* has been a subject of debate among scientists. An example in the literature is seen in Maroyi (2014) and Muchugi *et al*. (2008), where *W. ugandensis* is characterised as a single species, whereas Leonard and Viljoen (2015) identifies *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis*.

Warburgia salutaris is the only species whose range reaches southern Africa from eastern Africa (Maroyi, 2013). The species occurs along southern and eastern Africa, including Lesotho, eSwatini, Malawi, Mozambique, Zimbabwe and South Africa (Maroyi, 2014, Figure 1.1). The species is found in savannas, evergreen montane forests and along the coast in sandveld forests (Maroyi, 2013). The tree has many common names, such as "*ishibhaha*" in Zulu and "*mulanga*" in Venda but is commonly known as the "*pepper-bark tree*" (Maroyi, 2014). The present limited geographic distribution and low abundance of *W. salutaris* increases its vulnerability to human exploitation and human-induced habitat conversion and degradation (Maroyi, 2013) and has been listed as endangered (with a population reduction of over 70%, based on three criteria: direct observations, a decline in area and extent of occupancy and human exploitation, A1acd) on the International Union for Conservation of Nature and Natural Resources (IUCN) Red List (IUCN, 2023). *Warburgia salutaris* is frequently cultivated in South Africa. However, the demand for the bark, leaves and roots of the species exceeds the rate at which they grow (Botha *et al*., 2004).

Warburgia salutaris is an evergreen tree five to 10 m in height, but can reach 20 m in some situations (Palgrave, 1977), with a maximum stem diameter of 30 cm. The pepper-bark tree is able to coppice rapidly in the face of high levels of bark harvesting (Botha *et al*., 2004) and Maroyi (2014) states that the tree has a slow growth rate in the wild relative to cultivation sites. Flowers are small relative to the other species, and fruit that measure between 1.5 to 2.5 cm in diameter (Leonard and Viljoen, 2015). Individuals of the genus are self-incompatible, but also exhibit clonal (asexual) reproduction (Glennon *et al*., 2023; Senkoro *et al*., 2020; van den Bosch, 2020; van den Bosch *et al*., 2023). Clonality could lead to high genetic diversity through the independent somatic mutations of individuals within a ramet and increased allelic diversity, heterozygosity and polymorphisms (Meloni *et al*., 2013). Senkoro *et al* (2020) shows that two populations of *W. salutaris* in Mozambique exhibits random mating, which

could explain the high within and among species genetic diversity (also seen in Glennon *et al*., 2023 and Senkoro *et al*., 2020).

1.1.6. Conservation of Warburgia

African countries have recognised *Warburgia* as a conservation priority and some conservation and management efforts have been implemented (Botha *et al*., 2004; McMullin *et al*., 2014). Notably, in Kenya, the genus has become a priority for conservation and research efforts by the Kenya Forestry Research Institute (KEFRI) and the World Agroforestry Centre (ICRAF, McMullin *et al*., 2014; Wamalwa *et al*., 2006). Additionally, organisations such as the Agricultural Research Council, SANParks and SANBI have produced *W. salutaris* seedlings and have made them available for distribution to communities that depend on the tree (van den Bosch, 2020). McMullin *et al.* (2012) showed that the most identified trade species was *W. ugandensis* through conducting interviews with traditional medicine traders in Kenyan traditional markets. The authors recommended that future studies focus on the threat of harvesting natural populations of priority species, evaluating and promoting sustainable production of trade species, and considering cultivating priority species on small-scale farms. Currently, South Africa leads in conservation research of *W. salutaris*. The country documents the trade and supply of the species to estimate the degree of trading; and therefore harvesting; and the impact that this has on *W. salutaris* populations (Botha *et al*., 2004; McMullin *et al*., 2014). Such data has allowed South Africa to develop and implement management strategies for the sustainable use of *W. salutaris* (Botha *et al*., 2004; McMullin *et al*., 2014).

Although other African countries have put some conservation and management measures in place regarding *Warburgia*, there is still not enough being done to prevent the unsustainable use of these trees. South African legislation has been unable to suppress the illegal trade of medicinal species (Botha *et al*., 2004). The country also lacks adequate protection of natural populations of *W. salutaris*, with poaching further reducing numbers. Consumer value of traditional medicine has also prevented the use of medicine in tablet form, as consumers are more inclined to use traditional medicine species in their natural form (Botha *et al*., 2004). In addition, many traditional medicine traders are unaware of how threatened *Warburgia* species are because they are readily available to them through imports from various African countries (including South Africa). The lack of knowledge about the conservation status of *Warburgia* species, reflected by a lack of population trend data and the need for updating IUCN statuses

(IUCN, 2023), has placed more pressure on the trees. McMullin *et al.* (2014) highlights the inadequacy of the assessment of some of the *Warburgia* species - reflected by a lack of research regarding the potential for cultivation and trade of these species - and states that their statuses should be re-evaluated to allow for more appropriate conservation methods to be implemented.

1.1.7. Conservation strategies

The commercialisation of wild plant species places high pressure on the integrity of natural populations, especially when demand is high, which is the case for *Warburgia* species (Botha *et al*., 2004; Senkoro, 2021). In the past, it had been assumed that exploiting non-timber forest products, including bark, had a low impact on the environment; however, it has been recently highlighted that this can have detrimental effects on the biology and genetic diversity of populations (Baldauf *et al*., 2013). For example, a reduction in the genetic diversity of *Himatanthus drasticus* (Mart.) Plumel (Apocynaceae, common name "*janaguba*") was detected in populations that experienced high levels of bark harvesting in Brazil (Baldauf *et al*., 2013). The literature has highlighted several potential conservation strategies of trees in general, mainly *in situ* and *ex situ* approaches. *In situ* and *ex situ* conservation approaches focus on maintaining species diversity within or outside of their natural distributions, respectively (Maxted, 2013).

In situ conservation methods maintain the genetic variation of taxa at their existing locations, either in the wild or in farming systems. The most appropriate technique is creating nature reserves, whereby the minimum number of individuals of a species that can maintain genetic diversity is contained and protected in an enclosed area (Maxted, 2013). Nature reserves are effective in conserving large amounts of taxa, including recalcitrant-seeded species, at low costs (Pritchard *et al*., 2012; Wyse and Dickie, 2017), however they require continued management and monitoring and that the conserved species are not immediately available for human exploitation. Farmer- and community-based conservation involves the maintenance of economically important species by community members (Botha *et al*., 2004; Maxted, 2013). This method encourages farmers and community members to grow, maintain, and harvest their own populations of species, however, it may require assistance in the form of subsidy and monitoring (Maxted, 2013).

On the other hand, *ex situ* methods collect samples of a taxon to preserve in living collections, field gene banks, or botanical gardens or as samples of seeds or tissue explants. *Ex situ* botanical gardens are advantageous because they have more freedom to conserve wild species that are relatively overlooked by other conservation agencies. Space, however, is a constraining factor to this method, especially in cases where botanical garden populations must provide alternative harvesting sites to natural populations. In addition, some botanical gardens are quite expensive to implement and maintain as they need to be regulated to the specific climatic and/or environmental specifications of the species that the hold (Maxted, 2013). Seed storage conservation methods involve collecting plant material from individuals or populations to be stored, usually at sub-zero temperatures, which is feasible for short- to long-term storage. This method, however, is sensitive to the nature of the seed, since recalcitrant seeds are compromised by drying and freezing (Maxted, 2013; Senkoro, 2021). An alternative method that is suitable for recalcitrant-seeded species is *in vitro* conservation, which establishes tissue cultures on nutrient agar and stores them under conditions of slow or suspended growth. Unfortunately, this method requires costly technology; therefore, the most suitable method for long-term *in vitro* conservation is cryopreservation of tissue or *in vitro* culture, where tissue samples are used to enhance the number of individuals in the wild or to alleviate the pressure on wild populations (Maxted, 2013; Senkoro, 2021).

1.2. Rationale

Due to *Warburgia* species' popularity in traditional medicine, all are unsustainably harvested from their natural distributions, which has led to them becoming threatened (Maroyi, 2014; Senkoro *et al*., 2020). In addition, having fragmented and limited distributions, *Warburgia* species are more vulnerable to overharvesting and climate change, as populations numbers are too few to respond or adapt to these pressures timeously (Moyo *et al*., 2015). *Warburgia* species are further threatened by habitat degradation and loss, which is the result of land conversion for urbanisation and agriculture (Maroyi, 2014; McMullin *et al*., 2014). In addition, global change predictions for many sub-Saharan plant species will anticipate future range contractions due to the predicted increase in extreme climatic events (Danaher *et al*., 2022; McClean *et al*., 2005). Maxted (2013) highlights that although there are several factors that threaten biodiversity; the greatest threat is human-facilitated climate change. Considering the economic importance of and dependence on medicinal plant species, it is valuable to characterize their distributions and identify how they react to climate change to inform

protocols for how to conserve them for continued use (Kailash *et al*., 2022; Sharifian *et al*., 2022; Williams *et al*., 2000). Developing and implementing conservation interventions requires knowledge of the species to be conserved. Creating ideal conservation strategies requires a multidisciplinary approach, including geographic, social, economic, cultural and political dimensions.

Previous studies on *Warburgia* primarily focused on the ethnobotanical uses of the genus. Some focused on the traditional and pharmacological uses of the genus (Maroyi, 2014; Senkoro, 2021), while others highlight the biological activities, ethnobotany, phytochemistry and antifeedant compounds of *Warburgia* species (Kubo *et al*., 1976; Leonard and Viljoen, 2015; Msomi, 2017). Through amplified fragment length polymorphisms (AFLPs), Muchugi *et al*. (2008) revealed the genetic structuring of the members of the genus and McMullin *et al*. (2014) gives strategies for the sustainable use of the genus. Other studies focus on one or more *Warburgia* species and their phytochemistry (Drage *et al*., 2014; Kioy *et al*., 1990; Mohanlall and Odhav, 2009) and genetic structuring (Muchugi *et al*., 2012; Senkoro *et al*., 2020). Glennon *et al*. (2023) focused on the reproductive implications of genetic structure and diversity of *W. salutaris* in the Kruger National Park (KNP), South Africa. Finally, the reproductive ecology of *W. salutaris* in KNP was extensively examined and gave a possible reason for the species' low reproductive output in KNP, which was that seeds were aborted due to the tree's reliance on clonal growth to persist in KNP (van den Bosch *et al*., 2023). Despite the wealth of knowledge on the ethnobotanical and pharmacological uses of the genus, there is a gap in understanding how environmental variables and climate change influence the distribution of these medicinally important yet highly threatened *Warburgia* species.

Ecoinformatic approaches (i.e., species distribution modelling) identify the geographical distribution of a species and potentially suitable but uninhabited areas. In addition, areas predicted to be highly suitable may contain undiscovered populations of the target species. Projecting distribution maps into the future aids in identifying range declines, expansions and shifts, which is key in developing suitable conservation strategies (Senkoro, 2021; Sony *et al*., 2018). This study will inform conservation by using SDMs to ensure more accurately reported distributions of *Warburgia* species across Africa, as well as predicting current distributions, and future distributions, based on climate change, of *W. salutaris* (a southern African species of *Warburgia*). Finally, the geographic distributions of current and future

environmental niches of *W. salutaris* will be compared to infer the vulnerability of the species to a changing South African environment.

1.3. Aim and objectives

The aim of the study is to model the distributions and environmental niches of *Warburgia* species at continental (eastern and southern Africa) and local (South Africa; *W. salutaris* only) scales to determine habitat requirements and to identify areas that comprise potentially suitable habitat in support of conservation initiatives.

The following objectives were undertaken to address the aim of the study:

To model and assess the distributions of *Warburgia* species in eastern and southern Africa to identify potential habitats and address taxonomic confusion (coinciding with the ecological species concept, *W. ugandensis* subspecies only, Chapter 2).

To compare environmental variables for the different subspecies of *W. ugandensis* to determine if they are ecologically different species.

To assess the current distribution and predict the future distributions of *W. salutaris* under different climate change scenarios in South Africa (Chapter 3).

1.4. References

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1.5. Appendices

Appendix 1

Table S1.1. IUCN Red List abbreviations, categories and criteria.

Abbreviation	Category	Criteria
NE	Not Evaluated	-
DD	Data Deficient	-
LC	Least Concern	-
NT	Near Threatened	-
VU	Vulnerable	A. Population size reduction B. Geographic range C. Small population size and decline D. Very small or restricted population E. Quantitative analysis
EN	Endangered	
CR	Critically Endangered	
EW	Extinct in the Wild	-
EX	Extinct	-

Appendix 2

Table S1.2. Characteristics of *Warburgia* species (J Store, 2023; Leonard and Viljoen, 2015; Maroyi, 2014).

Species	*W. elongata*	*W. salutaris*	*W. stuhlmannii*	*W. ugandensis* subsp. *longifolia*	*W. ugandensis* subsp. *ugandensis*
Common name	Mkaa Msokonoi	Pepper-bark Ishibhaha Mulanga	Karambusi Mkaa	Uganda Greenheart	Uganda Greenheart
Distribution	Tanzania	Lesotho South Africa eSwatini Malawi Mozambique Zimbabwe	Tanzania Kenya	Tanzania	Uganda Tanzania Kenya Ethiopia
IUCN status	CR B2ab(iii, v)	EN A1acd	VU B1ab(iii) + 2ab(iii)	CR B2ab(iii) possibly extinct	N/A
Tree/shrub	Shrub	Tree	Tree	Tree	Tree
Maximum height (m)	6	10	15	42	42
Leaf morphology	Simple Alternate	Lanceolate Alternate Glossy dark green Pellucid Oil glands	Oblong Elliptic Glossy dark green	Simple Alternate	Simple Alternate
Bark morphology	Grey – brown Smooth Scattered red-brown lenticels	Smooth white-grey when young Rough brown when mature	Yellow when young Black-grey with irregular patches when mature	Yellow when young Black-grey with irregular patches when mature	Dark brown-black with rectangular patches
Seed type	Recalcitrant	Recalcitrant	Recalcitrant	Recalcitrant	Recalcitrant
Salient features	Elongated ellipsoid fruit	Small flowers	Smallest fruit	Green to purple berry of 5cm in diameter	Green to purple berry of 5cm in diameter

Table S1.3. Habitat characteristics of *Warburgia* species across Africa.

Species	Habitat	Reference
W. elongata	Coastal regions, forests	Leonard and Viljoen, 2015
	Lowland riverine, groundwater forest	Lovett *et al*., 2006
	Lowland coastal riverine, swamp forest	Maroyi, 2013
W. stuhlmannii	Dry lowland forest, woodland	Lovett *et al*., 2006
	Coastal forest, open woodland	Maroyi, 2013
	Dry lowland forest	Maroyi, 2014
*W. ugandensis**	Eastern African highlands	Dharani, 2011; Muchugi *et al*., 2008
	Dry montane forest	Kairu *et al*., 2014
	Lowland forest, groundwater forest, dry montane forest	Lovett *et al*., 2006
	Lowland rainforest, evergreen forest, swamp forest	Maroyi, 2013
W. salutaris	Dry rocky hillside, dry thicket, wet forest	Burrows *et al*., 2018; Schmidt *et al*., 2002
	Evergreen forest, lowland scrub, sandveld forest	Maroyi,2013
	Bushveld, forest	Moll, 1981; Pooley, 1993
	Evergreen forest, wooded ravine	Botha *et al*., 2004; Palgrave, 1977

*included at the species level rather than the subspecies level due to a lack of differentiation between the subspecies in the literature.

Table S1.4. Characteristics and environmental variables that are associated with the habitat types where *Warburgia* species occur.

Habitat	Characteristics	Reference
Bushveld/deciduous woodland/ savanna	Trees are spreading and do not grow tall; lower altitudes; low rainfall; dry Altitude between 400 and 900 masl; summer rainfall (between 700 and 1000 mm); average summer temperature 26 - 28 C; fertile, clay soils	Pooley, 1993 Thomas and Grant, 1998
Lowveld bushveld	Flat to undulating terrain from 350 - 500 m; overlying granite and gneiss (coarse, sandy soils); dense bush in uplands, tree savanna in valleys and dense riverine woodland on riverbanks; flat plains; e.g., KNP	Schmidt *et al*., 2002
Mopane bushveld	Tall and dense vegetation; granite geology; 300 - 700 masl altitude	Schmidt *et al*., 2002
Coastal lowland forest	Tall and more diverse than dune forest	Pooley, 1993
Forest	Closed canopy; several vegetation strata	Schmidt *et al*., 2002
Dry Kloof forest/ dry ravine forest	Dry forest; granite (coarse, sandy soils) and rhyolite (clay soils) underlying geology; 300 - 1000 m altitude	Schmidt *et al*., 2002
Dry forest	Fairly open, low canopy; multiple strata	Moll, 1981
Montane forest	Occurs in gorges; up to altitudes of 1900 masl; common in warmer, wetter Drakensberg; less common in southern Drakensberg; affected by extreme climatic conditions; trees grow tall	Pooley, 1993
Riverine forest	Low rainfall; tall forest; part of river system Permanent or seasonal water; deep, fertile soils Restricted to rivers in escarpment foothills and lowveld; generally below 800 masl; thinned out forest (may observe some savanna species in this habitat)	Pooley, 1993 Jacana, 1997 Schmidt *et al*., 2002
Swamp forest	Found near the coast; high water table; contains plants that can grow in waterlogged soil; subtropical; high rainfall Water-logged soils; low oxygen in soil; dense canopy	Pooley, 1993 Thomas and Grant, 1998
Rocky outcrops	Slopes are well-drained; shallow soils	Thomas and

		Grant, 1998
Sandveld	Level plains between sandstone outcrops and hills; limited to northern KNP and small area of Mozambique border	Schmidt *et al*., 2002
Thicket	Low, dense woody species; diverse Low-lying; disturbed soil; soil and rainfall unsuitable for trees	Moll, 1981

2. *Chapter 2: Modelling* **Warburgia** *distributions across eastern and southern Africa*

Abstract

The ability to measure environmental distributions and niches aids in identifying potential habitats for vulnerable species, thereby supporting their population growth and conservation initiatives. *Warburgia* (Canellaceae) is a genus of trees and shrubs endemic to Africa and contains four species, one of which has two subspecies. The genus is popular in the traditional medicine market for its ability to treat a multitude of ailments. Consequently, its high demand has led to the overharvesting of the species within the genus, resulting in all the species being threatened by extinction. The aim of this study was to model and assess the distributions of *Warburgia* species in eastern and southern Africa to identify potential habitats and invoke the ecological species concept to address taxonomic confusion using species distribution modelling (SDM). SDMs were conducted using occurrence data for the four *Warburgia* species and two subspecies and climate data. The study also estimated niche overlap using the niche equivalency and similarity tests to examine whether the two subspecies of *W. ugandensis* should be considered as a single species. Results showed that the distributions of *Warburgia* species are restricted and mainly determined by climatic factors that potentially influence water stress and sensitivity to desiccation. Sites for potential propagation of *Warburgia* species include the areas around the Great Lakes of Africa and Mt. Kenya. Results also revealed that the two subspecies of *W. ugandensis* should be sunk in accordance with the ecological species concept since their climatic niches are identical. This study outlines possible ecological implications for taxonomy of these medicinally important species.

Keywords: conservation, niche comparisons, overharvesting, species distribution modelling, traditional medicine

2.1. Introduction

Predictive models of geographic distributions of species according to their climatic and environmental requirements are useful tools in many aspects of ecology (Čengić *et al*., 2020; Phillips *et al*., 2006). They have been used to study alien invasions, climate change impacts, anthropogenic impacts on threatened taxa and the spatial ranges of biodiversity (Barbet-Massin *et al*., 2012; Koldasbayeva *et al*., 2022; Miller, 2010; Phillips *et al*., 2006; Variawa, 2017). Recent advancements in species distribution modelling could allow for the detection of how anthropogenic effects change biodiversity patterns (Čengić *et al*., 2020; Guisan and Thuiller, 2005; Koldasbayeva *et al*., 2022). With the use of species distribution modelling, the geographical ranges and environmental niches of species can be quantified, and conservation efforts can be directed appropriately (Barbet-Massin *et al*., 2012; Guisan and Thuiller, 2005; Jha *et al*., 2021). Notably, SDMs have implications for taxonomy, and can be used to characterize ecological niches which is beneficial in distinguishing closely related species from each other (Eisenring *et al*., 2016).The ability to quantify environmental niches aids in finding new suitable habitats for vulnerable species, which could assist in increasing population numbers (i.e., more individuals and covering a greater range) and conservation efforts (Johnson and Gillingham, 2005; Sharifian *et al*., 2022).

Warburgia is a well-known genus in the traditional medicine market, with 80% of Africans relying on traditional medicine as their primary source of healthcare (Maroyi, 2014; Moyo *et al*., 2015). Species within the genus are known to treat 37 human and animal ailments, which has resulted in a high demand for the stem bark and roots. Consequently, overharvesting of the genus has devastated wild populations across Africa (Dokata *et al*., 2023; McMullin *et al*., 2014; Senkoro, 2021) and the need for conservation interventions is imperative. This study is intended to characterise *Warburgia* species and address taxonomic confusions by assessing the environmental variables that may distinguish them, as well as mapping their distributions which will aid in conservation decisions.

2.1.1. Importance of taxonomy in conservation

Taxonomy is a multidisciplinary branch of science that uses features of a species, including its distribution, biology and ecology to delineate a taxonomic group. Taxonomy is foundational to all biology and ecology, including biodiversity conservation (Dubois, 2003; Ely *et al*., 2017; Mace, 2004). Describing a species is cardinal in conservation biology;

however, it is often overlooked. Many cases have documented species lost to synonymization and misidentification (Dubois, 2003; Ely *et al*., 2017; Ferreira *et al*., 2016; Iglésias *et al*., 2010). A detrimental case of taxonomic confusion is seen in Brazil, where two plant species of the *Hypericum* genus, *Hypericum ternum* A. St. Hil. (Thorny Hypericum) and *H. cavernicola* L. B. SM., were misidentified and led to local extinctions of *H. ternum* (Ely *et al*., 2017). This occurred because of taxonomic confusions between the two species, whereby the species in need of conservation interventions – *H. ternum* – was mistaken for *H. cavernicola*; hence conservative action was misinformed (Ely *et al*., 2017). Dubois (2003) and Ely *et al*. (2017) underscore the importance of taxonomic knowledge and how it influences conservation decisions. Misidentifications and a lack of knowledge about taxonomy may lead to choosing inappropriate priority areas for conservation and high risk species may be further threatened as a result of not being prioritised for conservation. Ely *et al*. (2017) argues that the lack of distribution modelling of taxa has created a gap in taxonomic knowledge. Mapping species distributions is pivotal in taxonomy and conservation, as taxa cannot be conserved if their habitat conditions are not known (Ely *et al*., 2017; Serena *et al*., 2020). In addition, mapping species' environmental niches informs conservation decisions by identifying their preferred habitats and how these habitats may evolve through time due to varying environmental pressures. This type of analysis can also be applied to assessing the environmental variables and niches that may aid in reassessing the taxonomy of species.

2.1.2. Taxonomic features of **Warburgia** *species*

For the development of sustainable management strategies for conservation, a comprehensive taxonomic identification and understanding of the genetic variation of a species is needed (Muchugi *et al*., 2008). The six genera of Canellaceae form a monophyletic group (Salazar and Nixon, 2008). One of the synapomorphies shared by the family is the aromatic nature of the bark and the peppery taste of the leaves. Other synapomorphies shared by the family include monadelphous stamens, campylotropous ovules and parietal placentation (Salazar and Nixon, 2008). There is some confusion regarding the taxonomy of species within *Warburgia*, with some authors listing different species as synonyms. For example, some studies refer to *W. salutaris* and *W. ugandensis* as synonyms due to their many shared morphological traits (Muchugi *et al*., 2008; Palgrave, 1977).

Muchugi *et al*. (2008) sought to define the phylogenetic relationships of *W. salutaris*, *W. stuhlmannii* and *W. ugandensis* using amplified fragment length polymorphisms (AFLPs).

The study showed that *W. ugandensis* is monophyletic, which supports it as a distinct taxon. Muchugi *et al.* (2008) further show that *W. ugandensis* is separated into two distinct groups. This finding has been subject to debate amongst scientists. These data support the presence of two subspecies, *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* (Muchugi *et al*., 2008). In addition, it has been argued that *W. salutaris* and *W. stuhlmannii* are synonyms of each other; given that genetic data suggests they are closely related, even though they are geographically separate (Muchugi *et al*., 2008; Pooley, 1993; Schmidt *et al*., 2002). The low degree of morphological distinction between *W. salutaris* and *W. stuhlmannii* may have arisen as a result of selection, caused by differing ecological pressures that they face in their distinct distributions (Muchugi *et al*., 2008). Moreover, Maroyi (2014) states that *W. ugandensis* is found in lowland rainforest habitats, which are characterised by having hot and dry conditions, whereas Muchugi *et al.* (2008) highlights that the preferred habitat type for *W. ugandensis* is the eastern African highland, which experiences cooler and wet conditions. These observations may be the reason behind the taxonomic confusion of the *Warburgia* genus and may explain why some scientists recognise that *W. ugandensis* comprises two subspecies, while others do not. For example, Maroyi (2014) and Muchugi *et al*. (2008) characterise *Warburgia* as a genus having four species, whereas Leonard and Viljoen (2015) identify five taxa in the genus.

Naidoo and Lamb (2006) used DNA sequencing and fingerprinting techniques to study the genetic diversity of the genus across Africa. The authors presented the possibility of one taxonomic unit in southern Africa consisting of *W. stuhlmannii* and two taxonomic units in north-eastern Africa. Here, we aim to address this confusion by examining the climatic niches of *Warburgia* species to determine the taxonomic differentiations of the genus (using the ecological species concept). In this study, we consider *Warburgia* as a genus comprising five taxa: *W. elongata*, *W. salutaris*, *W. stuhlmannii*, *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis*.

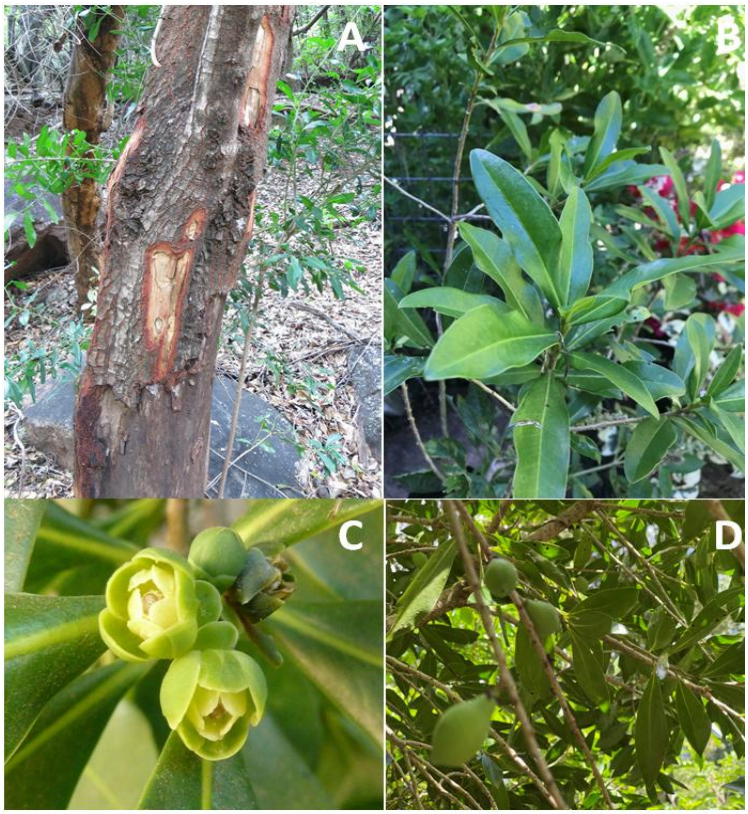

Figure 2.1. Image of *Warburgia salutaris* with evidence of bark stripping (A); *W. salutaris* shoots (B); *W. salutaris* flowers (C); and *W. ugandensis* leaves and fruit (D). Source: iNaturalist. Photo credit: R. Van Huysteen, Presha, M. Kriek, P. Kahumbu.

2.1.3. Aim and objectives

This study aimed to characterise the environmental niche of *Warburgia* species and compare the environmental variables of the two subspecies of *W. ugandensis* to test whether these warrant taxonomic distinctions based on the ecological species concept. The first objective was to construct SDMs for the five *Warburgia* taxa across eastern and southern Africa. The second objective was to extract environmental variables and compare the environmental niches of the two subspecies of *W. ugandensis* to determine whether they should remain separate taxonomic units or be combined.

2.2. Methods

2.2.1. Study area and species data

The study area covers 19 countries in eastern and southern Africa, specifically South Africa, Lesotho, Eswatini, Botswana, Zimbabwe, Mozambique, Zambia, Malawi, Tanzania, Rwanda, Burundi, Uganda, Kenya, Ethiopia, Somalia, Namibia, Angola, Democratic Republic of Congo (DRC) and South Sudan, and spans approximately 12 544 484 km² of surface area, with a mean elevation of 1000 masl. The study area includes the current natural distribution of *Warburgia* (obtained using locality data from GBIF, iNaturalist and SAEON), and buffer countries around the countries with known occurrence. The study area comprises five major climate regions, namely rainforest, grassland, semiarid, desert and humid subtropical zones. The four species and two subspecies of *Warburgia* inhabit a variety of habitat types, including lowland forests, montane forests, coastal and riverine systems and savannas (Pooley, 1993; Thomas and Grant, 1998).

Occurrence data for *W. elongata*, *W. salutaris*, *W. stuhlmannii*, *W. ugandensis*, *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* were downloaded from the Global Biodiversity Information Facility (GBIF; https://www.gbif.org/) repository and iNaturalist (https://www.inaturalist.org/). A further 226 *W. salutaris* localities were obtained from Dr. Dave Thompson at SAEON. Obtained occurrence information was georeferenced and validated using Google Earth (9.190.0.0) and herbaria records, and points that were found in unlikely locations (e.g., two localities found in the United States of America and two points found in Kirstenbosch Botanical Garden, Western Cape and East Cape Safari, Eastern Cape, which are likely cultivated and not representative of natural distributions), points recorded before the year 1970, and duplicates were removed. 374 occurrences remained after filtering (*W. elongata* = 9, *W. salutaris* = 295, *W. stuhlmannii* = 19, *W. ugandensis* = 24, *W. ugandensis* subsp. *longifolia* = 7 and *W. ugandensis* subsp. *ugandensis* = 20) and were used for analyses.

2.2.2. Climate variables

Nineteen variables were reviewed as potential predictors of the distributions of *Warburgia* species (Table S2.1). Climate data for the period of 1970 – 2000 were obtained from Chelsa version 2.1 (https://chelsa-climate.org/) at 30-arc second (~1km) resolution from the Hadley Global Environment Model 2-Atmosphere Ocean (HADGEM2-AO) circulation model (Martin *et al*., 2011) and was cropped to the study area, which covers the documented natural distribution of the five *Warburgia* species. Due to the large extent of the study area, although informative in species distribution modelling, fine-scale variables such as NDVI would not

have given meaningful results. To prevent overparameterization of the SDMs, over correlated variables were excluded using Pearson's pair-wise correlation coefficient tests between all the variables. Variables that were highly correlated ($r > |0.7|$) were removed from the dataset. In cases where two variables were highly correlated, the variable least correlated to the other variables was retained. The seven variables that remained from the 19 variables after processing were: Maximum Temperature of the Warmest Month (Bio05), Temperature Annual Range (Bio07), Mean Temperature of the Wettest Quarter (Bio08), Mean Temperature of the Coldest Quarter (Bio11), Precipitation of the Wettest Quarter (Bio16), Precipitation of the Driest Quarter (Bio17) and Precipitation of the Warmest Quarter (Bio18). These remaining climate variables were used in the distribution modelling of all the species to allow for comparisons of the distinguishing variables of each species and are included in other studies that predict species distributions of *Warburgia* species (e.g., Senkoro, 2021; van Breugel *et al*., 2011). Pre- and post-processing of the climate data layers were performed in R Studio (version 4.2.2, R Core Team, 2022) and ArcMap (version 10.6, ESRI, 2011).

2.2.3. Constructing distribution and niche models

2.2.3.1. Model building and interpretation

SDMs extract the environmental variables associated with the occurrence of a species and generate an environmental distribution for the species projected into geographic space (Guisan and Thuiller, 2005; Miller, 2010; Pecchi *et al*., 2019). The biomod2 package (Thuiller *et al*., 2012) in R Studio (version 4.2.2, R Core Team, 2022) was applied to ascertain the probable distribution of the five *Warburgia* species, as well as to identify the environmental variables that govern the distribution/preferred habitats of the species. There are ten algorithms available within the package (Table S2.2). After a primary run was done using all ten algorithms, the best performing algorithms were retained (AUC and TSS values ≥ 0.5). This included the generalised linear model (GLM), generalised additive model (GAM), generalised boosting model (GBM), surface range envelope (SRE), artificial neural network (ANN) and random forest (RF). Thereafter, an ensemble model was created for each species. An ensemble model uses a combination of algorithms to produce an improved model, when compared to individual algorithms (Hao *et al*., 2019; Hao *et al*., 2020). Habitat suitability ranges between 0 and 1, where $0 \leq$ suitability < 0.25 represents unsuitable, $0.25 \leq$ suitability < 0.5 represents low suitability, $0.5 \leq$ suitability < 0.75 represents moderate suitability and $0.75 \leq$ suitability < 1 represents high suitability.

To accommodate the lack of absence data, we used one run of ten thousand randomly selected pseudo-absences/background samples for one model evaluation. Using a selection of random pseudo-absences from the area around the occurrence points of the species provides the modelling algorithms with background samples that have a parallel predisposition as the actual presence/occurrence data (Phillips and Dudík, 2008). The pseudo-absence and presence datasets were split into 80% training data and 20% testing data for each model performed, and variable importance of the models was determined using three permutations per modelling algorithm. This step addressed any overfitting concerns that may have arose from the small sample sizes of occurrence data.

The remaining model parameters were set to default in the biomod2 package (Thuiller *et al*., 2012). Model predictive performance was assessed using area under the receiver operating curve (AUROC or AUC) and true skill statistic (TSS) values (Hanley and McNeil, 1982; Allouche *et al*., 2006), which range from 0 to 1, with values closer to one representing better predictive models. All statistical analyses for producing the SDMs were performed in R Studio (version 4.2.2, R Core Team, 2022) using the biomod2 package (Thuiller et al., 2021).

2.2.3.2. Calculating niche overlap of *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* in environmental space

Assessing environmental niche characteristics can be used to identify the differences between closely related taxa (Aguirre-Gutierrez *et al*., 2015). A statistical framework was applied to describe geographical comparisons of the environmental niches of the two subspecies of *W. ugandensis*. The degree of shared environmental niche space was calculated between *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* using an ordination technique where a kernel smoothing method is applied over species presences plotted onto an environmental space with a resolution of r x r cells (Broennimann *et al*., 2012). This is used to estimate the environmental niche occupied by the species and the environmental variables along the first two axes of each cell in the dataset. The smoothing technique used removes biases caused by spatial resolution of the input data and over- and under-estimations of species densities to produce a more reliable representation of suitable environmental conditions for the species. The application of this method was based on a principal component analysis, which is an ordination technique regulated across the full environmental space of the study area (referred to as PCA-env). Niche overlap was calculated by extracting environmental data from background points, created by implementing 20km buffers around

the species occurrence points, and then applying the Schoener's *D* statistic (Di Cola *et al*., 2017; Schoener, 1970; Warren *et al*., 2008; Warren *et al*., 2010). Schoener's *D* statistic ranges between 0 (no overlap) and 1 (complete overlap). The classification format described by Rödder and Engler (2011) was used to infer niche overlap, where $0.0 \leq D < 0.2$ represents no or very low overlap, $0.2 \leq D < 0.4$ represents low overlap, $0.4 \leq D < 0.6$ represents intermediate overlap, $0.6 \leq D < 0.8$ represents high overlap and $0.8 \leq D < 1.0$ represents very high overlap or identical niches. Ultimately, the species occurrence densities in the environmental niche space were plotted onto principal component (environmental) axes using a principal component analysis (PCA) to assess the differences in niche dimensions between *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis*.

Niche equivalency and niche similarity tests were performed to determine statistical confidence in niche overlap values (Warren *et al*., 2008; Di Cola *et al*., 2017). The niche equivalency test determines whether two environmental niches are identical when occurrences are randomly shuffled between two ranges and the niche overlap is recalculated. A null distribution was created by repeating this 100 times, which was compared to the observed *D* to make inferences on statistical significance. The null niche equivalency hypothesis is rejected when the observed niche indices are significantly lower than the null distribution niche indices (Broennimann *et al*., 2012). Due to the rigidity of the niche equivalency test, rejecting the identical niche hypothesis does not necessarily imply that the two niches involved are divergent (Variawa, 2017). The statistical niche similarity test compares *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* environmental niches to determine if one niche can predict the other niche better than a null or randomly generated niche, while accounting for varying background spaces. Rejecting the null hypothesis indicates that the niches are more similar or different than expected by chance (Variawa, 2017). All niche analyses were conducted in the ecospat package (Broennimann *et al*., 2016) in R Studio (version 4.2.2, R Core Team, 2022).

2.3. Results

2.3.1. Model evaluation indices and variable importance

The commonly used AUC and less commonly used TSS values indicated good model performance across all species (Table 2.1). AUC varied from 0.950 to 1.000 and TSS varied from 0.870 to 1.000. Models performed well across all taxa.

Table 2.1. Area under the ROC curve (AUC) and true skill statistic (TSS) values that demonstrate species distribution model performance for *Warburgia elongata*, *W. salutaris*, *W. stuhlmannii*, *W. ugandensis*, *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis.*

Species	AUC	TSS
W. elongata	0.959	0.958
W. salutaris	0.980	0.980
W. stuhlmannii	0.870	0.870
W. ugandensis	0.940	0.940
W. ugandensis subsp. *longifolia*	1.000	1.000
W. ugandensis subsp. *ugandensis*	0.950	0.950

Based on the ensemble model, temperature annual range (Bio07) was the most influential environmental variable in shaping the distribution of *W. elongata*, whereas mean daily air temperatures of the coldest quarter (Bio11) was the most important variable governing the distribution of *W. salutaris* (Table 2.2). *Warburgia stuhlmannii* is influenced by mean monthly precipitation of the driest quarter (Bio17). *Warburgia ugandensis* and *W. ugandensis* subsp. *ugandensis* are constrained by mean daily maximum air temperature of warmest month (Bio05), with *W. ugandensis* subsp. *longifolia* being influenced by the mean monthly precipitation amount during the warmest quarter (Bio18).

The variable importance of the individual algorithms (Table 2.C) somewhat corresponds with that of the ensemble models (Table 2.2), with temperature annual range (Bio07) constraining the distribution of *W. elongata*. The distribution of *W. stuhlmannii* was governed mainly by the mean precipitation of the driest quarter (Bio17). *Warburgia ugandensis* subsp. *longifolia* and *W. salutaris* distributions were driven mainly by the mean monthly precipitation of the warmest quarter (Bio18). Interestingly, the most influential variable shaping the distributions of *W. ugandensis* and one of its subspecies, *W. ugandensis* subsp. *ugandensis* differ between the ensemble and individual algorithms, with the individual algorithms showing that they are mainly influenced by annual air temperature range (Bio07), rather than maximum air temperature of the warmest month (Bio05).

Table 2.2. Environmental variable importance scores (mean and standard deviation shown for the three permutations, standard deviation in brackets) for *Warburgia elongata*, *W. salutaris*, *W. stuhlmannii*, *W. ugandensis*, *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* ensemble species distribution models. Bold variable scores in the grey cells represent the most important variable for each taxon. Bio05—Max Temperature of the Warmest Month; Bio07—Temperature Annual Range; Bio08—Mean Temperature of the Wettest Quarter; Bio11—Mean Temperature of the Coldest Quarter; Bio16—Precipitation of the Wettest Quarter; Bio17—Precipitation of the Driest Quarter; Bio18—Precipitation of the Warmest Quarter.

Species	Bio05	Bio07	Bio08	Bio11	Bio16	Bio17	Bio18
W. elongata	0.069 (0.002)	**0.844 (0.013)**	0.293 (0.006)	0.171 (0.003)	0.118 (0.004)	0.561 (0.006)	0.264 (0.007)
W. salutaris	0.105 (0.006)	0.097 (0.003)	0.171 (0.005)	0.146 (0.002)	0.488 (0.016)	0.059 (0.004)	**0.639 (0.016)**
W. stuhlmannii	0.478 (0.011)	0.517 (0.015)	0.801 (0.005)	0.393 (0.009)	0.218 (0.007)	**0.870 (0.015)**	0.370 (0.004)
W. ugandensis	**0.545 (0.009)**	0.202 (0.004)	0.099 (0.001)	0.139 (0.007)	0.102 (0.002)	0.268 (0.005)	0.275 (0.009)
W. ugandensis subsp. *longifolia*	0.112 (0.011)	0.621 (0.018)	0.438 (0.007)	0.509 (0.004)	0.263 (0.007)	0.635 (0.009)	**0.723 (0.017)**
W. ugandensis subsp. *ugandensis*	**0.668 (0.020)**	0.281 (0.015)	0.131 (0.001)	0.121 (0.001)	0.203 (0.003)	0.134 (0.005)	0.184 (0.007)

2.3.2. Current Warburgia *distribution in eastern and southern Africa*

Under the current climatic state, there was little highly suitable habitat (suitability: 0.75 – 1.0) available for *W. elongata*. The ensemble distribution model predicted moderate to high suitability (0.5 – 1.0) in the coastal regions of East Africa, spanning from Somalia down to Mozambique. Low to moderate suitability (0.25 – 0.5) suitability was predicted between 7°N and 15°S, characterised by a low annual air temperature range of approximately 10°C. The ensemble predicted a considerable area of high habitat suitability for *W. salutaris* in southern Africa, specifically in South Africa, Mozambique and Zimbabwe, which is characterised by a

mean monthly precipitation of the warmest quarter of approximately 60 mm. *Warburgia stuhlmannii* was predicted to have a restricted distribution with high suitability along the coasts of Kenya and Tanzania and low suitability in a limited portion of the DRC, which both have a low mean monthly precipitation of 22 mm in the driest quarter of around. The distribution of *W. ugandensis* was predicted to span a large part of the study area, with high habitat suitability in the DRC, Uganda, inland Kenya and Tanzania, Angola and Ethiopia, characterised by a moderate maximum air temperature of the warmest month between 20 – 25 °C. Low to moderate habitat suitability spanned throughout the eastern part of the study area. *Warburgia ugandensis* subsp. *longifolia* had the geographically smallest predicted distribution of all the species, with high habitat suitability in small portions of Angola, the DRC, Uganda and the coast of Tanzania, which are characterised by a low to moderate mean precipitation in the warmest quarter of 55 mm. Lastly, *W. ugandensis* subsp. *ugandensis* was predicted to have the largest geographic range of all the species, with a low to moderate habitat suitability spanning most of the study area. The species exhibits high habitat suitability in Ethiopia, Kenya, Uganda and Tanzania and, unexpectedly, the east coast of South Africa. The areas of high habitat suitability of *W. ugandensis* subsp. *ugandensis* are characterised by a moderate maximum air temperature in the warmest month of 20 – 25 °C.

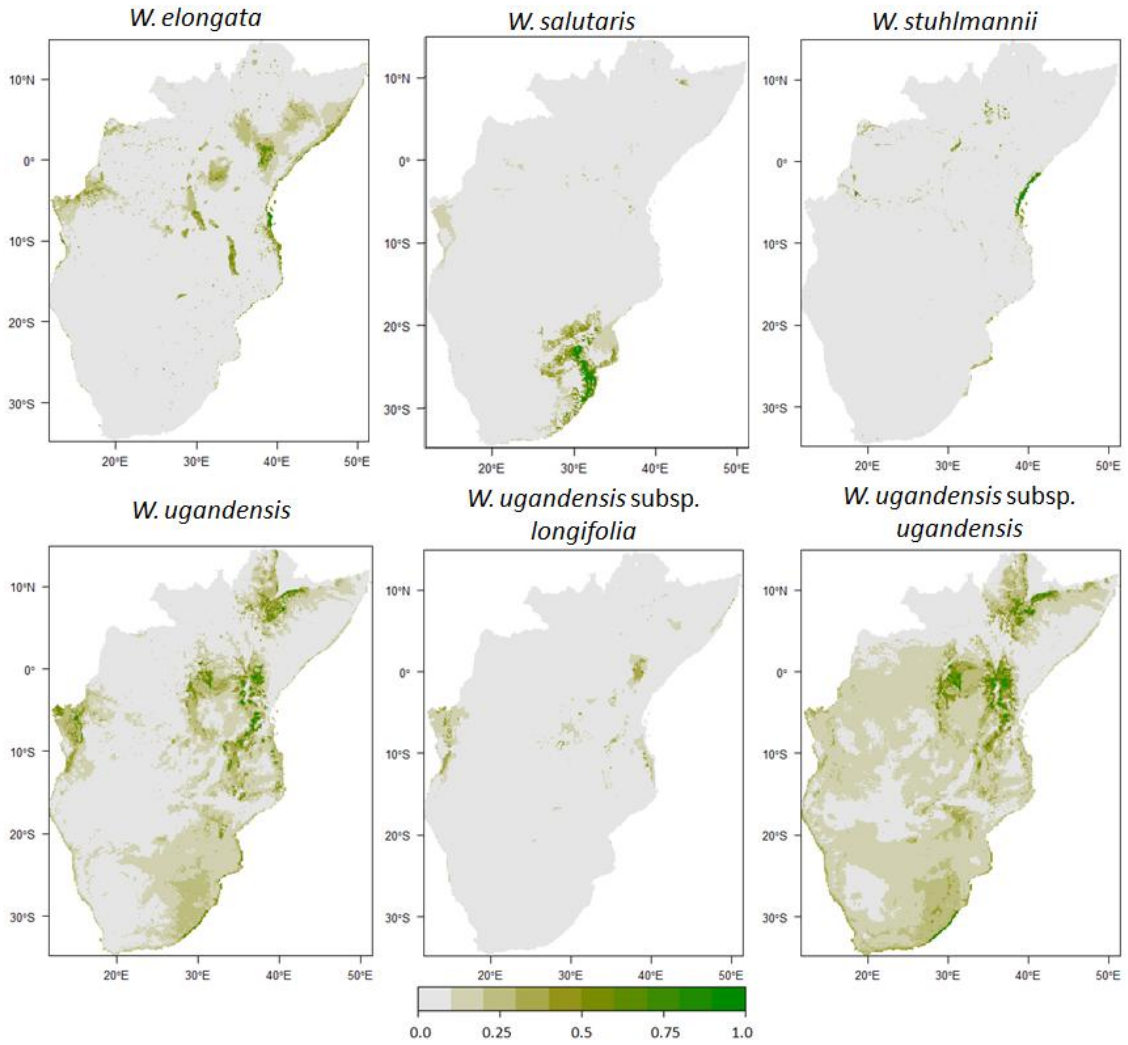

Figure 2.2. Distribution of *Warburgia elongata*, *W. salutaris*, *W. stuhlmannii*, *W. ugandensis*, *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* predicted using ensemble modelling based on seven current climatic environmental variables. Habitat suitability ranges from unsuitable (grey) to highly suitable (dark green).

2.3.3. Environmental niche overlap and similarity of W. ugandensis *subspecies*

Warburgia ugandensis subsp. *ugandensis* has the broader environmental niche (Figure 2.3b) between the two subspecies of *W. ugandensis*, and therefore spans a larger geographical area (Figure 2.2). The environmental niches of *W. ugandensis* subsp. *ugandensis* and *W. ugandensis* subsp. *longifolia* overlap, with the former fitting almost entirely into the latter's niche (Figure 2.3c). This implies that *W. ugandensis* subsp. *ugandensis'* niche is the broader of the two niches.

Interestingly, *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* showed low overlap, with only 24.9% niche overlap. In addition, the comparisons of *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* show that their environmental niches were more similar than expected by random chance (P = 0.0495) and are equivalent (P = 0.727; Table 2.3). Although contradictory to results for the niche equivalency and similarity tests, the low niche overlap value is likely the result of the limited occurrence records for the *W. ugandensis* subspecies used for the analyses.

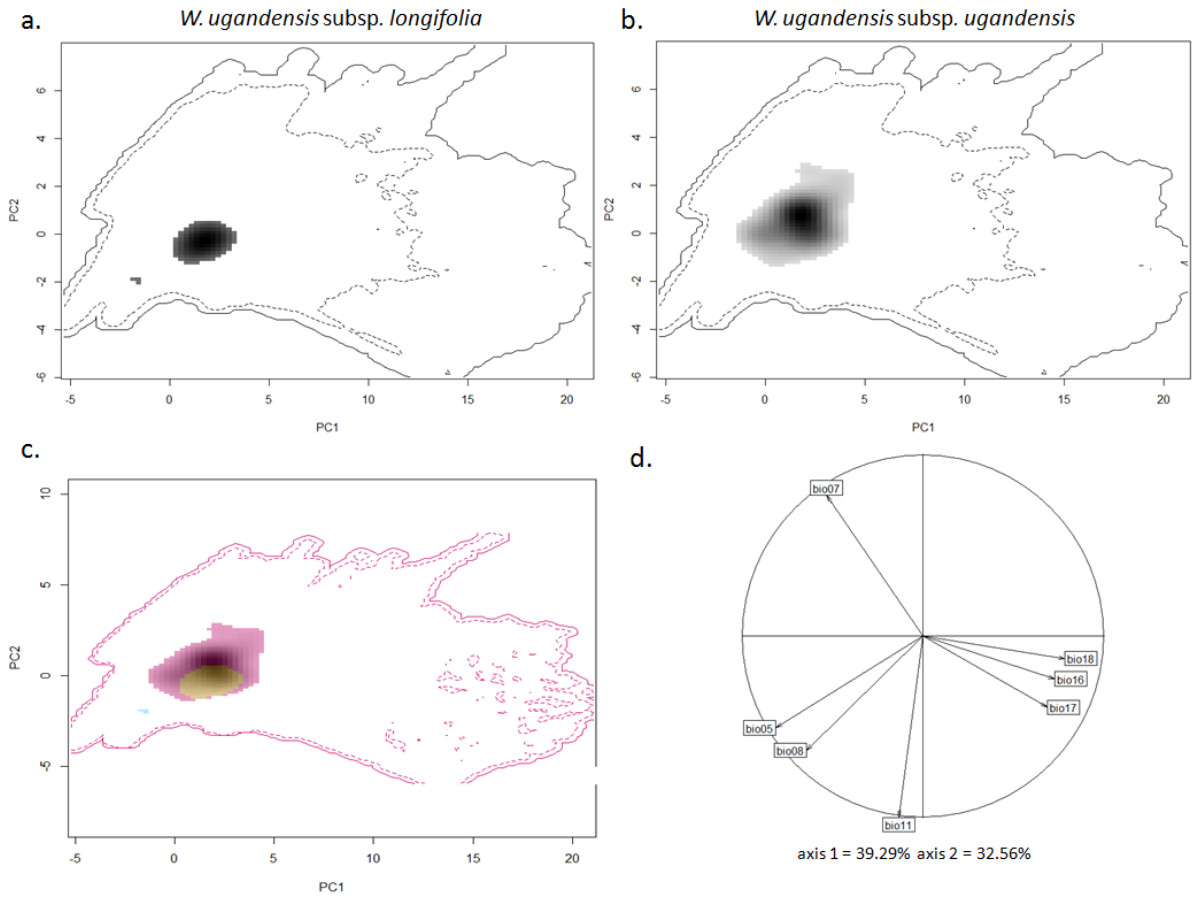

Figure 2.3. Environmental niches of *Warburgia ugandensis* subsp. *longifolia* (a) and *W. ugandensis* subsp. *ugandensis* (b) produced by the principal component analysis (PCA-env). The PCA-env plots represent the niches of the two species in the two main axes/environmental variables of the complete study area. The solid line delineates 100% of the available environmental space and the dashed line indicates 50% of the background space. The strength of the shading represents the density of occurrence. Dynamic niche overlap plot showing the overlap of the environmental niches of *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* (c). The blue area represents the *W. ugandensis* subsp. *longifolia* range and the pink area shows the *W. ugandensis* subsp. *ugandensis* range. The yellow area represents niche overlap. Variable correlation circle showing the contribution of variables for loading the two main PCA-env axes and the percentage of inertia explained by those axes (d).

Table 2.3. Niche overlap (*D*), similarity and equivalency of *Warburgia ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis*. Significant similarity tests are in bold.

Pairwise comparison	*D* statistic	Niche similarity	Niche equivalency
W. ugandensis subsp. *longifolia* → *W. ugandensis* subsp. *ugandensis*	0.249	**0.0495**	0.727
W. ugandensis subsp. *ugandensis* → *W. ugandensis* subsp. *longifolia*	0.249	**0.0495**	0.727

2.4. Discussion

Studying spatial patterns of species occurrence is critical in understanding species' ecological requirements (Srivastava *et al*., 2019). This study used SDMs to define the primary climate variables determining the current distribution and predicted habitat suitability of *Warburgia* species, and to specifically test whether these warrant taxonomic distinction of the two subspecies of *W. ugandensis*.

In this study, ensemble SDMs were used to analyse the environmental variables influencing the distributions of *Warburgia* species. In addition, we used comparative niche modelling on the climatic niches of two *Warburgia* subspecies (*W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis*), that have been a topic of debate regarding their taxonomic classification, to gain insights into the taxonomy of the genus. It was found that the ensemble SDMs accurately predicted the known geographic distributions of each *Warburgia* species, and that the genus is generally limited by temperature variables.

2.4.1. Distributions and key environmental predictors of **Warburgia** *species' habitat*

The different *Warburgia* species have restricted and mutually exclusive geographic distributions, with the slight exception of *W. elongata* and *W. ugandensis* that overlap in a small portion of Kenya. Results also show that the distributions of *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* overlap, however *W. ugandensis* subsp. *ugandensis* is much more widespread than *W. ugandensis* subsp. *longifolia*, which is

supported by numerous studies (e.g., Leonard and Viljoen, 2015; Maroyi, 2014; Muchugi *et al*., 2008).

The predicted distributions of *W. elongata*, *W. stuhlmannii* and *W. ugandensis* span small areas of high suitability ($0.75 \leq$ suitability ≤ 1) outside their geographic distributions. *Warburgia elongata* is endemic to Tanzania and tracks annual air temperature ranges of approximately 12°C (Figure 2.2). New populations could therefore be established, or undiscovered populations could be found, around the forested slopes of Mount Kenya (with possible assistance from Mount Kenya National Park, Nyongesa and Vacik, 2019) and around the Great Lakes of Africa. *Warburgia stuhlmannii* has a restricted range along the east coast of Tanzania; however, the ensemble model predicted high habitat suitability along the east coast of Kenya in addition to the coast of Tanzania. Fungomeli *et al*. (2020) developed a database of the tree species found in the coastal forests of Kenya. *Warburgia* did not fall into the database; however, the species is likely to thrive along the coastal forests of Kenya, with the help of forest reserves found in the area (Fungomeli *et al*., 2020). *Warburgia ugandensis* (and similarly *W. ugandensis* subsp. *ugandensis*) naturally occurs in Tanzania, Kenya and Uganda; however, the ensemble predicted high habitat suitability in Ethiopia as well (Williams *et al*., 2004). Populations may occur along the Ethiopian Highlands, which consists primarily of grasslands and montane forests. It is important to note that the genus may not be in the database due to the sensitive nature of the species' localities. The ensemble model predicted high habitat suitability for *W. salutaris* in eastern South Africa, Lesotho, eSwatini, southern Mozambique and south-eastern Zimbabwe. In addition to their primary influencer variables, *Warburgia* species' ranges are likely restricted due to a lack of dispersal and not necessarily because there is a lack of suitable habitat (Senkoro *et al*., 2021).

The ensemble models predicted restricted areas of highly suitable habitat for *Warburgia* species in the study area. Predictions of highly suitable areas were generally in accordance with the ground observation of the distributions of the species, which are concentrated within eastern and southern Africa. Projections made by the models in this study show that there are areas of suitable habitat outside of the central regions, however much of these areas have low suitability (suitability < 0.5). *Warburgia* species may still be able to successfully occupy these seemingly out-of-range areas. For example, an extra-limital *W. salutaris* population growing outside of Pietermaritzburg, South Africa, exhibits good health and abundant seed production (D. Thompson, Nov. 2023, pers. comm.). The projections that are made outside of

the central distributions of the species may confirm the ecologically generalist nature of *Warburgia* species, as they can occupy a broad environmental niche (Maroyi, 2014).

The most important variable driving the distribution of *W. elongata* is annual range of air temperature (Bio07), ranging between 13 to 15°C in the east coast of Tanzania. Mean monthly precipitation amount of the driest quarter (Bio17) was the most impactful variable for *W. stuhlmannii*, whereas mean daily air temperature of the coldest quarter (Bio11) was the most constraining factor of *W. salutaris*. Desiccation sensitivity at the seed and seedling stage - a common feature of woody forest species - are generally associated with water stress (Pritchard *et al*., 2022; Subbiah *et al*., 2019; Walck *et al*., 2011; Wyse and Dickie, 2017). Having the ability to delay germination due to seed dormancy allows species to germinate during favourable conditions, ensuring their growth and success. If a species has nondormant seeds however, such as *Warburgia* species, seeds must germinate relatively quickly following dispersal (Walck *et al*., 2011). Temperature and precipitation affect soil characteristics, and thus seed persistence in soil, especially for desiccation-sensitive seeds (Walck *et al*., 2011). It is possible that these species' distributions are highly constrained by water stress, as their persistence is dependent on a narrow but relatively warm temperature range and intermediate rainfall.

Mean daily mean air temperature of the coldest quarter (Bio11) was the most constraining factor of *W. salutaris*. The mean daily mean air temperature of winter of the species' southern African distribution ranges between 15 and 20°C. Although mature *W. salutaris* trees are quite hardy, young trees and seedlings are sensitive to frost (Mbambezeli, 2018). The most influential variable of *W. ugandensis* was mean daily maximum air temperature of the warmest month (Bio05), with *Warburgia ugandensis* subsp. *longifolia* being driven by mean monthly precipitation amount of warmest quarter (Bio18) and *W. ugandensis* subsp. *ugandensis* by mean daily maximum air temperature of the warmest month (Bio05). This is likely due to photosynthesis and stomatal conductance. Kinyamario *et al*. (2008) showed that *W. ugandensis* had significantly higher photosynthetic and stomatal conductance rates in shaded environments, compared to fully lit environments. Since incoming solar energy can be used as a proxy for temperature, that study coincides with *W. ugandensis* being constrained by temperature of the warmest month (when incoming solar energy is highest). In addition, Kinyamario *et al*., (2008) found that stomatal conductance of *W. ugandensis* is lowest in full sunlight areas. Seedlings perform poorly in high radiation environments, as the species

readily reaches maximum photosynthetic rates at low light intensities, which then decreases when there is a flux in photon density (Kinyamario *et al*., 2008; Pritchard *et al*., 2022).

Overall, *W. ugandensis*, has a reduced water use efficiency in full sunlight. This is due to the species having low photosynthetic rates, low stomatal conductance and therefore low transpiration rates in full sunlight conditions (Kinyamario *et al*., 2008). Therefore, mean monthly precipitation amount of warmest quarter (Bio18) is the most important variable influencing the distribution of *W. ugandensis subsp. longifolia*, as stomatal conductance and respiration rates are negatively correlated to rainfall, especially in warm temperatures (i.e., summer or the warmest quarter). Kairu *et al*. (2014) showed that *W. ugandensis* occurs mainly in drier regions, such as the dry montane forests of Mt. Kenya. Their study found that there is a strong negative correlation between rainfall and tree size proxies. The species thrives in low rainfall areas; hence its limiting factor is rainfall in the summer rainfall region, when rainfall is highest. The species could survive in intermediate forests; however, these regions are usually susceptible to land use change (Kairu *et al*., 2014).

2.4.2. Niche overlap, equivalency and similarity of W. ugandensis *subsp.* longifolia *and* W. ugandensis *subsp.* ugandensis

This study aimed to characterise and distinguish the species of *Warburgia*, particularly, *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* using applications of the ecological species concept. Using ecological niche theory to identify or differentiate species is prevalent in the literature (e.g., Bedson *et al*., 2021). One example shows how two sister oak species in the U.S.A. have a shared geographic space but have diverged ecologically due to different flowering times. Quantifying these two sister species' niches have indicated that they are separate species due to niche divergence (Cavender-Bares and Pahlich, 2009), which is the process by which a species occupies an ecological niche that differs from its original or ancestral one. Niche conservatism is the tendency of species to retain their ancestral ecological niche preferences over time (Vaissi and Rezaei, 2022; Wiens and Graham, 2005). Consequently, related species occupy more divergent niche space than by random chance, but individuals or populations of the same species will share more similar niche space than by random chance (Vaissi and Rezaei, 2022).

2.4.3. Niche conservatism in Warburgia

Warren *et al*. (2008) reports on two opposing hypotheses regarding niche conservatism. On one hand, closely related species occupy significantly diverse ecological niches, suggesting that varying selection likely drove speciation (Warren *et al*., 2008). On the other hand, speciation occurs over geographic (not ecological) dimensions, which is later followed by ecological differentiation (Peterson, 2011; Warren *et al*., 2008; Wiens and Graham, 2005). This could be the case for the two subspecies of *W. ugandensis*, which show slight geographic differences, but a high degree of overlap of their ecological states. The morphological differentiation in their boles (main wooden axis of a tree) and bark colour could be the result of edge effects, which are the differing environmental conditions experienced at the boundaries between habitat patches. This could result in the speciation of those individuals found on edges (de Paula *et al*., 2016). In addition, Wamalwa *et al*. (2006) found evidence for genetic variation in different Kenyan populations of *W. ugandensis*, suggesting that the species is on the verge of diversification. Analyses used in this study to interpret the environmental niches of the two subspecies of *W. ugandensis* revealed that the niches of *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* were equivalent or identical (D = 24.9%, P>0.05) and more similar than expected by random chance (D = 24.9%, P<0.05). It is possible that the niche overlap value is quite low, due to the limited occurrence records for the species. These findings suggest that the environmental niches exhibit strong niche conservatism, so much so that we suggest sinking the subspecies of *W. ugandensis*, but still recognise that the species may be in the process of diverging even in the absence of significant ecological niche differentiation (Wamalwa *et al*., 2006).

2.5. Conclusion

This study used species distribution modelling and environmental niche indices to identify suitable habitats for the threatened *Warburgia* genus and characterise the environmental factors to inform the current taxonomy of the genus. Overall, it was found that *Warburgia* species' distributions are primarily constrained by environmental factors that influence their desiccation-sensitive seeds, such as annual temperature range and winter temperatures. Notably, there are suitable habitats around the Great Lakes of Africa and Mt. Kenya for *ex situ* conservation strategies which do not have any recorded occurrences for *W. ugandensis* and *W. elongata*. The results also suggest that the two *W. ugandensis* subspecies should be lumped together in accordance with the ecological species concept. From a conservation viewpoint, it will be useful for future studies to improve field surveys of these threatened

species and update the occurrence point database. Additionally, further data collection and validation could improve the robustness of the findings of this study. This study highlights the efficacy of using ENMs to address species diversification in accordance with the ecological species concept.

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2.7.Appendices

Appendix 1

Table S2.1. Chelsa (version 2.1) climate variables used in environmental analyses. Climate variables used in SDM production are highlighted.

Code	Variable	Unit of measurement
Bio01	Mean Annual Ait Temperature	°C
Bio02	Mean Diurnal Ait Temperature Range	°C
Bio03	Isothermality	°C
Bio04	Temperature Seasonality	°C/100
Bio05	**Maximum Temperature of the Warmest Month**	**°C**
Bio06	Minimum Temperature of the Coldest Month	°C
Bio07	**Temperature Annual Range**	**°C**
Bio08	**Mean Temperature of the Wettest Quarter**	**°C**
Bio09	Mean Temperature of the Driest Quarter	°C
Bio10	Mean Temperature of the Warmest Quarter	°C
Bio11	**Mean Temperature of the Coldest Quarter**	**°C**
Bio12	Annual Precipitation	mm
Bio13	Precipitation of the Wettest Month	mm
Bio14	Precipitation of the Driest Month	mm
Bio15	Precipitation Seasonality	mm
Bio16	**Precipitation of the Wettest Quarter**	**mm**
Bio17	**Precipitation of the Driest Quarter**	**mm**
Bio18	**Precipitation of the Warmest Quarter**	**mm**
Bio19	Precipitation of the Coldest Quarter	mm

Table S2.2. Modelling algorithms available in the biomod2 package (Thuiller *et al*., 2012) in R Studio (R Core Team, 2022). Algorithms used in SDM production are highlighted.

GLM	**Generalised Linear Model**
GAM	**Generalised Additive Model**
GBM	**Generalised Boosting Model**
SRE	**Surface Range Envelope**
CTA	Classification Tree Analysis
ANN	**Artificial Neural Network**
MARS	Multiple Adaptive Regression Splines
RF	**Random Forest**
FDA	Flexible Discriminant Analysis
MAXENT.Phillips	Maximum Entropy

Appendix 2

Table S2.3. Environmental variable importance scores for *Warburgia elongata*, *W. salutaris*, *W. stuhlmannii*, *W. ugandensis*, *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* species distribution models under the current climate scenario for all algorithms used. Bold variable scores in grey cells represent the most important variable for each taxon. Bio05—Max Temperature of the Warmest Month; Bio07—Temperature Annual Range; Bio08—Mean Temperature of the Wettest Quarter; Bio11—Mean Temperature of the Coldest Quarter; Bio16—Precipitation of the Wettest Quarter; Bio17—Precipitation of the Driest Quarter; Bio18—Precipitation of the Warmest Quarter.

Species	Model	Bio05	Bio07	Bio08	Bio11	Bio16	Bio17	Bio18
W. elongata	GLM	0.000	**0.957**	0.140	0.000	0.000	0.473	0.000
	GAM	0.543	0.916	0.483	0.361	0.687	0.677	**0.981**
	GBM	0.075	**0.821**	0.627	0.098	0.058	0.243	0.510
	SRE	0.235	**0.755**	0.563	0.504	0.197	0.306	0.739
	ANN	0.131	**0.899**	0.009	0.012	0.391	0.677	0.733
	RF	0.300	0.611	**0.644**	0.603	0.413	0.642	0.606
W. salutaris	GLM	0.689	0.366	0.718	**0.817**	0.108	0.184	0.171
	GAM	0.360	0.459	0.319	0.555	0.586	0.094	**0.704**
	GBM	0.017	0.029	0.321	**0.637**	0.043	0.231	0.489
	SRE	0.350	0.404	0.272	0.454	**0.474**	0.461	0.446
	ANN	0.525	0.353	0.618	**0.862**	0.674	0.166	0.746
	RF	0.045	0.160	0.279	0.334	0.220	0.049	**0.391**
W. stuhlmannii	GLM	0.838	**0.925**	0.879	0.792	0.459	0.639	0.371
	GAM	0.444	0.712	0.930	0.733	0.747	**0.960**	0.752
	GBM	0.004	0.006	0.902	0.092	0.045	**0.962**	0.291
	SRE	0.206	0.507	**0.896**	0.671	0.563	0.824	0.474
	ANN	0.762	0.846	0.905	0.781	0.285	**0.952**	0.524
	RF	0.587	0.628	0.726	0.267	0.521	**0.859**	0.648
W. ugandensis	GLM	**1.000**	0.000	0.238	0.000	0.000	0.155	0.000
	GAM	0.425	**0.662**	0.395	0.473	0.462	0.660	0.502
	GBM	0.123	**0.562**	0.027	0.315	0.173	0.388	0.512

	SRE	0.217	0.491	0.261	0.401	0.413	**0.564**	0.492
	ANN	0.363	0.036	0.478	**0.600**	0.261	0.512	0.555
	RF	0.423	0.422	0.282	0.433	0.263	0.190	**0.529**
	GLM	0.000	**0.893**	0.061	0.000	0.059	0.543	0.545
W.	GAM	0.900	0.875	0.214	0.936	0.706	0.740	**0.940**
ugandensis	GBM	0.000	0.435	0.033	0.093	0.129	**0.845**	0.789
subsp.	SRE	0.135	**0.776**	0.109	0.324	0.216	0.626	0.598
longifolia	ANN	0.027	0.117	0.811	0.845	0.851	0.812	**0.943**
	RF	0.016	0.244	0.123	0.155	0.048	0.192	**0.281**
	GLM	**0.997**	0.000	0.000	0.000	0.043	0.009	0.000
W.	GAM	0.246	**0.709**	0.402	0.697	0.573	0.675	0.522
ugandensis	GBM	0.320	**0.497**	0.172	0.057	0.332	0.225	0.316
subsp.	SRE	0.233	0.445	0.277	0.379	0.384	**0.522**	0.396
ugandensis	ANN	0.785	**0.874**	0.725	0.786	0.866	0.397	0.873
	RF	0.501	0.421	**0.564**	0.493	0.442	0.468	0.418

Appendix 3

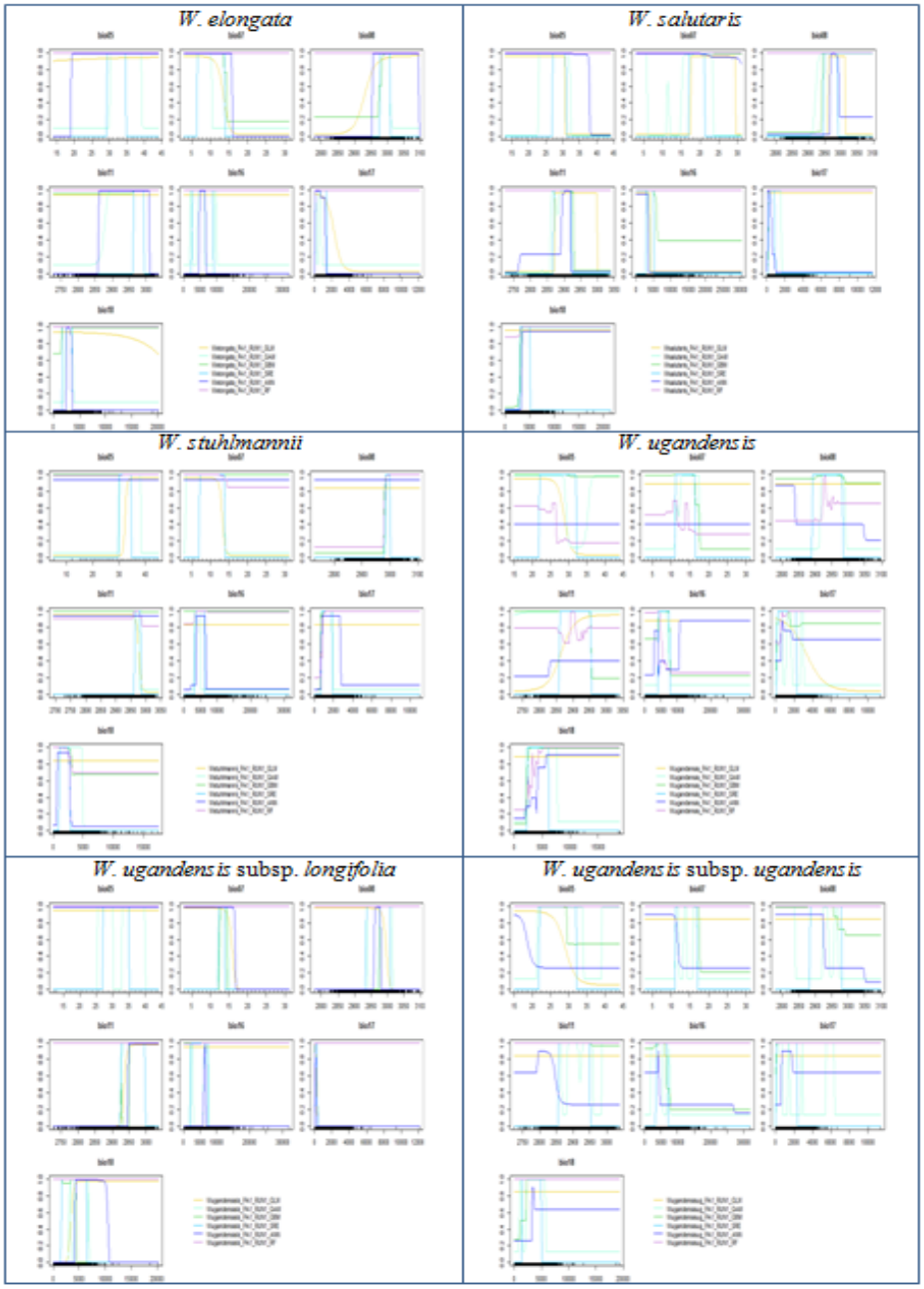

Figure S2.1. Ecological response curves for *Warburgia elongata*, *W. salutaris*, *W. stuhlmannii*, *W. ugandensis*, *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* showing the ranges of climatic conditions that are suited to the species' distributions. The x-axis represents the range of climatic variables across the study area and the y-axis represents suitability of a specific variable when all other variables are set to their mean.

Appendix 4

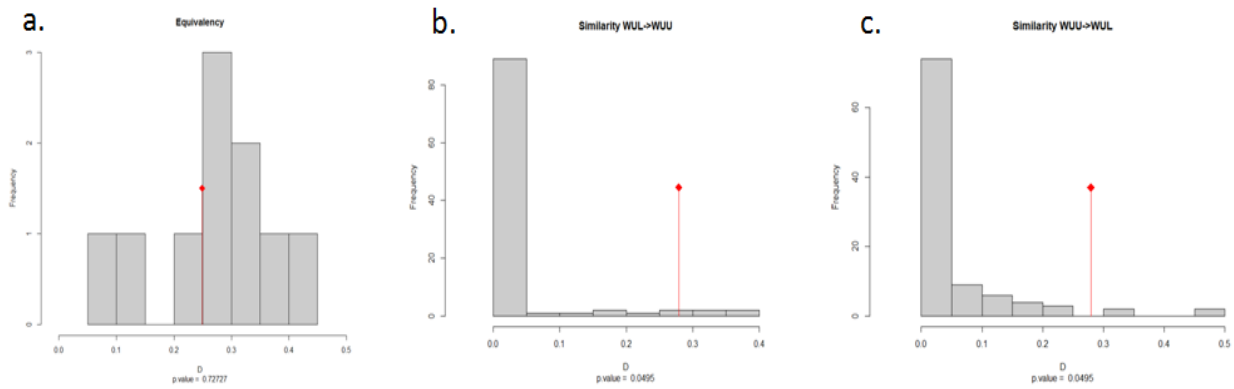

Figure S2.2. Equivalency (a) and similarity tests (b and c) between *Warburgia ugandensis* subsp. *longifolia* (WUL) and *W. ugandensis* subsp. *ugandensis* (WUU).

3. *Chapter 3: Assessing the current distribution and predicting the future distributions of* **Warburgia salutaris** *under different climate change scenarios in South Africa*

Abstract

Understanding where species occur and predicting where they may migrate to as a response to climate change is essential in conservation biogeography. *Warburgia salutaris*, commonly known as the pepper-bark tree, faces a decrease in suitable habitat due to the threat of climate change. The disjunct range of the tree and over-harvesting by humans for the traditional medicine market in South Africa has raised concern about the persistence of this ethnobotanically-important species. This study characterises the current (2000) and future (2070) environmental niches of *W. salutaris* and uses species distribution models (SDM) to project current and future distributions and to identify the most influential climatic variables governing the species' distribution. SDMs were conducted with species occurrence data of *W. salutaris* as well as current and future climate data under two emission scenarios (RCP 4.5 and 8.5). The study also used indices, such as overlap, expansion, stability and unfilling to examine how the environmental niche of *W. salutaris* shifts under different climate change scenarios. The most influential climatic variables of the distribution of *W. salutaris* were maximum temperature of the warmest month, annual temperature range, mean temperature of the wettest and coldest quarters, and monthly precipitation of the wettest, driest, and warmest quarters. The distribution of *W. salutaris* was predicted to contract in eastern Limpopo and parts of Mpumalanga and expand along eastern KwaZulu-Natal. Overall, the distribution of *W. salutaris* contracts from the Indian Ocean coastal belt and grassland biomes and into the savanna biome. The results show that conservation strategies, such as identifying new cultivation sites and protected areas, and prioritizing the monitoring and management of sensitive *W. salutaris* populations, are necessary for the conservation of *W. salutaris* in the near future.

Keywords: climate change, conservation, niche modelling, species distribution modelling, traditional medicine

3.1. Introduction

Before the introduction of allopathic medicine, commonly known as the 'pepper-bark tree', the main form of health care was traditional medicine (Mander *et al*., 2007). Although allopathic medicine has become more accessible, approximately 27 million South Africans still depend on and prefer traditional medicine as their primary source of healthcare (Mander *et al*., 2007; Williams *et al*., 2013). This includes plant, animal and/or mineral-based medicines to maintain the health and well-being of people (Richter, 2003). Traditional medicine practices generally involve the collection, processing and trading of plant parts, including leaves, barks, roots and bulbs. Mander *et al*. (2007) found that South Africa consumes 70,000 tonnes of plant material in the form of traditional medicine per year. Unfortunately, the harvesting of certain plant parts, like the bark and roots, has detrimental effects to the survival of many medicinally important species (McMullin *et al*., 2014). This, along with the extremely high demand for traditional medicine, has resulted in many plants being unsustainably harvested. South Africa has documented local (e.g., *Warburgia salutaris* (G. Bertol.) Chiov., commonly known as the pepper-bark tree) and provincial (e.g., *Siphonochilus aethiopicus* (Schweif.) B. L. Burt., commonly known as wild ginger*)* extirpations due to unsustainable harvesting, and even a case of extinction in the wild (*Encephalartos woodii* Sander, commonly known as Wood's cycad, Williams *et al*., 2013). Other threats to the survival of medicinal plant species include land use change and climate change (Castle *et al*., 2014).

Climate change is occurring at a concerning rate and brings a host of new challenges to traditional conservation methods, which involves establishing and preserving new protected areas, and migration corridors. The changing climate is anticipated to alter the distribution of suitable habitats of numerous species, which requires adaptive conservation strategies to consider and address these shifts (Austin and Van Niel, 2011; Lyam *et al*., 2022; Williams *et al*., 2005). Sub-Saharan Africa is expected to experience higher than average warming levels, according to the Intergovernmental Panel on Climate Change (IPCC, 2014). Southern Africa is predicted to experience dramatic declines in rainfall, increasing aridity and extreme heat events (Serdeczny *et al*., 2017). It has also been predicted that a changing climate will bring fluctuating rainfall patterns, increased temperatures and an increase in the frequency in extreme climatic and weather events. South Africa's rich floral diversity is likely to be extremely vulnerable to varying pressures, including climate change, habitat degradation and

harvesting (Daya and De Crom, 2017; DEA, 2013; SANBI *et al*., 2013). DEA (2013) conducted assessments to identify how climate change and land-use change/anthropogenic affects will influence South African biomes. The study found that the Indian Ocean coastal belt, the forest and grassland biomes are the most vulnerable to climate change as they are targeted for urbanisation and agricultural expansion.

3.1.1. Study species

*Warburgia salutaris*belongs to the genus *Warburgia* (Canellaceae). It is an evergreen tree endemic to the savanna, Indian Ocean coastal belt, and grassland biomes of southern Africa (Mucina *et al*., 2006; Rutherford *et al*., 2006). The pepper-bark tree has numerous uses, including timber, firewood, mulch, resin, and most importantly, traditional medicine (Leonard and Viljoen, 2015; Maroyi, 2013; Maroyi, 2014; Senkoro, 2021; Van Wyk and Wink, 2018). It is highly popular for its wide range of socio-economic uses and as a result, has become threatened due to unsustainable harvesting and has experienced population declines and local extirpations (Botha *et al*., 2004; Maroyi, 2014; Moyo *et al*., 2015; Senkoro, 2021; Van den Bosch, 2021; Van den Bosch *et al*., 2023). In addition, climate change poses the threat of reduced habitat suitability and range shifts and has an exaggerated effect on threatened species with small, fragmented populations (Cheng *et al*., 2023; Gwate *et al*., 2023; Senkoro, 2021). The species has been deemed a priority species for enhanced management in South Africa and neighbouring countries, however, many traditional medicine vendors were not aware of its Red List of Threatened Species ranking. The ease of obtaining the bark and other parts of *W. salutaris* from medicinal markets without any negative consequences is likely the reason for this lack of awareness (Botha *et al*., 2004; Senkoro, 2021).

Further complicating the vulnerability of *W. salutaris* is the way the tree is used for traditional medicine. Healers primarily require the bark of mature trees, which renders trees susceptible to disease and death, and populations then have relatively high proportions of individuals in small size classes (< 1.5 m, Botha *et al*., 2004). In addition, *W. salutaris* has a relatively low reproductive output, due to low fruit production, seed predation and the inability for its recalcitrant seeds to be stored (van den Bosch, 2021; van den Bosch *et al*., 2023; Senkoro, 2021). It has been found that South African populations of *W. salutaris* rely heavily on clonal reproduction and resprouting. This is problematic as it limits the amount of

genetic diversity within populations, making them more susceptible to the detrimental effects of climate change (Glennon *et al*., 2023).

Maroyi (2013), Maroyi (2014) and Leonard and Viljoen (2015) give extensive overviews of the importance and uses of this species. Botha *et al*. (2004) studied the effects of commercialisation and harvesting on the species and Drewes *et al*. (2001) assessed alternative harvesting options. Other studies focused on the reproductive ecology of the species (van den Bosch *et al*., 2023) and the genetic structure and diversity of *W. salutaris* in Kruger National Park (KNP, Glennon *et al*., 2023). Although a distributional study has been done on *W. salutaris* (Senkoro *et al*., 2020), it does not include the largest population of the species (i.e., KNP).

3.1.2. Conservation status

The species has been listed as an endangered species (A1acd) on the IUCN Red List in South Africa and Malawi due to a reduced area of occupancy (AOO), reduced extent of occurrence (EOO), reduced habitat quality and overexploitation (IUCN, 2023). Mozambique and eSwatini have listed the species as vulnerable and critically endangered respectively, with Zimbabwe deeming the tree extinct in the wild (Botha *et al*., 2004; Maroyi, 2013).

The historical distribution of *W. salutaris* spans from Kenya and Tanzania, down to Mozambique and Zimbabwe and into South Africa and eSwatini. The South African distribution was extensive, with the species occurring widely in KwaZulu-Natal, Mpumalanga and Limpopo. Unfortunately, the majority of the South African *W. salutaris* populations have become extirpated, resulting in at least a 50% decline in the country's wild population. Although some healthy subpopulations occur in the Limpopo and Mpumalanga provinces of South Africa, most subpopulations have faced extirpations due to bark overexploitation (Scheepers *et al*., 2011; Williams *et al*., 2008).

Strides in the conservation of *W. salutaris* have been taken, especially in South Africa. Tissue culture trials were performed by various botanical organisations in Africa, which were successful and produced over 40 000 seedlings available for redistribution to traditional healers (van den Bosch *et al*., 2023). In addition, studies highlight the utility of leaves instead of bark as a more sustainable harvesting method (Drewes *et al*., 2001; Senkoro, 2021). There is a lack of data regarding the driver variables of the species' distribution and how these

variables, and consequently the species' distribution, will be affected by climate change. This type of analysis can be undertaken using species distribution models (SDMs).

SDMs are used in climate change projections and conservation planning. They play a fundamental role in selecting potential reserves and protected areas that can mitigate extinction risks caused by climate change and human exploitation (Seo *et al*., 2009). SDMs have become widely used in predicting how species distributions change (e.g., contract, expand, or shift) to adapt to changing environmental conditions (Austin and Van Niel, 2011). This study uses two approaches to characterise the current and future distributions of *W. salutaris*: a geographical approach that exhibits the temporal transferability of SDMs and an approach that estimates niche overlap indices.

This study aimed firstly to assess the current South African distribution of *W. salutaris* and identify those variables that govern it. The study then aimed to predict the future distribution under two climate change scenarios to assess how climate change will influence the species' distribution. The first objective was to construct SDMs to predict current and future potential areas where *W. salutaris* may occur in South Africa under different climate change scenarios. The second objective was to identify and compare the environmental variables, from various modelling algorithms, that define the distributions of *W. salutaris* under different climate change scenarios. Lastly, the third objective was to compare the geographic distributions of current and future environmental niches of *W. salutaris* to assess changes in the species range as a response to climate change. The findings aim to enhance our understanding of the species' environmental niche requirements and aid in locating potential areas for cultivation of the species, as well as to offer initial insights into the effects of climate change on the species.

3.2.Methods

3.2.1. Study area and species data

The study area covers the eastern part of South Africa, which includes the natural South African distribution of *W. salutaris* – the only *Warburgia* species occurring in southern Africa (Maroyi, 2013; Maroyi, 2014). In South Africa, *W. salutaris* occurs in the Limpopo, Mpumalanga, Gauteng, and KwaZulu-Natal provinces. Some of the last and largest remaining wild populations occur in KNP (Glennon *et al*., 2023). Additionally, two reproductively successful populations occur in Eston in the KwaZulu-Natal interior (van den

Bosch, 2021). The study area spans three of the nine South African biomes: savanna, Indian Ocean coastal belt and grassland (Rutherford *et al*., 2006). The extent of the study area was selected to encompass the known South African distribution of *W. salutaris*, with additional buffer space to accommodate potential shifts in the species' distribution due to climate change.

Occurrence data for *W. salutaris* were downloaded from the Global Biodiversity Information Facility (GBIF; https://www.gbif.org/) repository, with additional localities for Limpopo and Mpumalanga obtained from Dr. Bronwyn Egan (University of Limpopo), Dr. Mervyn Lotter (Mpumalanga Tourism and Parks Agency) and Dr. Dave Thompson (SAEON). Where required, occurrence information was georeferenced and validated using Google Earth (9.190.0.0), and points that were found in unlikely locations (e.g., Kirstenbosch National Botanical Garden, Western Cape) as well as duplicates were removed. A total of 295 unique occurrences remained after filtering and were used in the analysis of the South African distribution of the species.

3.2.2. Climate and environmental variables

Current (1970–2000) climate data were obtained from Chelsa version 2.1 (https://chelsa-climate.org/) at 30-arc second (~1km) resolution from the HADGEM2-AO circulation model and cropped to South Africa (excluding the Prince Edward Islands) using ArcMap (version 10.6, ESRI, 2011).

CHELSA climate also offers datasets of future (2070) climate projections for four different emission scenarios. The future climate scenarios are based on Representative Concentration Pathways (RCPs) that explore various plausible changes in climate based on anthropogenic activity (van Vuuren *et al*., 2011). These RCPs are models that predict future climatic states under various greenhouse gas emission scenarios. The four RCP scenarios are: RCP 2.6, RCP 4.5, RCP 6.0 and RCP 8.5. RCP 4.5 emissions are expected to peak in 2040, followed by a decline, and is thought to be the most probable emission scenario, whereas RCP 8.5 emissions are expected to have a continuous increase throughout the 21st century. These emission scenarios encompass a highly probable (4.5) and a worst-case scenario (8.5, Meinshausen *et al*., 2011), and were used in this study. Soil surface wetness (surface to 5cm below) data for the study area were obtained from NASA's Data Access Viewer (https://power.larc.nasa.gov/data-access-viewer/) for the year 2020.

The climate and environmental variables were chosen due to the relatively broad distribution of *W. salutaris* in eastern South Africa. Fine scale variables such as soil pH were not included as these are too spatially variable to have produced informative results. Although altitude (or elevation) influences species distributions (Hof *et al*., 2012), it was not included in this study as it has a strong correlation with climate, and would have been a confounding factor.

To prevent overparameterization of the SDMs, highly correlated variables were removed. This was achieved by conducting Pearson's pair-wise correlation coefficient analyses on all 19 variables and excluding those with a correlation of 0.7 or higher ($r > |0.7|$). Where two variables were highly correlated, the variable with the weaker correlation to other variables was retained. After the Pearson's pair-wise correlation coefficient analyses, seven climate variables of the initial 20 climate and environmental variables remained and were used in subsequent modelling: Maximum Temperature of the Warmest Month (Bio05), Annual Temperature Range (Bio07), Mean Temperature of the Wettest Quarter (Bio08), Mean Temperature of the Coldest Quarter (Bio11), Precipitation of the Wettest Quarter (Bio16), Precipitation of the Driest Quarter (Bio17) and Precipitation of the Warmest Quarter (Bio18, Table S3.1).

3.2.3. Model building and interpretation

SDMs, or habitat suitability models (HSMs), identify environmental variables linked to a species' occurrence and create a geographical representation of the species' distribution (Guisan and Thuiller, 2005; Miller, 2010; Pecchi *et al*., 2019). The biomod2 package in R Studio (Thuiller *et al*., 2012, R Core Team, 2022) was used to determine the likely distribution of *W. salutaris* and identify the environmental factors that make up its preferred habitats. All ten algorithms available in the biomod2 package (Table S3.2) were employed in preliminary analyses, and the top-performing ones were retained (AUC and TSS values $\geq$ 0.5): generalised linear model (GLM), generalised additive model (GAM), generalised boosting model (GBM), surface range envelop (SRE), artificial neural network (ANN) and random forest (RF). Subsequently, an ensemble model was created, which combined these algorithms to improve predictive accuracy compared to when using individual algorithms (Hao *et al*., 2019; Hao *et al*., 2020). The range of habitat suitability lies between 0 and 1, where $0 \leq$ suitability < 0.25 represents not suitable, $0.25 \leq$ suitability < 0.5 represents low

suitability, $0.5 \leq$ suitability < 0.75 represents moderate suitability and $0.75 \leq$ suitability < 1 represents high suitability.

Studies comparing different SDM methods showed that models using both presence and absence data tend to outperform models using only presence data. Since true absences are widely unavailable for many species, artificial absences, or pseudo-absences are created to use in modelling (Barbet-Massin *et al*., 2012). Absence and pseudo-absence data are necessary in distribution modelling because they compare the environmental predictors of occurrence points compared to non-occurrence points, which give a better understanding of the environmental conditions the species prefers and where it does and does not occur (Barbet-Massin *et al*., 2012). In the modelling process, ten thousand randomly sampled pseudo-presences or background points were used for a single model evaluation. The pseudo-absences were selected from the area surrounding the species' occurrence to ensure they have the same predisposition as the actual presence data. Both the presence and pseudo-absence datasets were divided into 80% for training data and 20% for testing, which were used to calculate the model performance. The variables' importance were assessed by conducting three permutations for each modelling algorithm.

The remainder of the model parameters were kept at default settings for the biomod2 package (Thuiller *et al*., 2012). The predictive performance of the models was assessed using area under the receiver operating curve (AUROC or AUC) and true skill statistic (TSS) values, which range from 0 to 1, with higher values representing better performance. Three separate models were constructed for the current climate state and the future climate states (RCP 4.5 and RCP 8.5), which were trained with the current conditions and then projected into the future.

3.2.4. Range size change

The SDMs were then manipulated to create binary maps, which were used to examine changes in the geographic extent of the current and future distributions under both emission scenarios (i.e., RCP 4.5 and RCP 8.5). The binary maps' thresholds remained at the default setting for the biomod2 package, utilizing TSS optimization (Thuiller *et al*., 2012). 'Loss', 'gain', 'stable' and 'unsuitable' were used as terms to represent changes in the species distributions (Pang *et al*., 2021). Loss represented the geographic area that originally inhabited the species but became unsuitable after the respective climate change scenario. Gain referred to the geographic area that was not originally suitable for the species but

became inhabited by the species after climate change. Stable referred to the geographic areas where the species persisted as the climate changed. Unsuitable referred to the geographic space that was not originally suited to the species and will remain unsuitable (Pang *et al*., 2021).

3.2.5. Calculating niche overlap between current and future distributions of W. salutaris

The study employed environmental niche models in combination with future climate predictions to assess the vulnerability of *W. salutaris* to extinction, habitat loss and distribution changes. We used a statistical framework, specifically a principal component analysis (PCA-env), to compare the current and future (RCP 4.5 and RCP 8.5) environmental niches of the species (per Broennimann *et al*., 2012). The degree of overlap, ranging from 0 (no overlap) to 1 (complete overlap), between the current and future niches was calculated using a kernel smoothing method and quantified using Schoener's *D* statistic. Schoener's *D* statistic was then classified as per Rödder and Engler (2011): $0.0 \leq D < 0.2$ indicating no or very low overlap, $0.2 \leq D < 0.4$ indicating low overlap, $0.4 \leq D < 0.6$ indicating intermediate overlap, $0.6 \leq D < 0.8$ indicating high overlap and $0.8 \leq D < 1.0$ indicating very high overlap or identical niches Lastly, we plotted the species occurrence density in the environmental niche space onto principal component axes using a principal component analysis (PCA) to assess the differences in niche dimensions between the current and future niches.

We then conducted niche equivalency and niche similarity tests to assess niche overlap values given by Schoener's *D* statistic (Warren *et al*., 2008; Di Cola *et al*., 2017). The niche equivalency test determined whether the current and future niches are identical by shuffling occurrence between two ranges and recalculating niche overlap. A null distribution, created through 100 repetitions of shuffling occurrences, was compared to the observed *D* value to assess statistical significance. Rejection of the null hypothesis occurred when observed niche indices were significantly lower than those in the null distribution. It is noted that rejecting the niche equivalency test does not necessarily imply niche divergence, due to the stringent nature of the test. The niche similarity test compared the current and future niches of *W. salutaris*, and assessed whether one niche could predict the other niche better than a null niche (Variawa, 2017). All analyses of ecological niches were performed using the ecospat package (Broennimann *et al*., 2016) through R Studio (version 4.2.2, R Core Team, 2022).

3.2.6. Overlap of highly suitable habitat for W. salutaris and protected areas in South Africa

A map was constructed to identify the overlap of protected areas with highly suitable habitat ($0.75 \leq$ suitability ≤ 1.0) by overlaying the future species distribution maps of *W. salutaris* to have a representation of the area within which the tree can potentially become established. Polygons representing protected areas, including nature reserves, game reserves, heritage parks, strictly protected areas and protected environments, were then overlaid onto the highly suitable areas.

3.3.Results

3.3.1. Model evaluation indices and variable importance

Model performance was good for all emission scenarios, with AUC ranging from 0.936 to 0.965 and TSS ranging from 0.935 to 0.965.

Table 3.1. Area under the ROC curve (AUC) and true skill statistic (TSS) values showing species distribution model performance of *Warburgia salutaris* under current and future (RCP 4.5 and RCP 8.5) climate scenarios.

Emission scenario	AUC	TSS
Current	0.9356	0.9351
RCP4.5	0.9647	0.9647
RCP8.5	0.9639	0.9641

The current ensemble distribution of *W. salutaris* was determined by the mean temperatures of the coldest quarter (Bio11, Table 3.2), however the leading environmental variable from the individual algorithms were inconclusive (Table S3.3). The future distributions of *W. salutaris* were inhibited by annual air temperature range (Bio07), which coincides with the most influential environmental variable predicted from the individual modelling algorithms.

Table 3.2. Environmental variable importance scores (mean and standard deviation shown for the three permutations) for *Warburgia salutaris* ensemble species distribution models under current and future climate scenarios. Bold variable scores in the grey cells indicate the most important variable for each taxon under current and future climate scenarios. Bio05—Max Temperature of the Warmest Month; Bio07—Temperature Annual Range; Bio08—Mean

Temperature of the Wettest Quarter; Bio11—Mean Temperature of the Coldest Quarter; Bio16—Precipitation of the Wettest Quarter; Bio17—Precipitation of the Driest Quarter; Bio18—Precipitation of the Warmest Quarter.

Emission Scenario	Bio05	Bio07	Bio08	Bio11	Bio16	Bio17	Bio18
Current	0.194 (0.002)	0.146 (0.001)	0.094 (0.001)	**0.550 (0.021)**	0.146 (0.002)	0.159 (0.002)	0.287 (0.007)
RCP 4.5	0.249 (0.006)	**0.614 (0.013)**	0.078 (0.001)	0.202 (0.002)	0.263 (0.006)	0.116 (0.003)	0.084 (0.002)
RCP 8.5	0.329 (0.005)	**0.752 (0.019)**	0.222 (0.005)	0.284 (0.002)	0.069 (0.001)	0.116 (0.001)	0.302 (0.003)

3.3.2. W. salutaris *distribution under climate change*

The ensemble model predicted high habitat suitability ($0.75 \leq$ suitability ≤ 1.0) for *W. salutaris* in the provinces of Limpopo, Mpumalanga and KwaZulu-Natal, all characterised by variable habitats, such as coastal regions, lowveld, highveld, montane forests and mountainous regions (Figure 3.1).

The east coast of South Africa was predicted to become more suitable for *W. salutaris* under both future scenarios. For the more probable RCP future scenario, habitat suitability was expected to decrease along the north-eastern boundary of Limpopo. The same pattern was predicted for the more extreme scenario, however to a lesser extent. Under RCP 4.5, but more so under RCP 8.5, the western part of KNP was expected to become more suitable for the species. Overall, the current distribution of *W. salutaris* was predicted to decrease by 22.90% under the RCP 4.5 scenario and decrease by 19.53% under the RCP 8.5 scenario by 2070 (Table 3.3).

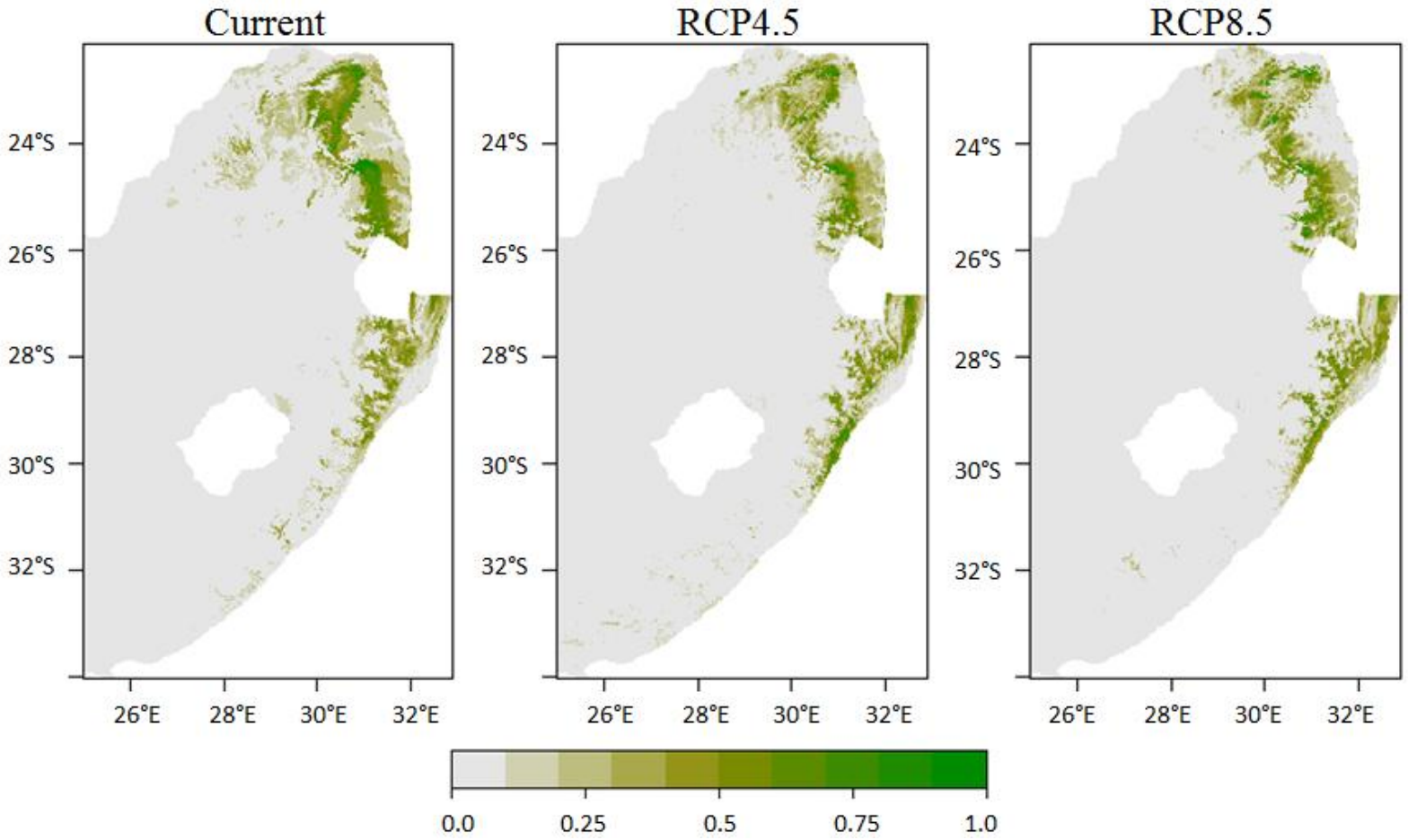

Figure 3.1. Distribution of *Warburgia salutaris* predicted using ensemble modelling based on current and future (RCP 4.5 and RCP 8.5) climatic environmental variables. Habitat suitability ranges from not suitable (grey) to highly suitable (dark green).

Table 3.3. Overall predicted range size changes of *Warburgia salutaris* under future scenarios (RCP 4.5 and RCP 8.5) for all models.

Emission scenario	Loss (%)	Gain (%)	Species range change (%)
RCP 4.5	50.413	22.404	-22.901
RCP 8.5	59.228	39.695	-19.533

3.3.3. Environmental niche overlap and similarity

The PCA plots displayed how current and future ranges of *W. salutaris* are distributed along principal climate niche axes. The niche of *W. salutaris* is quite narrow but geographically widespread along the eastern part of South Africa. The environmental niche of *W. salutaris* was predicted to contract by 2070 under both RCP 4.5 and RCP 8.5 (Figure 3.2).

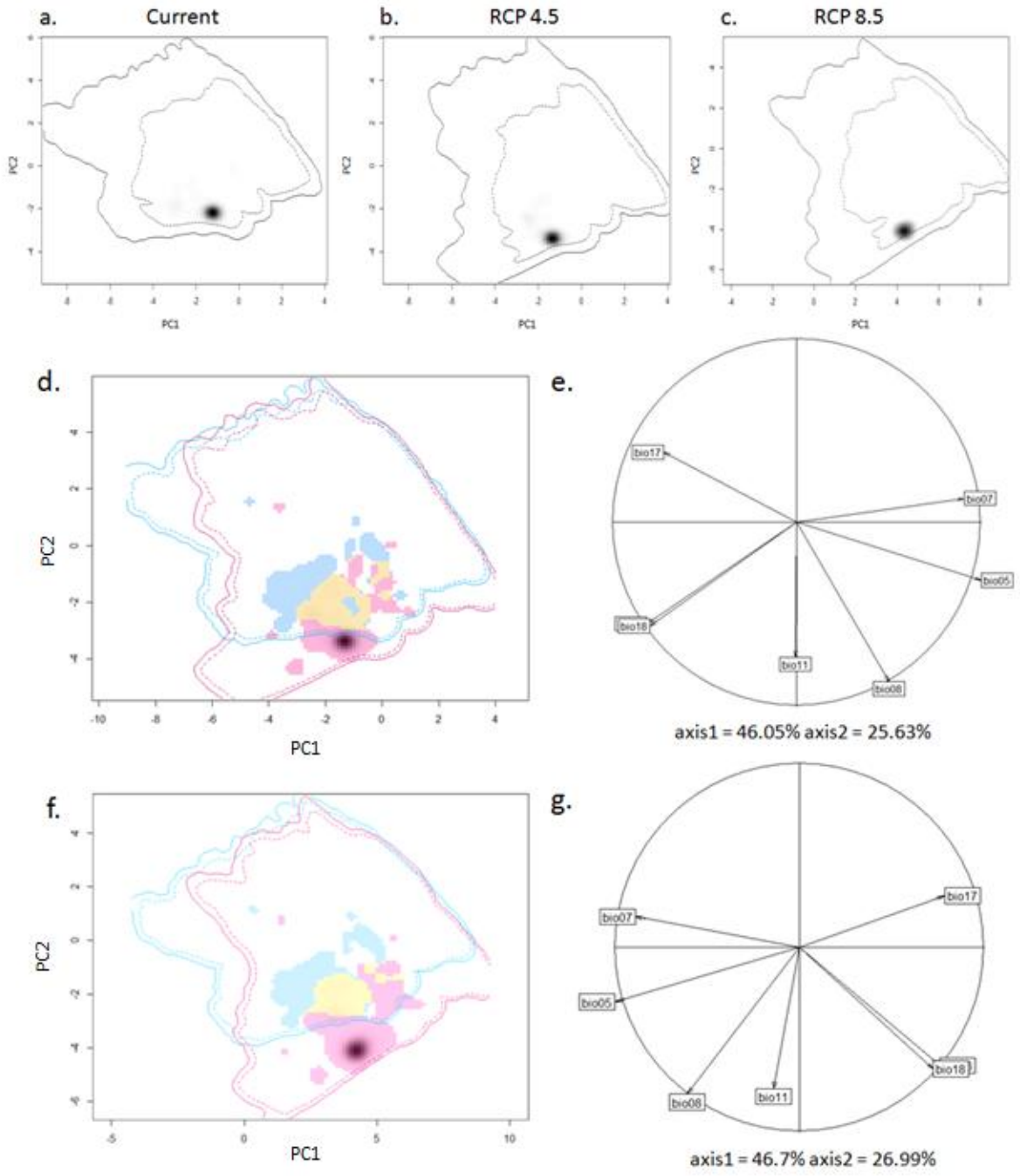

Figure 3.2. Current (a) and future (b, RCP 4.5 and c, RCP 8.5) environmental niches of *Warburgia salutaris* produced by the principal component analysis (PCA-env). The PCA-env plots represent the current and future niches of *Warburgia salutaris* in the two main axes/environmental variables of the complete study area. The solid line delineates 100% of the available environmental space and the dashed line indicates 50% of the background space. The strength of the shading represents the density of occurrence. Dynamic niche overlap of the current and future (d, RCP 4.5 and f, RCP 8.5) niches of *W. salutaris*. The blue area represents the current range, and the pink area shows the future range. The yellow area

represents niche overlap. Variable correlation circles showing the contribution of variables for loading the two main PCA-env axes and the percentage of inertia explained by those axes for RCP 4.5 (e) and RCP 8.5 (g).

3.3.4. **W. salutaris** *environmental niche similarity*

Interestingly, the comparisons of the current to future niches show that the niches are not more similar/different than expected by random chance; however, the reverse comparisons show that the future niches are more similar to the current niches than expected by random chance. In addition, the current and future niches (RCP 4.5 and RCP 8.5) of *W. salutaris* are equivalent to each other, according to the niche equivalency tests ($P > 0.05$).

Table 3.4. Niche similarity of *Warburgia salutaris* under current and future climate emission scenarios (RCP 4.5 and RCP 8.5). Significant *P*-values of similarity tests are in bold.

Pairwise comparisons	Niche similarity *P*-value	Niche equivalency *P*-value
Current → RCP 4.5	0.198	0.188
RCP 4.5 → Current	**0.020**	1.000
Current → RCP 8.5	0.366	1.000
RCP 8.5 → Current	**0.020**	1.000

3.3.5. Niche overlap between **W. salutaris** *current and future environmental niches*

The current and RCP 4.5 environmental niches showed low overlap (2.8%) and the current and RCP 8.5 environmental niches showed very limited overlap (0.3%, Table 3.5). Although seemingly contradictory to results from the niche equivalency and similarity tests, the low niche overlap value is likely the consequence of the future climate changing across *W. salutaris*' current range.

Analysis of the niche overlap between current and future climate scenarios showed that the current and RCP 4.5 environmental niches were stable; however, this was not the case for RCP 8.5. In addition, we found that *W. salutaris* is more inclined to expand into new ecological niches (niche expansion: Current → RCP 4.5: 0.880; Current → RCP 8.5: 0.918)

than to vacate its existing niche (niche unfilling: Current → RCP 4.5: 0.439; Current → RCP 8.5: 0.127).

Table 3.5. Niche overlap dynamics between current and future (RCP 4.5 and RCP 8.5) environmental niches of *Warburgia salutaris*. Significant *P*-values are in bold. Values range from low (0) to high (1).

Pairwise comparisons	Niche overlap (*D*)	Expansion	Stability	Unfilling
Current → RCP 4.5	0.028	0.880	0.120	0.439
Current → RCP 8.5	0.003	0.918	0.082	0.127

3.3.6. Overlap of highly suitable habitat for W. salutaris and protected areas in South Africa

The overlap between protected areas in eastern South Africa and highly suitable area predicted for *W. salutaris* by 2070 is quite low, and is concentrated along the western edge of KNP, in the interior of Mpumalanga and along the eastern coast of KwaZulu-Natal.

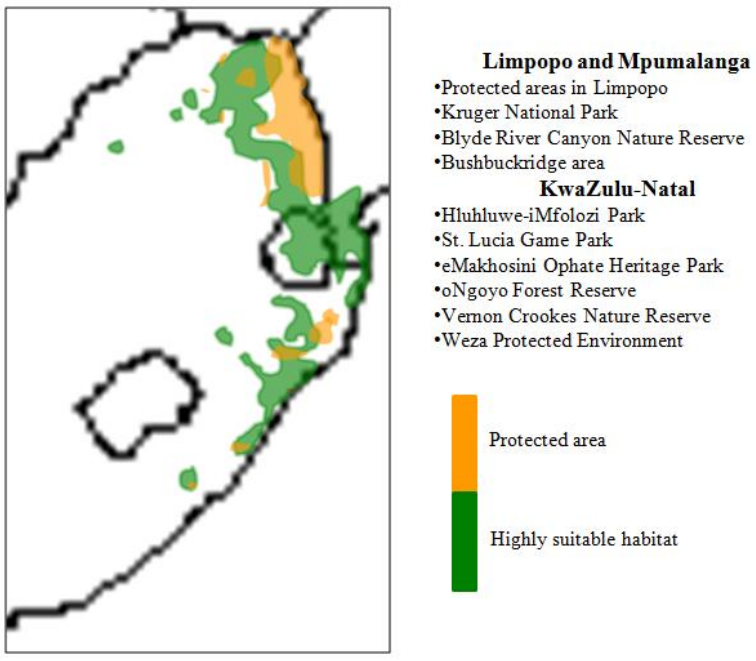

Figure 3.3. Highly suitable habitat (dark green) predicted for *Warburgia salutaris* in South Africa (predicted for both RCP 4.5 and RCP 8.5) overlaid with protected areas (names on the right) for potential propagation (orange)

3.4.Discussion

3.4.1. Current and future distributions and key environmental predictors of **W. salutaris***' habitat*

Of the seven environmental variables used to infer the ensemble models, the most influential variable of *W. salutaris*' current distribution was winter temperature; and that of its future distribution (for both climate scenarios) was annual temperature range. These findings were expected as research has reported the sensitivity of the species to low temperatures and frost, especially when young (Becking, 2015; Mbambezeli, 2018). Consequently, the distribution of *W. salutaris* is limited to areas with relatively high winter temperatures (15 – 20 °C) and an annual temperature range of 12 – 17 °C.

Warburgia salutaris produces flowers from autumn to winter and this may expose flowers to detrimentally low temperatures (Mbambezeli, 2018). In addition, fruit are produced from winter to early summer, which again, may expose them to frost. Temperature fluctuations are predicted to decrease under RCP 4.5 but increase under RCP 8.5 within the predicted geographic distributions of *W. salutaris*. In addition, these findings highlight that the climatic requirements of *W. salutaris* are particularly important because the species produces recalcitrant seeds during the months where seasonality is relatively low. Kioko *et al*. (2003) highlights this by reporting that in uncommon events where seeds of *W. salutaris* do reach maturity without human intervention; these seeds rapidly lose their viability due to their initially high water content gradually equilibrating to ambient relative humidity. The specifications of *W. salutaris*' distribution are therefore likely due to it being a frost and desiccation sensitive species (Becking, 2015; Kioko *et al*., 2003; Mbambezeli, G., 2018; Senkoro, 2021).

The ability of plants to disperse is pivotal for their survival, as it determines whether they can reach suitable habitats when faced with climate change (Variawa, 2017). *Warburgia salutaris*' seeds are dispersed passively, by birds (Senkoro *et al*., 2020), and possibly by baboons (Van Wyk, 1984). Dispersal mechanisms for *W. salutaris* are not well understood, but seeds could potentially spread to new suitable habitats via birds, baboons, or human assistance. *Warburgia salutaris* has been considered the most endangered plant species in South Africa (Kioko *et al*., 2003), due to the high levels of bark collection and low seedling recruitment in addition to its climatic limitations. The low level of seedling recruitment is due

to a range of reasons, such as: pre-dispersal predation by unidentified insect larvae; short distance pollination; the need for immediate germination of seeds after dropping off the tree and water stress (Hannweg *et al*., 2015; Kioko *et al*., 2003; van den Bosch *et al*., 2023; Senkoro, 2021). Furthermore, the pepper-bark tree displays late-acting self-incompatibility, resulting in the abortion of many seeds and fruit (van den Bosch *et al*., 2023).

Climate change effects on tree communities have been reported worldwide (DEA, 2013; Senkoro, 2021; Twala *et al*., 2023). South African biomes vary in their vulnerability to climate change, and the Indian Ocean coastal belt and grassland biomes are highly vulnerable (DEA, 2013). This study forecasts range adaptations for *W. salutaris* under the different future climate change scenarios. Under both future climate scenarios, overall range contractions were predicted for *W. salutaris*, with RCP 8.5 having the more optimistic outcomes. The largest contractions were predicted in the western regions of the Vhembe and Mopani districts, Limpopo. Opposing findings have been reported by Senkoro (2021) however, whereby *W. salutaris* distribution was mapped in southern Mozambique under all four climatic states (i.e., RCP 2.6, RCP 4.5, RCP 6.0 and RCP 8.5) and range expansion was predicted under all future scenarios.

Results from both emission scenarios (but more so for RCP 8.5) predict an increase in habitat suitability and range expansions along the east coast of KwaZulu-Natal, which is the Indian Ocean coastal belt biome. This finding may have been predicted because the temperature range of that area is expected to increase in the future regardless of climate scenario, resulting in the area shifting from the Indian Ocean coastal belt biome into the savanna biome. Currently, the mean daily mean air temperature of winter in the natural distribution of the species is between 15 – 20 °C, which stabilizes in 2070 at approximately 20 °C. This range shift may eliminate the risk of frost and low temperatures, which will be beneficial to the species (Mbambezeli, 2018). This finding offers new insights into the climatic preferences of *W. salutaris*, as it tracks warmer temperatures. It was also found that habitat suitability decreases in patches of Mpumalanga and eastern Limpopo but increases in the western interior of Limpopo for both future scenarios.

The conservation of threatened biodiversity is commonly achieved through protected areas (Yu *et al*., 2014). Protected areas include national parks, game reserves, heritage parks and nature reserves (Prendergast *et al*., 1999). Protected areas are an essential *ex situ* conservation

technique, as they conserve large amounts of biodiversity at low costs through management and monitoring (Maxted, 2013; Pritchard *et al*., 2012; Yu *et al*., 2014). Despite their effectiveness in conserving threatened taxa, protected areas are not designed to accommodate changes in species' distributions caused by climate change. In extreme cases, the effects of climate change may even drive populations of endangered trees out of protected areas; therefore, conservation biologists should safeguard these populations occurring within protected areas (Yu *et al*., 2014) as well as look at other methods such as afforestation to mitigate the effects of climate change.

The ensemble model predicted a decrease in habitat suitability for *W. salutaris* in the eastern part of KNP for both future climate scenarios. Since KNP is home to some of the largest *W. salutaris* populations, efforts should be taken to conserve these valuable populations as the climate changes. Climate change affects endangered trees in different ways, including decreased genetic diversity, higher susceptibility to pests and disease and extinction (Caffarra *et al*., 2012; Yu *et al*., 2011; Yu *et al*., 2014). Sensitive populations of *W. salutaris*, such as those in KNP, should therefore be prioritised for long-term monitoring and management. Additionally, areas that were predicted to become more suitable for *W. salutaris* outside of KNP should be evaluated for the acquisition and expansion of the existing protected area (Yu *et al*., 2014) to provide corridors to allow the tree to track its niche (e.g., Bushbuckridge and Blyde River Canyon Nature Reserve, Figure 3.3).

Conservation efforts and propagation should also be concentrated in areas where habitat suitability is predicted to increase, for example an orchard in Eston (KwaZulu-Natal, approximately 45km from Durban, van den Bosch *et al*., 2023). Eston orchard is an example of an extra limital population of *W. salutaris*. The region in which Eston occurs is currently marginally suitable ($0.5 \leq$ suitability < 0.75); however, it contains two healthy and reproductively successful populations and is predicted to become more suitable by 2070. Propagation of *W. salutaris* trees should therefore be concentrated in areas that are currently moderately suitable but will increase in suitability in the future. These areas include the western boundaries of KNP (in the eastern regions of the Mopani and Vhembe districts). On western boundary of KNP lie many areas that are suitable for potential conservation of *W. salutaris*, including the Blyde River Canyon Nature Reserve and the Bushbuckridge (Mapulaneng) area. The Blyde River Canyon Nature Reserve has some records of *W. salutaris* already; therefore a *W. salutaris* plantation could potentially be established there.

Bushbuckridge is an area that has experienced land use change, and is made up of human settlements, nature reserves and agricultural land (Thornton, 2012). The area also contains large areas of communal rangelands (Dovie *et al.*, 2004), which may make it more complex to establish *W. salutaris* plantations; however, we could engage local communities and employ the use of *in situ* conservation strategies, such as greenhouses and other infrastructure to aid in these plantations (Scott-Shaw, 1999). Ayodeji (2020) shows that the communities of Bushbuckridge, especially the rural communities, rely heavily on forests for a variety of uses. They also show positive attitudes towards conserving important tree species and are willing to adopt conservation practices. Other areas where *W. salutaris* plantations could potentially be established include protected areas in eastern KwaZulu-Natal, such as Vernon Crookes Nature Reserve, Weza Protected Environment and St. Lucia Game Park (Figure 3.3.). We should therefore concentrate *in situ* conservation efforts in these areas to increase *W. salutaris* populations.

3.4.2. Comparative niche analysis (niche overlap, equivalency and similarity)

Niche conservatism pertains to a species' inclination to maintain its ancestral ecological specifications (Wiens *et al.*, 2010). There are numerous studies that show that despite climatic and environmental changes, many species exhibit continued niche conservatism over time (Peterson, 2011; Wiens *et al*., 2010). The persistence of niche conservatism generally restricts species' geographic ranges and hold significant implications for conservation efforts (Variawa, 2017; Wiens *et al*., 2010). Here, the data show that *W. salutaris* is unlikely to undergo significant changes in environmental niche occupancy. The niche equivalency assessment showed that the current and future environmental niches of *W. salutaris* are equivalent. The niche similarity assessment showed that the comparisons of the future and current environmental niches (RCP 4.5 → Current and RCP 8.5 → Current) exhibited more shared niche space than randomly expected. Interestingly, the inverse of these comparisons (Current →RCP 4.5 and Current → RCP 8.5) show that the current and future niches of the species do not share more environmental niche space than randomly expected. These results are not necessarily contradictory and instead show that overall, the current and future environmental niches of the species are equivalent. Interestingly, *W. salutaris* displays both niche conservatism and high environmental niche expansion ability (Table 3.5). Although *W. salutaris*' niche is narrow, the range of climatic variables that make up its niche is widespread in eastern South Africa. We see a high niche expansion value potentially because this set of

climatic variables (or climatic niche) is predicted to become more widespread across South Africa. Consequently, *W. salutaris* maintains its narrow yet widespread niche in response to climate change.

3.5.Conclusion

Overall, this study found that the current and future distributions of *W. salutaris* in South Africa are primarily determined by climatic variables that impact water-stress and sensitivity to desiccation and frost. *Warburgia salutaris* has a restricted range, likely due to its lack of dispersal ability due to its physiological constraints, such as its sensitivity to low temperatures and wide temperature ranges. *Warburgia salutaris* populations are increasingly threatened by land use change, overharvesting, and climate change. Although the environmental niche of *W. salutaris* will be conserved throughout a changing climate, its distribution will decrease by 2070 under both climate change scenarios and shift from the Indian Ocean coastal belt and grassland biomes and move increasingly into the savanna biome. Conservation efforts should focus primarily on propagating *W. salutaris* in areas where habitat suitability is expected to increase, particularly in eastern KwaZulu-Natal and central and western Limpopo. Additionally, populations of *W. salutaris* occurring in areas that are expected to experience a decrease in habitat suitability should be prioritized for long-term monitoring and managing (i.e., KNP populations). This study emphasizes the importance of using species distribution and niche modelling to prepare conservation strategies in response to climate change.

3.6.References

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3.7.Appendices

Appendix 1

Table S3.1. Chelsa (version 2.1) climate variables. Climate variables used in SDM production are highlighted.

Code	Variable	Unit of measurement
Bio01	Mean Annual Air Temperature	°C
Bio02	Mean Diurnal Air Temperature Range	°C
Bio03	Isothermality	°C
Bio04	Temperature Seasonality	°C/100
Bio05	**Maximum Temperature of the Warmest Month**	**°C**
Bio06	Minimum Temperature of the Coldest Month	°C
Bio07	**Temperature Annual Range**	**°C**
Bio08	**Mean Temperature of the Wettest Quarter**	**°C**
Bio09	Mean Temperature of the Driest Quarter	°C
Bio10	Mean Temperature of the Warmest Quarter	°C
Bio11	**Mean Temperature of the Coldest Quarter**	**°C**
Bio12	Annual Precipitation	mm
Bio13	Precipitation of the Wettest Month	mm
Bio14	Precipitation of the Driest Month	mm
Bio15	Precipitation Seasonality	mm
Bio16	**Precipitation of the Wettest Quarter**	**mm**
Bio17	**Precipitation of the Driest Quarter**	**mm**
Bio18	**Precipitation of the Warmest Quarter**	**mm**
Bio19	Precipitation of the Coldest Quarter	mm

Table S3.2. Modelling algorithms available in the biomod2 package (Thuiller *et al*., 2012) in R Studio (R Core Team, 2022). Algorithms used in SDM production are highlighted.

GLM	**Generalised Linear Model**
GAM	**Generalised Additive Model**
GBM	**Generalised Boosting Model**
SRE	**Surface Range Envelope**
CTA	Classification Tree Analysis
ANN	**Artificial Neural Network**
MARS	Multiple Adaptive Regression Splines
RF	**Random Forest**
FDA	Flexible Discriminant Analysis
MAXENT.Phillips	Maximum Entropy

Appendix 2

Table S3.3. Environmental variable importance scores for *Warburgia salutaris* species distribution models under current and future climate scenarios for all models. Variable scores in bold represent the most important variable for each taxon under current and future climate scenarios. Bio05—Max Temperature of the Warmest Month; Bio07—Temperature Annual Range; Bio08—Mean Temperature of the Wettest Quarter; Bio11—Mean Temperature of the Coldest Quarter; Bio16—Precipitation of the Wettest Quarter; Bio17—Precipitation of the Driest Quarter; Bio18—Precipitation of the Warmest Quarter.

Climate scenario	Model	Bio05	Bio07	Bio08	Bio11	Bio16	Bio17	Bio18
Current	GLM	0.378	0.343	0.158	**0.838**	0.072	0.205	0.273
	GAM	0.457	0.575	0.277	**0.706**	0.533	0.241	0.683
	GBM	0.009	0.060	0.047	**0.489**	0.018	0.088	0.238
	SRE	0.463	**0.583**	0.363	0.567	0.394	0.144	0.389
	ANN	0.224	0.387	0.372	**0.882**	0.619	0.390	0.975
	RF	0.208	0.101	0.093	0.125	0.223	**0.361**	0.262
RCP 4.5	GLM	0.848	**1.000**	0.313	0.558	0.533	0.263	0.000
	GAM	0.729	**0.919**	0.425	0.461	0.393	0.293	0.299
	GBM	0.004	0.162	0.042	**0.276**	0.063	0.080	0.214
	SRE	0.340	**0.569**	0.379	0.508	0.519	0.215	0.432
	ANN	0.057	0.541	0.059	0.308	**0.742**	0.246	0.403
	RF	0.068	0.058	0.048	0.060	0.167	**0.210**	0.111
RCP 8.5	GLM	0.869	**1.000**	0.334	0.512	0.022	0.257	0.747
	GAM	0.714	**0.884**	0.480	0.515	0.514	0.243	0.606
	GBM	0.013	0.131	0.056	**0.279**	0.046	0.040	0.252
	SRE	0.321	**0.525**	0.373	0.509	0.498	0.203	0.424
	ANN	0.451	**1.000**	0.873	0.534	0.509	0.367	0.710
	RF	0.097	0.148	0.057	0.075	0.045	**0.239**	0.166

Appendix 3

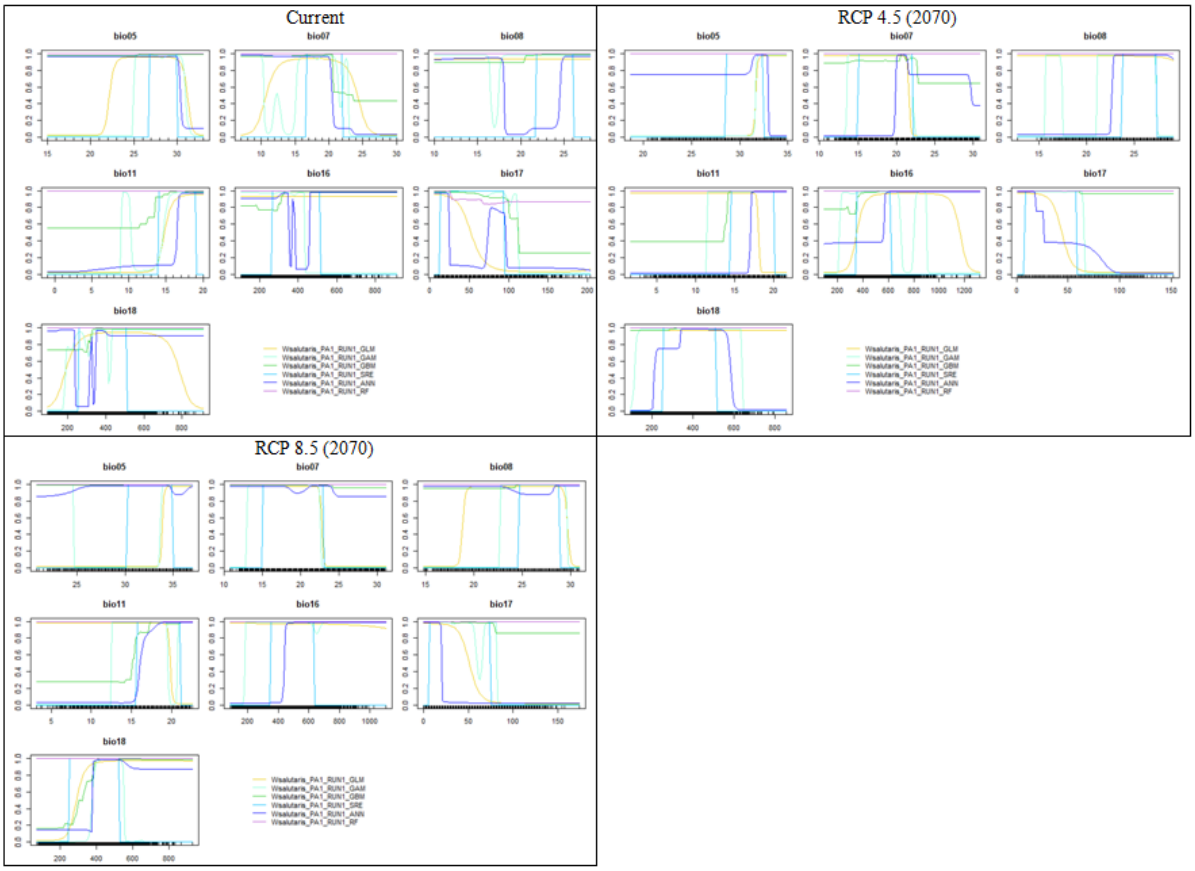

Figure S3.1. Ecological response curves for the current and future climate scenarios of *Warburgia salutaris* showing the ranges of climatic conditions that are suited to the species' distributions. The x-axis represents the range of climatic variables across the study area and the y-axis represents suitability of a specific variable when all other variables are set to their mean.

Appendix 4

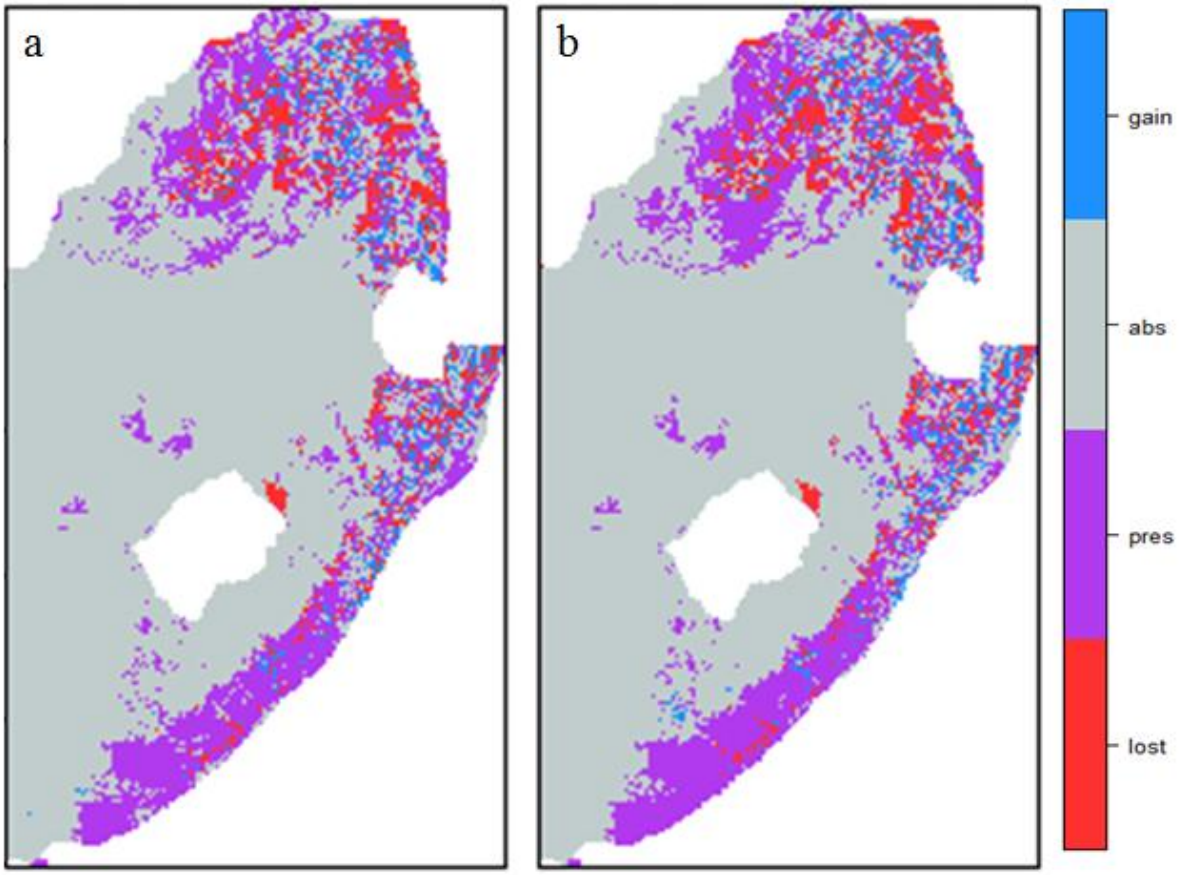

Figure S3.2. Future range size change predictions of *Warburgia salutaris* under RCP 4.5 (a) and RCP 8.5 (b). Purple represents stability, red represents loss, blue represents gain and grey represents unsuitability.

4. Chapter 4: Conclusion

4.1.General discussion

The restricted populations, unsustainable harvesting, and habitat loss of *Warburgia* have resulted in the genus becoming highly threatened in Africa (Leonard and Viljoen, 2015; Maroyi, 2014). Conservation efforts have been implemented for two of the four *Warburgia* species – *W. salutaris* and *W. ugandensis* – to reduce the demand on the few natural populations that are still present, although vulnerable. Previous studies have assessed the biology, ethnobotany, social uses, genetics and distribution of at least one of the *Warburgia* species (Becking, 2015; Kioko *et al*., 2003; Leonard and Viljoen, 2015; Maroyi, 2013; Maroyi, 2014; Mbambezeli, G., 2018; Muchugi *et al*., 2008; Naidoo and Lamb, 2006; van den Bosch *et al*., 2023). To build on this previous body of knowledge, it was necessary to assess the current and future distributions and driver climatic variables of *Warburgia* species in eastern and southern Africa to investigate potentially important factors for the conservation of this threatened genus of medicinal trees. This approach required creating species distribution maps of *Warburgia* species in eastern and southern Africa and South Africa, characterising the ranges of suitable habitats, and identifying the environmental variables that are responsible for determining their distributions, as well as how distributions of *W. salutaris* could change under projected climate scenarios. The results from this work inform the taxonomy of *W. ugandensis* with its two subspecies and can have implications for the conservation efforts for *Warburgia* species.

4.2.Key findings

This study highlights the significance of using species distribution modelling as an approach to aid the conservation of important medicinal species. Results showed that at the continental scale, distributions of *Warburgia* species are limited by environmental factors that likely influence their recalcitrant seeds and that the subspecies of *W. ugandensis* should be considered the same taxon in accordance with the ecological species concept. The only South African *Warburgia* species, *W. salutaris*, will not experience significant changes in its climatic niche in response to future climate change. We also found that *in situ* conservation by propagation is possible for all of the *Warburgia* species (except *W. salutaris*) around the Great Lakes of Africa and Mt. Kenya. For *W. salutaris*, *in situ* conservation was found to be suitable in western Limpopo and eastern KwaZulu-Natal.

4.2.1. Taxonomic classification of W. ugandensis

The two subspecies of *W. ugandensis* should be considered as a single taxon in accordance with the ecological species concept as they occupy the same environmental niche. The subspecies concept was first proposed by Mayr (1942), which gave a method of quantifying variation within a species. It was initially based on genetic and geographic distinctions, but later became more complex, considering various distinctions (e.g., ecological, phylogenetic, Wilson and Brown, 1953), all of which *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* do not meet.

The taxonomy of the genus *Warburgia* has been a subject of controversy amongst authors (Leonard and Viljoen, 2015; Muchugi *et al*., 2008). *Warburgia* was initially described in the 1800s. In the early 1900s, *W. ugandensis* samples were collected from the Kibale district (Kenya) and were initially incorrectly described. Later, more *W. ugandensis* samples were collected in Tanzania, which were found to be a subspecies of *W. ugandensis*. However, the differences between the taxa were not well investigated, and were only an expression of the geographical isolation between the two sampled populations (Leonard and Viljoen, 2015). Muchugi *et al*. (2015) investigated the legitimacy of *W. ugandensis* containing two distinct subgroups.

The morphology of the *W. ugandensis* subspecies are very similar, with the only distinctions seen in the bark and bole. *Warburgia ugandensis* subsp. *longifolia* has an intermediate sized bole and yellow or black-grey bark that splits into irregular patterns. *Warburgia ugandensis* subsp. *ugandensis*, however, has a small to intermediate sized bole with dark brown to black-grey bark splitting into rectangular patterns. Phylogenetic and molecular studies (e.g., Muchugi *et al*., 2008; Wamalwa *et al*., 2006) show that various geographically distinct populations of *W. ugandensis* form genetically distinct clusters, based on migratory patterns across the Great Rift Valley. To have a holistic understanding of whether *W. ugandensis* has diversified, or is in the process of doing so, we need to consider multiple lines of evidence that support or refute it. This study, therefore, found that on the basis of the ecological species concept, scientists should sink the subspecies of *W. ugandensis*.

Knautia drymeia Heuff. (Caprifoliaceae, Dipsacoideae) is a perennial herb found in Europe (Rešetnik *et al*., 2016). The species comprises polyploids which are disjunctly distributed. One group occurs in the northern Apennine Peninsula to the north-western part of the Balkan

Peninsula, whereas another group occurs in the south-eastern area of the Balkan Peninsula and the southern Carpathians. AFLPs were used to explore the genetic structure of *K. drymeia*, and confirmed that diagnosable subspecies cannot be recognised. Instead, *K. drymeia* is a morphologically and genetically variable species with no infraspecific taxa (Rešetnik *et al*., 2016). *Warburgia ugandensis* can however be considered as a genetically and geographically variable species, rather than a species containing two distinct subgroups.

4.2.2. Distribution of Warburgia *species*

The distributions of *Warburgia* species are limited by low temperatures and rainfall, which is likely due to their sensitivity to frost and desiccation-sensitive recalcitrant seeds. Climate is considered as a key influencer in plant species distribution (Körner *et al*., 2016; Vetaas, 2002). Sub-Saharan Africa is a narrow land mass bordered by oceans, characterised by having a tropical and subtropical climate. The various ocean currents and land and sea distributions lead to a regionally complex and variable climate in the region (Reason, 2017).

Many tree speciesare limited by low temperatures. This may be due to lower temperatures having a more direct effect on vital ecophysiological processes than warmer temperatures, which may affect survival (Körner *et al*., 2016). Vetaas (2002) highlights that the natural range limits of many tree species often match absolute minimum temperatures.

The term “recalcitrant’ was first introduced by Roberts (1973), given for seeds that are shed at relatively high water contents and are sensitive to desiccation. There have been many species recorded that produce recalcitrant seeds, for example *Aporosa lindleyana* (Wight) Baill. (Berjak and Pammenter, 2004). The production of recalcitrant seeds has been favoured in environments that are humid and do not experience great seasonality (Berjak and Pammenter, 2004; Subbiah *et al*., 2019; Tweddle *et al*., 2003). Although some species in temperate and dry environments do produce recalcitrant seeds (e.g., *Boscia senegalensis* (pers.) Lam.), such examples are rare, especially in Africa, as this may be due to the generally dry conditions of the continent (Berjak and Pammenter, 2004; Tweddle *et al*., 2003).

Desiccation-sensitive seeds are likely the remnants of a more mesic past (Subbiah *et al*., 2019). The global incidence of recalcitrant-seeded species equates to 8% of extant species and are primarily forest species occurring in the mesic and sub-tropics. There have been debates on whether recalcitrant seeds are ancestral or evolutionary mechanisms; however, it

likely evolved from a desiccation tolerant state from the end of the Miocene (Subbiah *et al*., 2019). Being affiliated with the tropics, many South African recalcitrant-seeded species have adapted to shedding their seeds during the rainy season. In addition, species with desiccation-sensitive seeds have adapted to temperate and drier climates by having hard and/or waxy seed coats to reduce the likelihood of drying out. Subbiah *et al*. (2019) highlights a causal relationship between recalcitrance and habitat type. Therefore, we see *Warburgia* species' distributions being highly influenced by water stress and sensitivity to desiccation during the seed and seedling stages.

4.2.3. W. salutaris*' response to climate change*

Warburgia salutaris' ecological niche is conserved and its geographical distribution shifts as South Africa's climate gets hotter. Africa, especially sub-Saharan Africa is one of the most vulnerable regions to climate change (Serdeczny *et al*., 2017). African temperatures are expected to experience a 1.5°C increase for the low climate change scenario (RCP2.6, which is the most unlikely scenario) and a 5°C increase for the most extreme scenario by 2100 (RCP8.5, Serdeczny *et al*., 2017). For the most probable emission scenarios, African temperatures are expected to increase by approximately 2.1°C (Coulibaly *et al*., 2018). Additionally, precipitation is expected to face drastic declines in southern Africa (Serdeczny *et al*., 2017). South Africa's climate is significantly influenced by the position of Madagascar (Reason, 2017) and annual temperatures have risen by approximately 1.5 times the global average (0.65°C) over the past 50 years (Ziervogel *et al*., 2014). An approach to assess how changing climates influence biodiversity is assessing the geographical shifts in niches (DEA, 2013).

South Africa consists of nine biomes (Rutherford *et al*., 2006). Each biome provides habitats to various communities of plants and animals, specifically adapted to the existing environment and climate (DEA, 2013). The Department of Environmental Affairs of South Africa reports that the Indian Ocean coastal belt and grassland biomes are priority biomes for conservation and are highly vulnerable to climate change. Under intermediate and high-risk climate change scenarios, these biomes will likely be replaced by the savanna biome.

For *W. salutaris*, the conversion of the Indian Ocean Coastal Belt and grasslands biomes into savanna will cause range shifts from inland Limpopo and Mpumalanga to the eastern coast of KwaZulu-Natal. An example of a genus displaying niche conservatism is the Mediterranean

legume genus *Medicago*. Yang *et al*., (2022) showed that throughout multiple climate change events, the genus *Medicago* is still limited by warmer temperatures. Essentially, *W. salutaris*' narrow environmental niche will be conserved; however, its geographic distribution will shift to track the savanna biome.

4.3.Lessons: **Warburgia** *distribution and human activity*

The occurrence of *Warburgia* species has been disconnected from some areas of suitable habitat because of human activity. In the case of *Warburgia*, the primary activities that cause this decoupling between occurrence and suitable habitat are overharvesting, land use change and climate change. While we understand the well-studied impacts of overharvesting of *Warburgia* species, the effects of land use and climate change are not well explored. The population of sub-Saharan Africa is expected to reach 2 billion by 2050, and close to 4 billion by 2100 (Serdeczny *et al*., 2017). The predicted population explosion will result in a higher demand for resources, leading to expanses of natural land becoming converted to other land uses. For *W. ugandensis*, areas of high suitability have been converted into agricultural land (Muchugi et al., 2008). Habitat fragmentation resulting from these land use changes are expected to hinder the survival of the species (Ries and Sisk, 2004). The forest and grassland biomes, within which the east African *Warburgia* populations and some of the southern African populations (i.e., *W. salutaris*) occur, are the most vulnerable biomes to land use change.

Climate change occurs at unprecedented rates due to anthropogenic activity (Serdeczny *et al*., 2017). This has resulted in extreme weather events, which have decimated valuable plant populations. For example, Kenya experienced great agricultural losses due to floods associated with intense El Niño events (Serdeczny *et al*., 2017). Predicted declines in precipitation in combination with the rising demand for water places further threats to African trees (Serdeczny *et al*., 2017). A South African example shows how human activity coupled with land use and climate change threatens plants. *Clivia miniata* (Lindl.) Verschaff., commonly known as the Natal lily, has experienced a loss in over 40% of individuals within the last 90 years due to excessive trading. Furthermore, climate change and land use change have been detrimental to populations. When modelling species distributions with variables relating to human activity and climate change, Groner *et al*. (2023) found that land use change was the most damaging variable to species' distributions. When variables depicting

overharvesting, land use change and climate change were combined, substantial declines in habitat suitability were predicted. These findings highlight how the occurrence of a species may become disjunct from suitable habitat in the Anthropocene. In essence, we found spans of areas that will become climatically suitable for *W. salutaris* propagation by 2070, but realistically unsuitable due to detrimental non climatic factors (summarised by Figure 4.1), such as land use change.

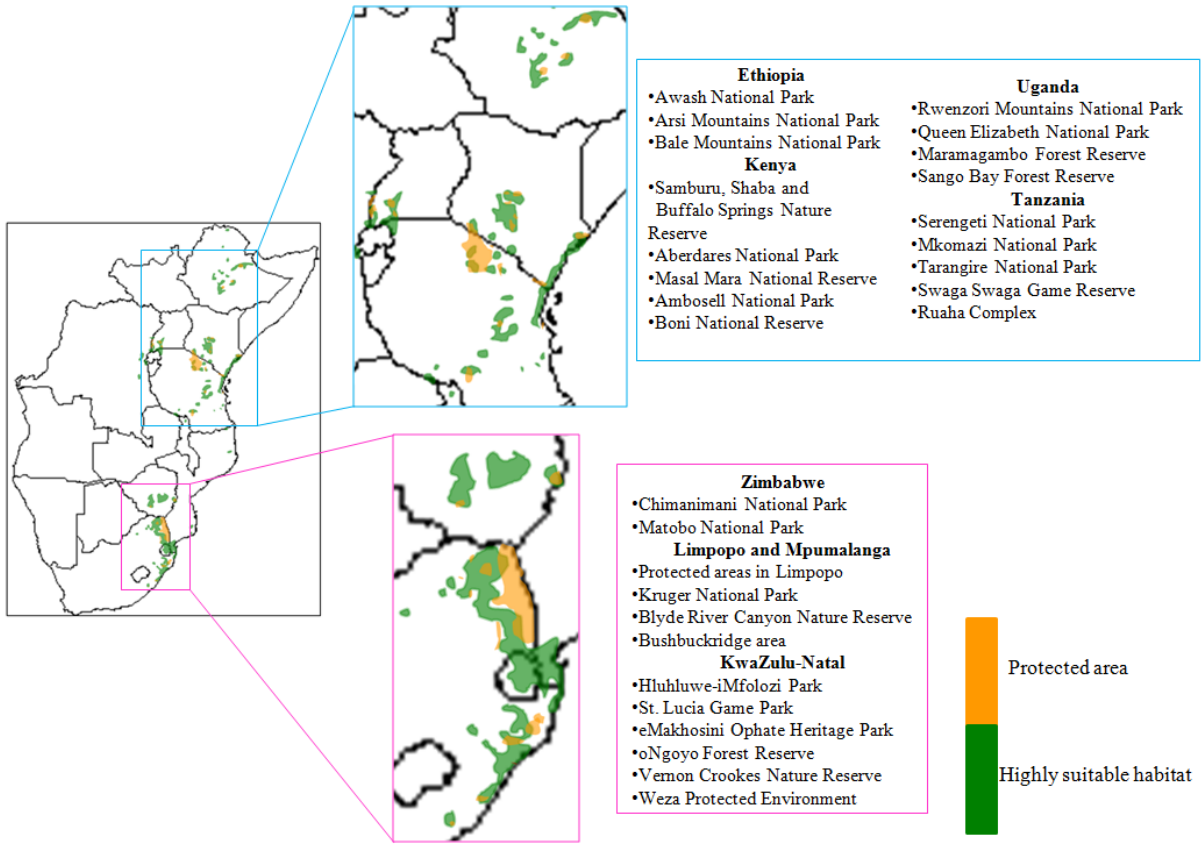

Figure 4.1. Map of highly suitable habitat ($0.75 \leq$ suitability ≤ 1) predicted for all *Warburgia* species by 2070 (dark green) overlaid with protected areas (orange, names on the right, ArcMap, version 10.6, ESRI, 2011) where *Warburgia* plantations could be established.

4.4.Limitations and future work

A known limitation to SDMs is the availability of both presence and absence data (Hipólito *et al*., 2015). Models can be misguided by presences of relict or refuge populations or sink populations and by absences in locations that are actually suitable but inaccessible due to dispersal constraints or lack of human observation. In contrast to widely distributed species, rare species with low abundance and/or restricted ranges are generally not appropriately

suited to distribution models (Hipólito *et al*., 2015). This is the case because occurrence data for many species is lacking due to restricted geographic distributions and insufficient or ineffective collecting, resulting in inadequate and over- or underestimated species distribution predictions (Elith *et al*., 2006; Hipólito *et al*., 2015). For *Warburgia*, occurrence data may be lacking due to the sensitive nature of localities. It should also be noted that there was a large sampling bias, with *W. salutaris* occurrences making up 79% of the total occurrence dataset for the genus. Furthermore, 98% of *W. salutaris*' localities were recorded in South Africa. In addition, Williams and Crouch (2017) emphasize that large areas of South Africa remain poorly collected, and that collecting locality data from at least one herbarium in every province that the species occurs in in addition to other databases (e.g., GBIF) is preferred for SDMs (V. Williams, Nov. 2023, pers. comm.). This highlights the uneven sampling across species and countries. To accommodate for these limitations, we performed preliminary model runs and assessed the performances of all modelling algorithms. Those that underperformed (i.e., algorithms that had low AUC or TSS scores) were removed from subsequent modelling.

When modelling niches of real species, biases in occurrence data may influence results even in cases with randomly generated background points. The complexity of environmental space cannot be controlled in niche identity tests and some algorithms might overly rely on sampling biases to estimate niches. Consequently, these tests are susceptible to Type 1 errors (Peterson, 2011). Our study analysed the environmental niches of *W. ugandensis* subsp. *longifolia* and *W. ugandensis* subsp. *ugandensis* and *W. salutaris* to aid in characterising the taxonomy of the genus and to investigate how climate change may influence individuals, respectively. Due to different populations experiencing differing environmental constraints, modelling the environmental niches of an entire taxon may over- or underestimate distribution and abundance under certain conditions. To overcome this, we modelled ecological niches at the subspecies level (highlighted in Zimmermann *et al*., 2010). This approach was particularly helpful for taxonomically classifying the two subspecies of *W. ugandensis*. It provided an improved understanding that these subspecies should be sunk, following the ecological niche species concept.

Predictions made by the SDMs of this study are based on abiotic factors to indicate habitat suitability alone; however, species abundance is affected by a collection of biotic and abiotic factors (Austin, 2007). Future studies should therefore consider biotic factors when modelling

distributions and niche for a more holistic understanding of species. For *Warburgia* species, distributions are based on environmental and anthropogenic factors; therefore, future studies should consider anthropogenic variables (e.g., land use change, harvesting) to achieve a more realistic understanding of where propagation should be employed.

4.5.Recommendations for conservation

Conservation efforts have been directed towards *W. salutaris* and *W. ugandensis* in parts of Africa, but other species within the *Warburgia* genus have received comparatively less attention. For example, Dokata *et al*. (2023) map the human threats posed to a population of *W. ugandensis* in a forest reserve in Kenya. They show how the individuals on the edge of the population (those closest to human settlement) are negatively affected by human presence due to bark harvesting and livestock grazing and use this to emphasize the need for conservation interventions. Senkoro (2021) maps the distribution of *W. salutaris* in southern Mozambique and proposes conservation strategies through propagating and distributing *W. salutaris* seedlings to traditional healers. In addition, efforts have been made to facilitate the conservation of *W. salutaris* in South Africa and Zimbabwe by botanical and agricultural organisations. These efforts include growing and distributing seedlings to traditional healers and communities that have a high demand for traditional medicine (Maroyi, 2013; Senkoro, 2021). Other studies have proposed using alternate plant parts compared to the bark (Drewes *et al*., 2001), as well as using cryopreservation for long-term storing of *W. salutaris* seeds (i.e., seed banks, Kioko *et al*., 2003).

In addition to propagating *Warburgia* trees around Africa (possible protected areas for propagation summarised in Figure 4.1), plant part substitution serves as an effective way to conserve *Warburgia* species. Drewes *et al*. (2001) evaluated the pharmacological compounds in the leaves and bark of *W. salutaris* and found that the levels of these compounds did not show any significant differences between the two plant parts. Zschocke *et al*. (2000) also tested the extracts of the bark and leaves, but also the twigs and immature bark. Both Drewes *et al*. (2001) and Zschocke *et al*. (2000) found that *W. salutaris* bark can be substituted by the aerial parts (leaves and twigs) in traditional medicine to achieve the same benefits. Kioy *et al*. (1990) and Zschocke *et al*. (2000) found that *W. salutaris*, *W. ugandensis* and *W. stuhlmannii* contain the same pharmacological compounds; hence this form of plant part substitution will most likely also apply to *W. ugandensis* and *W. stuhlmannii*.

Scheepers *et al*. (2011) found that although highly traded, traditional healers are unaware of the potential of substituting the bark of *W. salutaris* with its leaves. Traditional healers also reported that they import *W. salutaris* material since it is so rare and inaccessible in South Africa; hence they do not have access to the leaves of the tree. Local organisations, such as the South African National Biodiversity Institute (SANBI) and South African National Parks (SANParks) should aim to educate traditional medicine markets of the possibility of plant part substitution when distributing *W. salutaris* seedlings to them.

Warburgia species can also be conserved by supporting propagation from cuttings. Seedlings grown from these cuttings can then be distributed to the communities that rely on traditional medicine to reduce the levels of harvesting from wild populations. This method has been elucidated in the literature (Akwatulira *et al*., 2011; Senkoro, 2021; van den Bosch *et al*., 2023), but only for some of the *Warburgia* species. Propagating plants directly from seeds is generally the better option for conservation as it allows for higher genetic diversity; however, in the case of the pepper-bark tree, this is not always the most efficient solution due to high levels of seed predation (van den Bosch *et al*., 2023). Nevertheless, seeds that are protected from predation germinate successfully and promote greater genetic diversity in populations that are more likely to adapt to environmental changes. In cases where propagation from seeds is not efficient, clonal propagation should be considered. Clonal propagation involves using cuttings from a parent plant to produce a genetically identical plant. This method results in genetically identical ramets and may weaken the ability to reproduce sexually in the future (Glennon *et al*., 2023); however, this provides a relatively quick and effective way of producing seedlings for commercial use and can be applied to all *Warburgia* species (Akwatulira *et al*., 2011).

Protected areas, such as national parks, or reserves, offer a win-win solution for conservation and communities through shared management of the land and its resources (Scheepers *et al*., 2011). Nature reserves are *in situ* conservation applications that allow for species to maintain their genetic diversity in protected areas at low costs (Pritchard *et al*., 2012). Unfortunately, there is very limited overlap between suitable habitat for *Warburgia* species and protected areas (Figure 4.1). This could be addressed by establishing new protected areas that will be able to support *Warburgia* species. Additionally, carrying out supplemental planning to allow for range expansions and shifts of *Warburgia* species into areas of high suitability through migration corridors is also paramount.

4.6.Conclusion

This study used species distribution modelling and comparative niche modelling to model the distributions of *Warburgia* species at a continental (eastern and southern Africa) and local (South Africa) scales. The predicted distributions of *Warburgia* species closely match the actual known distributions of these species, and the results showed that they are likely driven by climatic variables associated with temperature and precipitation. This is likely due to their seeds that are highly sensitive to desiccation and can only thrive within specific temperature and precipitation ranges. Additionally, in line with the ecological species concept, the subspecies of *W. ugandensis* should be sunk as their environmental niches are identical.

Warburgia salutaris' sensitivity to frost and production of desiccation-sensitive seeds appear to be the main driving forces of its current and future distribution in South Africa. Under both climate change scenarios, the distribution of *W. salutaris* will contract from eastern Limpopo and expand along the eastern coast of KwaZulu-Natal by 2070. Findings in this study show that *W. salutaris* will likely conserve its narrow environmental niche, which will lead to shifts in its geographic range, as the climate changes over time. Populations occurring in areas that are expected to experience reductions in habitat suitability should be prioritized for long-term monitoring. Additionally, *W. salutaris* trees should be planted in areas that are expected to experience increased habitat suitability, such as eastern KwaZulu-Natal.

Identifying and assessing climatically suitable habitats and the factors that drive these habitats aids in understanding species' biogeography, aiding proactive conservation efforts. Additional research, specifically field surveys and ground-truthing and using anthropogenic predictor variables, would enhance our understanding of the distribution of *Warburgia* species. These findings highlight the significance of using species distribution modelling as an approach to pursue different conservation programs for important tree species.

4.7.References

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