South African Podocarpaceae distribution interpreted from a physiological and population genetics perspective.

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Declaration

I declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted by me before for any other degree, diploma or examination at any University or tertiary Institution. To the best of my knowledge, this document contains no material previously published or written by someone else, except for text that has been referenced.

T.C. Twala

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Dedication

I dedicate this thesis to my mother (Johanna Twala), father (Sthembiso Twala), brother (Simphiwe Twala) and daughter (Phila Twala).

Abstract

Podocarpaceae (podocarp) are the most diverse conifer family with a Southern Hemisphere distribution. Podocarps occur in Afrotemperate and Afromontane forests at high elevations that are cool and humid. Podocarps once dominated the forest canopy but due to their slower growth rate and photosynthetic rates they have are in competition with angiosperms which have faster growing and higher photosynthetic rate. Due to the competition between podocarps and angiosperms, Bond (1989) proposed that podocarps were excluded to nutrient poor and unfavourable environments due to their limited competitive ability. However, podocarps persist under the forest canopy until conditions become favourable. This power dynamic shifts with climate oscillations where podocarps distributions expand and dominate when conditions become cooler. Owing to podocarps being the most diverse and widespread conifers they make for a good study system.

This thesis focuses on understanding the climatic variables driving the current and future distribution of podocarps, how their seedling physiology may influence their ability to recruit and establish under climate change, and how this can influence their ability to disperse in their South African distribution. Ensemble species distribution modelling was used to characterise the current and future distribution of podocarps and identify the climatic variables that influence their distribution. The current and future environmental niche was quantified using environmental niche modelling. I found that variables predicting rainfall seasonality were the most important at determining the distribution of podocarps in South Africa. *Afrocarpus falcatus* and *P. latifolius* were predicted to have the largest geographic distribution, with *P. henkelii* and *P. elongatus* having restricted distributions. Both *A. falcatus* and *P. latifolius* were predicted to occur in the Limpopo, Mpumalanga, KwaZulu-Natal, Eastern Cape and Western Cape provinces of South Africa. *Podocarpus henkelii* was predicted to occur in the KwaZulu-Natal and Eastern Cape provinces. *Podocarpus elongatus* is endemic to the Western Cape Province. All four podocarps were predicted to expand to higher altitudes (up the escarpment) under climate change

and contract in its coastal distribution. Although *P. elongatus* was predicted to occupy the smallest geographic distribution it was predicted to have the widest environmental niche than the other species, which was predicted to contract under climate change. The environmental niche of *P. latifolius* and *P. henkelii* was predicted to remain stable. *Afrocarpus falcatus, P. latifolius,* and *P. henkelii* showed niche conservatism, however, *P. elongatus* under RCP 4.5 → current and the RCP 8.5 ↔ current niche comparisons showed niche divergence. *Podocarpus elongatus* was predicted to expand to an environment it currently does not occupy.

Ecophysiological and morphological experiments were conducted to understand how podocarp seedlings respond to drought and elevated temperatures. The experiments indicated that *P. henkelii* seedlings were more drought and heat tolerant than *A. falcatus* seedlings. Conditions are predicted to become hotter and drier in some parts of South Africa, and this study has shown that *P. henkelii* seedlings will be able to tolerate these conditions better than *A. falcatus* seedlings. Furthermore, this suggests that the distribution of *P. henkelii* is not constrained by its physiology but rather by other mechanisms such as competition, reproductive biology, and/or shade tolerance.

Microsatellites were used to inform us about possible podocarp dispersal patterns in *A. falcatus, P. latifolius* and *P. henkelii* in South Africa. The results suggested that podocarp populations in South Africa were shown to have higher genetic diversity than other podocarps globally, however these results may be due to the limited number of microsatellites used in this study, smaller population sizes in comparison to other studies and methods used to measure population structure and diversity. As expected, the geographically widespread species (*A. falcatus* and *P. latifolius*) are more diverse than the geographically restricted *P. henkelii.* Geographically distant *A. falcatus* and *P. henkelii* populations showed higher differentiation than geographically proximal populations. In *P. latifolius* South African populations, there was strong isolation by distance. Although the distribution of podocarps is disjunct, there is dispersal between populations.

Podocarps are resilient to climate change as was demonstrated by the work in this thesis, and by their paleodistribution expanding and contracting with climate oscillations. In this thesis I considered climate, ecophysiology and genetics as determinants of podocarps distribution. Under climate change, podocarps are predicted to expand to higher elevations to track favourable climatic conditions. Seasonal drought is the most important climatic determinant of podocarp distribution. The ability of these species to tolerate drought and heat stress suggests that the seedlings might be able to tolerate short periods of drought and heat stress, however prolonged exposure may lead to seedling mortality, but populations will then be maintained by adults. Populations show evidence of gene flow, indicating they will be able to persist through changing climates, as they have done in the past. This thesis has highlighted that the factors constraining podocarp distributions might be demographic, and future works should investigate the role of fire in podocarp seedling establishment and longevity, as well as their interactions with angiosperm competitors.

Keywords: climate change, dispersal, distribution, drought stress, ecophysiology, environmental niche, heat stress, morphology, podocarp, population genetics, species distribution modelling.

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Chapter 1 - General introduction

1.1. Research rationale

Bond (1989) argued that the faster growth rates and high plasticity of angiosperms at the seedling phase put them at a competitive advantage to gymnosperms (particularly conifers). These traits restrict gymnosperms to edaphically (nutrient-poor) and climatically (cold) suboptimal conditions where they are unable to out-compete angiosperms (Bond 1989; Cernusak *et al.* 2009). Podocarpaceae (podocarps) make for a study system because they are the most diverse conifer family which has a Southern Hemisphere distribution. Due to the various adaptations of podocarps such as root nodules and flattened leaves, podocarps can coexist with angiosperms in tropical and subtropical conditions. However, there is limited knowledge on why or how podocarps can coexist with angiosperms regardless of Bond's (1989) argument.

Podocarp distribution is well known, however there is limited knowledge on the factors and mechanisms (Geldenhuys 1993, 1994; Brodribb and Hill 1998; Negash 2003a; Adie and Lawes 2009) influencing the distribution and persistence of African podocarps. Numerous studies have emphasised the need for further understanding and investigation into factors influencing the distribution and persistence of tropical and subtropical podocarps (Brodribb and Hill 1997, 1998; Hill and Brodribb 1999; Wubet *et al.* 2003, 2006; Fetene and Beck 2004; Adie and Lawes 2011). Majority of the progress made in this field has been focused on understanding the seed anatomy and germination requirements, propagation, life-history, recruitment and growth dynamics of *Afrocarpus falcatus*, *Podocarpus cunninghamii, P. henkelii, P. latifolius, P. nubigena, P. totara* and *P. usambarensis* (Osborn 1960; Noel and van Staden 1976; Chamshama and Downs 1982; Dodd *et al.* 1989a, 1989b; Koen 1991; Geldenhuys and von dem Bussche 1997; Wilson and Owens 1999; Bloor and Grubb 2003; Negash 2003a, 2003b; Lawes *et al.* 2007; Strobl *et al.* 2011; Williams *et al.* 2011; Krepkowski *et al.* 2012).

There are four main podocarp species in South Africa: *Afrocarpus falcatus* (Thunb.) C.N.Page*,* *Podocarpus latifolius* (Thunb.) R.Br. ex Mirb*, P. henkelii* Stapf ex Dallim. & A.B.Jacks and *P. elongatus* (Aiton) L'Hér. Ex Pers. (Figure 1)*. Afrocarpus falcatus* and *P. latifolius* have a pan-African distribution and *P. henkelii* and *P. elongatus* have restricted distributions in South Africa. Significant breakthroughs have been made in regard to understanding how tropical and subtropical podocarps perform and persist under different environmental conditions (Lübbe and Geldenhuys 1991; Geldenhuys 1993; Brodribb and Hill 1997, 1998; Hill and Brodribb 1999; Kowalski and van Staden 1998, 2001; Bloor and Grubb 2003; Negash 2003a, 2003b; Adie and Lawes 2009; Krepkowski *et al.* 2012; Enright *et al.* 2022). The reproductive biology of *A. falcatus* has been identified as a factor controlling its distribution (Osborn 1960; Geldenhuys 1993; Negash 2003a), however once established it is able to persist. *Podocarpus latifolius* is a strong disperser, can establish in habitats when conditions are favourable and is shade tolerant (Geldenhuys 1993; Bussmann and Lange 2000; Adie and Lawes 2009; Adie and Lawes 2011). Little is known about the factors that could be restricting the distribution of *P. henkelii.* Like *A. falcatus*, *P. henkelii* distribution could be restricted by its reproductive biology (ability to disperse) considering the similarity in their seed anatomy. *Podocarpus henkelii* produces neotonous (recalcitrant) seeds which germinate in full sun and shade conditions; however, germination is delayed by the presence of the epicuticular wax, epidermis and epimatium (Noel and van Staden 1976; Dodd *et al.* 1989a, 1989b). In addition, the dispersal agent of *P. henkelii* is not well known, in the field, they appear to be gravity dispersed which could be a factor contributing to its restricted distribution. However, *P. henkelii* produces seeds similar to those of *A. falcatus* whose seeds are bird dispersed (Geldenhuys 1993), it is possible that *P. henkelii* seeds are also bird dispersed. In addition, the distribution of *P. henkelii* overlaps that of the Cape parrot (*Poicephalus robustus*) (Wirminghaus *et al.* 2000, 2002; Downs *et al.* 2014) which makes it a possible disperser.

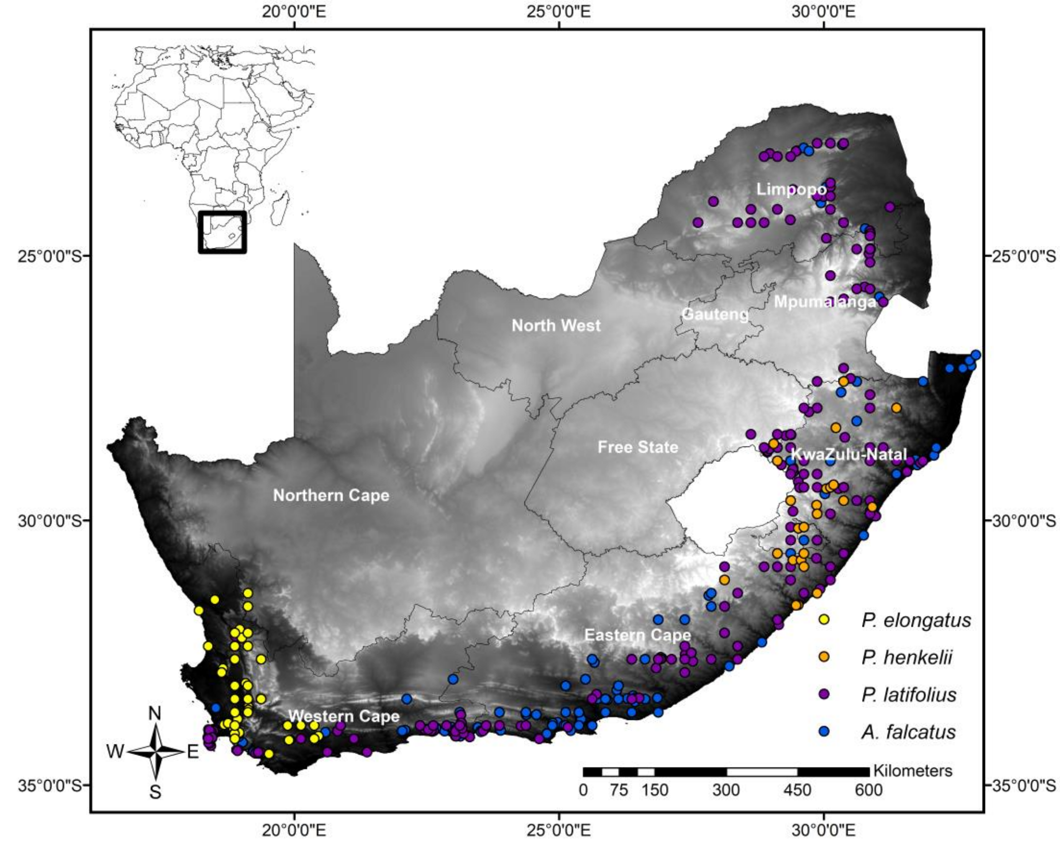

Figure 1. South African occurrences of *Afrocarpus falcatus, Podocarpus latifolius, P. henkelii* and *P. elongatus* that were downloaded from the Global Biodiversity Information Facility (http://gbif.org). The map is shaded according to elevation where darker grey indicates low elevation and light grey/white areas indicate high elevation (> 1800m.a.s.l) (Adapted from Twala *et al.* 2023).

It is fundamental that the ecophysiology and dispersal patterns of podocarps are well understood to adequately define how, why, and what enables podocarps to persist in their current and possibly their future distribution. This study investigates and highlights some of the environmental factors and processes influencing the distribution and persistence of southern African podocarps in their current distribution and how this might influence their future distribution.

1.2. Introduction

Gymnosperms were initially discovered in the rocks of the early Carboniferous (~359 MYA) – where they became the most dominant vegetation on the planet, however their success was overshadowed by the appearance of angiosperms in the early Cretaceous (~146 MYA) (Beck 1966; Scott 1974; Doyle 1978). When angiosperms diversified and occupied most of the landscape, several gymnosperm groups became extinct with remaining populations being restricted to the tropics (Kelch 1997; Barker *et al.* 2004). Modern tropical forests are dominated by angiosperms, yet gymnosperms (including conifers) persist. Podocarpaceae are the most diverse family of conifers with *Dacrydium* and *Podocarpus* being the most diverse genera (Kelch 1998). Podocarpaceae (podocarp) is considered a classical Gondwanan family and was a dominant component of Southern Hemisphere vegetation through the Tertiary period (~2.58 MYA) (Chalwell and Ladd 2005). Podocarp are restricted to wet montane forests (Hill and Brodribb 1999), which are exceptionally dry during winter. They are most abundant in mid- to high-elevations; however, they have also been found in forest types at low-elevations such as kerangas of Borneo (Aiba *et al.* 2004; Kitayama *et al.* 2011). The mechanisms that led to the displacement of podocarps to these suboptimal conditions are controversial (e.g., Brodribb 2011; Adie and Lawes 2011). Bond (1989) argued that faster growth rates of angiosperms place conifers at a competitive disadvantage at the regeneration stage restricting them to suboptimal conditions where they can out-compete angiosperms. Podocarps are viewed as stress tolerators; they are characterised by low mortality rates and slower growth rates. These traits enable podocarps to avoid competition from faster growing species through their location on suboptimal sites including, deep shade, nutrient-poor soils, poorly drained soils, and drought and cold prone sites. The persistence of podocarps to these suboptimal sites has been attributed to other “conservative” traits. For example, their narrow tracheid’s are less at risk of embolism in cold climates such as boreal regions (Sperry *et al.* 1994, 2006) and under drought conditions, and they produce tough, long-lived needle leaves are advantageous in nutrient-poor soils (Lusk *et al.*2003; Brodribb 2011).

1.2.1. Distribution and diversity of African podocarps

The Podocarpaceae is the most morphologically diverse family of conifers, but despite this their ecological and environmental range is smaller than most conifer families (Kelch 1997; Kelch 1998; Hill and Brodribb 1999; Biffin *et al.* 2011). Podocarps occur in tropical and subtropical regions (Kelch 1997; Barker *et al.* 2004). Based on anatomical and morphological differences, biogeographical data, as well as phylogenetic evidence, the family Podocarpaceae has been divided into 19 genera with 201 species (Khan *et al.* 2023). The seven main genera are *Acmopyle*, *Dacrydium, Microcachrys, Pherosphaera, Phyllocladus,* and *Saxegothaea*, which occur in Australasia, except *Saxegothaea* which is native to South America and *Podocarpus* which has the widest distribution. *Podocarpus, the* largest genus, contains approximately 100 species and is commonly referred to as yellowwood. Podocarps are widely distributed in mountain habitats in Africa (including Madagascar), Australia, and Central to South America, Indochina, and Oceania. *Afrocarpus* is a small genus that is native to Africa and Madagascar. In the Southern Hemisphere, the main centres of podocarp diversity are in Australasia, particularly Malesia, New Caledonia, New Zealand, and Tasmania (Kelch 1997; Barker *et al.* 2004; Khan *et al.* 2023). Africa (including Madagascar) only harbours a small fraction of Podocarpaceae diversity and is represented by two genera: *Afrocarpus* (6 species) and *Podocarpus* (7 species) (Farjon 2001). All African taxa were previously placed in the genera *Podocarpus*; however, subsequent studies have raised the section *Afrocarpus* to generic level (Kelch 1997; Conran *et al.* 2000; Barker *et al.* 2004; Knopf *et al.* 2012). There are five to seven podocarp species in Madagascar and due to its Gondwanan distribution in East Africa it appears to be the centre of African podocarp (*Afrocarpus* and *Podocarpus*) radiation (Adie and Lawes 2011). This suggests that podocarps extended their distribution from East Africa. The species that remain in Africa occur in regions that are considered refugia during extreme palaeoclimatic conditions (White 1981, 1993), and species that persist in Africa are likely to be exceptionally robust.

The distribution of mainland African podocarps is restricted to seven Afromontane archipelagos: (i) West Africa and Cameroon highlands, (ii) Ethiopian and Arabian highlands, (iii) western (Albertine) rift, (iv) eastern rift, (v) southern rift, (vi) eastern highlands, and (vii) Drakensberg & Cape Fold Mountains (Figure 2, White, 1981, 1993). *Afrocarpus* is native to Africa and is distributed in the Afromontane Forest belt of Africa, starting from the Ethiopian highlands and extending southwards to the Cape Fold Mountains. The genus is absent from West Africa except for *Afrocarpus mannii* a small tree endemic to the volcano of Pico de São Tomè in the São Tomè and Príncipe Island that grows at altitudes of 1450 – 2024 m in high montane cloud forests (Farjon 2013a). *Afrocarpus dawei* occurs in central and East Africa but is present from Ethiopia where it grows in swampy forests (Farjon 2013b). Like most podocarps, *A. dawei* has been overharvested for its timber and is near threatened (Farjon 2013b). *Afrocarpus gracilior* is a medium-sized tree that occurs in central and East Africa (Farjon 2013c). *Afrocarpus usambarensis* is a critically endangered tree – owing to logging – occurring over a large altitudinal gradient (1500 – 3000 m) (Farjon 2013d). The species occurs in central and East Africa but is absent in Ethiopia, where populations are rapidly declining due to deforestation and logging (Farjon 2013d). *Afrocarpus gaussenii* is endemic to the eastern plateau of Madagascar. *Afrocarpus falcatus* occurs in East Africa extending southwards into the Drakensberg and Cape Fold mountains (Farjon 2001, 2013e). The absence of *Afrocarpus* west of the Ethiopian and Arabian highlands suggests that *Afrocarpus* never extended farther westward (Adie and Lawes 2011). The westward expansion of *Afrocarpus* could be constrained by its reproductive biology (Adie and Lawes 2011). *Afrocarpus falcatus* is dioecious – like most podocarps – and is scattered at low density (Geldenhuys 1993; Figure 3). *Afrocarpus falcatus* is dispersed by bats, birds, baboons, and monkeys and is therefore unlikely to be limited by dispersal (Geldenhuys 1993; Negash 2003a); even across the grassy matrix but may be recruitment limited. In addition, the hard sclerotesta of *A. falcatus* delays germination by one or more years which exposes the seed to post dispersal damage (Geldenhuys 1993). Furthermore, the seed viability declines by more than 50% within 12 months of falling from the tree (Negash 2003a). Reduced seed viability and post dispersal mortality together with infrequent seed production make *A. falcatus* a poor coloniser. Although *A. falcatus* is not effective at colonising new habitats, once it establishes it persists well. These adaptations can be beneficial as delayed germination may be beneficial especially when conditions are not favourable.

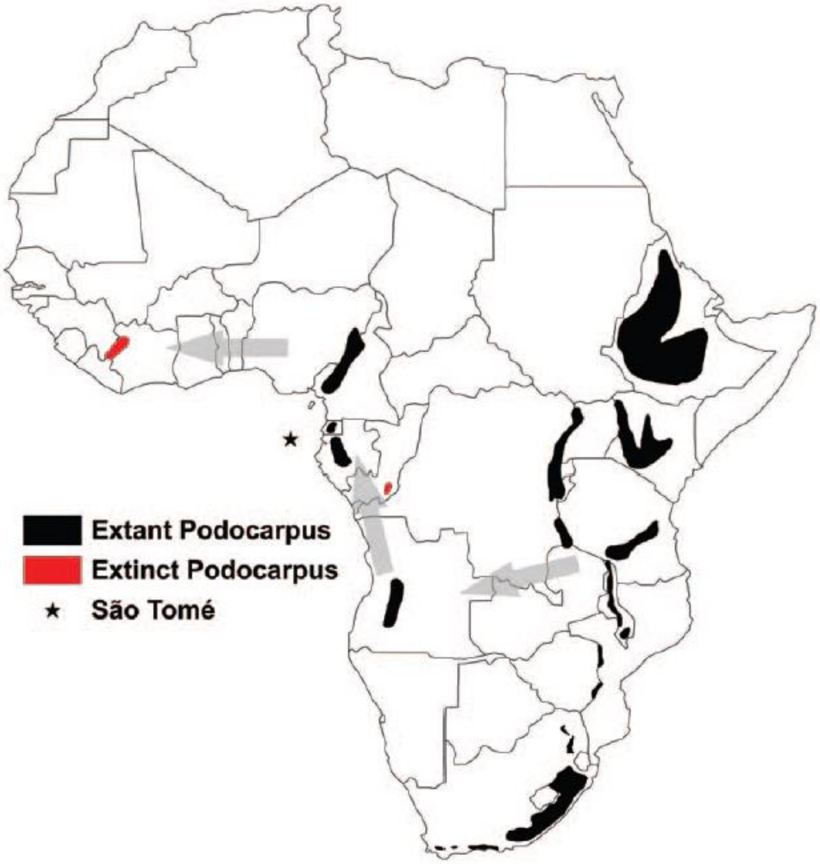

Figure 2. Podocarp distribution in Africa (adapted from Adie and Lawes 2011 who adapted the distributions from: Malaisse 1967; White 1981; Maley 1989; Maley *et al.* 1990; Elenga *et al.* 1991;

Malley 1993; Huntley and Matos 1994; Maley and Brenac 1998; Dupont *et al.* 2000; Parmentier *et al.* 2001).

Of the seven African *Podocarpus* species (*P. latifolius* and *P. milanjianus* are considered synonyms), four are endemic to Madagascar. The Madagascan species include *P. capuronii, P. humbertii, P. madagascariensis* and *P. rostratus* (Farjon 2001, 2013f, 2013g, 2013h, 2013i), and *P. elongatus, P. henkelii* and *P. latifolius* occur in South Africa (Farjon 2001, 2013j; Farjon *et al.* 2013; Figure 4 & 5). *Podocarpus* occurs throughout East and southern Africa but is absent in Ethiopia. The absence of *Podocarpus* in Ethiopia and West Africa is unusual considering many Afromontane species are found in this region. Regional extinction of *Podocarpus* is plausible considering the historical regional extinction of *Podocarpus* in West Africa (Dupont *et al.* 2000). *Podocarpus* was likely present in East Africa and dispersed to West Africa due to climatic cooling during the Pliocene (Morley 2011; Migliore *et al.* 2022). However, *Podocarpus* became extinct in West Africa owing to denudation of uplands following the mid-Cretaceous (Morley 2000). *Podocarpus latifolius* has isolated populations in West Africa. The species has an intermittent distribution restricted to highland areas that extend from the Cameroon highlands into central Angola, into East Africa where the species is distributed in the western-, eastern- and southern-rift regions, and southwards into eastern highlands of Zimbabwe and the Drakensburg and Cape Fold Mountains. *Podocarpus latifolius* is absent from the Ethiopian and Arabian highlands where *A. falcatus* is distributed.

1.2.2. Distribution and diversity of South African podocarps

In South Africa, there are four podocarp species: *Afrocarpus falcatus, Podocarpus elongatus, P. henkelii* and *P. latifolius*. *Podocarpus henkelii* is listed in the IUCN Red Data List as endangered with population sizes decreasing. The remaining three species have stable populations across their distribution and are considered of least concern (Farjon 2013j; Farjon *et al.* 2013; IUCN 2019). All podocarps receive special protection in South Africa under the National and Provincial legislation, due to logging which reduced population numbers (Adie *et al.* 2013). There are two widespread podocarp species in South Africa: *A. falcatus,* commonly known as the Outeniqua yellowwood, is distributed in Afromontane forests of South Africa – from Swellendam in the Western Cape to the midlands of KwaZulu-Natal. However, the species also has a coastal distribution in the Eastern Cape and KwaZulu-Natal and extending into subtropical regions of southern Mozambique (Farjon 2001, 2013e). *Podocarpus latifolius* occurs in the Limpopo province, the western Free State, and the midlands and coastal regions of KwaZulu-Natal and Eastern Cape, and Table Mountain in the Cape Peninsula (Farjon 2001). The other two species have geographically restricted ranges. *Podocarpus elongatus* is widespread in the Western Cape Province (Afrotemperate forests) and *P. henkelii* is distributed in the KwaZulu-Natal and Eastern Cape provinces where it is common in midlands forests and rare in the montane region (Farjon 2001, 2013a, *et al.* Farjon 2013).

1.2.3. Demographic constraints to podocarp distribution

1.2.3.1. Breeding system

All podocarps have wind-dispersed pollen, and many are dioecious – especially in the genus *Podocarpus* – resulting in generally lower tree densities in lowland tropical forests (Enright and Jaffré 2011). Pollen dispersed by wind must be transported from male to female plants across large distances over a landscape populated with competing plants, therefore, the sex ratio and adult spatial distribution is an important component of successful reproduction in podocarps (Nanami *et al.* 2005; Woldearegay and Bekele 2020). However, podocarps negate the negative effects of dioecy through synchronised high cone and pollen production to increase the chances of fertilisation. Dioecious plants are adaptable to changing environments because it ensures minimal inbreeding and opens the possibility of sex ratio adjustment. For example, when there are more females in a population reproductive success is increased whereas more males increase the potential for long-distance dispersal of genetic material.

1.2.3.2. Seed structure, anatomy, and morphology

Podocarp seed structure, anatomy, and morphology are diverse between and within genera. The breeding system of *A. falcatus, P. elongatus, P. henkelii* and *P. latifolius* are similar, i.e. dioecy and wind-pollinated, and all have fleshy fruits. Fleshy fruits predominate in tropical and subtropical forests and are an adaptation for animal dispersal (Howe and Smallwood 1982; Geldenhuys 1993; Parolin *et al.* 2013). However, their seed and fruit morphology is different (Noel and van Staden 1976; Dodd *et al.* 1989b; Geldenhuys 1993). Most podocarp seeds are enclosed by the epimatium which surrounds the stony seed coat (Noel and van Staden 1976; Dodd *et al.* 1989b; Geldenhuys 1993; Wilson and Owens 1999). The epimatium tends to prevent water uptake and allow rapid water loss (Noel and van Staden 1976). When cracked a resinous secretion occurs between the epimatium and sclerotesta (Noel and van Staden 1976; Dodd *et al.* 1989b; Geldenhuys 1993).

1.2.3.3. Seed dispersal and germination

Podocarp seeds are dispersed by animals (i.e., birds and small mammals), which are attracted to the single-seeded fleshy bright coloured fruit (Givnish 1980; Geldenhuys 1993; Negash 1995, 2002, 2003a). Podocarp seeds range in colour from olive-green to bluish reddish-purple (Rohwer and Bittrich 1990). Seed production ranges considerably among species, from almost annually in *P. henkelii* to almost every four to six years in *A. falcatus* (Rohwer and Bittrich 1990; Koen 1991; Geldenhuys 1993). Germination of some podocarps is slow ranging from two to thirteen months for *A. falcatus, P. henkelii* and *P. latifolius* with the seeds losing viability the longer they are stored (Dodd *et al.* 1989a, 1989b; Geldenhuys 1993; Teketay and Granstrom 1997; Bussmann and Lange 2000; Negash 2003a; Adie and Lawes 2009). Germination can be more rapid in some species with germination occurring within 20 – 60 days. Physical dormancy mechanisms such as an epimatium have been reported for *A. falcatus* (Geldenhuys 1993; Negash 2003), *P. henkelii* (Noel and van Staden 1976; Dodd *et al.* 1989b), and *P. latifolius* (Geldenhuys 1993). Dioecy, infrequent fruit production, loss of seed viability, post dispersal predation and delayed germination (Geldenhuys 1993; Negash 2003a) all reduce the chances of podocarps colonising new habitats. However, in South African podocarps reproductive success is sometimes significantly increased by high seed productivity (Midgley 1989).

1.2.4. Podocarp response to environmental factors

Environmental or abiotic stresses are the major regulators of plant growth and distribution. These environmental factors include temperature, light, water (drought) and nutrient availability. Each plant species exhibits a range of tolerance against these factors. When a plant has reached its tolerance limit it experiences stress and up-regulates stress response pathways. This leads to the survival of the stress-tolerant species and susceptibility of stress-sensitive species in the area under stress. Therefore, these factors cumulatively dictate the geography of plant distribution.

1.2.4.1. Light adaptations and stress tolerance

There are specific microclimatic conditions in the forest understory such as lower light availability, lower ground and average temperature, daily variation in temperature, lower fuel bed flammability and higher humidity (Bowman and Panton 1993; Biddulph and Kellman 1998; Hoffmann *et al.* 2005; Ibanez *et al.* 2013). Light is an important resource influencing plant establishment, growth, and survival in forest ecosystems (Adie and Lawes 2009; Kulakowski *et al.* 2017; Kupers *et al.* 2019). Light levels influence forest species specialisation from light-demanding, fast-growing, and short-lived pioneer species to shade-tolerant, slow-growing, and long-lived species.

Podocarps are slow-growing long-lived species which may put them at a competitive disadvantage under high productivity environments (Grime 1979; Loehle 1988; Bond 1989). However, this disadvantage relative to angiosperms may be reduced under a combination of low-light and low-nutrient conditions (Becker 2000; Pammenter *et al.* 2004; Adie and Lawes 2009). The less-efficient vascular system and not fully vascularized leaves of conifers enables them to dominate in low productivity sites where angiosperms are unable to persist (Bond 1989; Midgley and Bond 1989; Midgley 1991; Brodribb and Hill 1998, 1999, 2004; Brodribb *et al.* 2005; Becker 2000; Pammenter *et al.* 2004; Coomes *et al.* 2005; Brodribb 2011). Given these constraints, podocarps are the most successful of conifers, with a large number of species showing a range of leaf adaptations and shade tolerance. Shade tolerance is a common pervasive strategy utilised by most African podocarps (Midgley *et al.* 1995b; Adie and Lawes 2009). Podocarps are able to regenerate continuously through recruitment of shade-tolerant seedling in the understorey (Midgley *et al.* 1990; Lusk and Smith 1998; Adie and Lawes 2009). *Afrocarpus falcatus* and *P. latifolius* are shade tolerant species which can continuously establish beneath the canopy, produce straight poles and have bilaterally flattened leaves – which have a larger surface area to increase light capture – these traits are consistent with shade tolerance for conifers. In the absence of a persistent seed bank seedlings must persist as a seedling bank in the low-light environment of the forest understory until conditions for growth improve.

1.2.4.2. Nutrient adaptation and stress tolerance

The global distribution of conifers suggests a competitive advantage over angiosperms at nutrient-poor and arid environments (Ellenberg 1988; Bond 1989; Richardson *et al.* 2004; Coomes *et al.* 2005). Bond (1989) argued that conifers are excluded by their relatively slower seedling growth rates compared with angiosperms which restrict them to low productivity environments. Low-productivity environments may eliminate this competitive disadvantage and can result in greater nutrient and water use efficiency than angiosperms (Grime 1979). Some podocarp species are persistent in nutrient-poor soils such as *A. falcatus* which can persist in sandy and infertile soils given their ability to acquire and retain immobile nutrients (Coomes and Bellingham 2011). Podocarps possess traits that allow them to tolerant nutrient-poor soils and are successful competitors for below-ground resources.

Efficient capture of nutrients

Podocarps develop root nodules without any stimulus from microorganisms, although they have a symbiotic relationship with arbuscular mycorrhizal fungi (Baylis 1969; Russell *et al.* 2002). The root nodules increase the volume of the root cortex volume which results in greater fungal infection (Dickie and Holdaway 2011). The arbuscular mycorrhizal fungi provide an important mechanism to overcome growth constraints in nutrient-poor soils (Brundrett 2002). The fine roots have a low specific root length, and the associated fungal hyphae have a high area to mass ratio which makes them more efficient at foraging for immobile nutrients. Such fungi have a significant effect on seedling growth, particularly in phosphorus-deficient soils (Baylis 1969; Chen *et al.* 2005; Richardson *et al.* 2005; Lambers *et al.* 2008) and have the potential to influence regeneration success (Chen *et al.* 2005). Arbuscular mycorrhizal have been identified in *A. falcatus* and *P. latifolius* in Afromontane forests (Wubet *et al.* 2003; Hawley and Dames 2004).

Increase nutrient retention of long-lived leaves

Podocarps have long-lived leaves which are associated with species from nutrient-poor environments (Grime 1977; Chapin 1980; McGlone *et al.* 2004; Brodribb 2011). Plants reabsorb a fraction of nitrogen and phosphorous from leaves prior to leaf abscission (Aerts 1995). Long leaf lifespan reduces nutrient loss through leaf abscission during harsh conditions (Monk 1966). Therefore, long-lived leaves are advantageous in nutrient-poor environments where recapturing nutrients is costly (Aerts 1995). The leaves are protected from herbivory by significant quantities of terpenes (Brophy *et al.* 2000). Most podocarps have a leaf lifespan of >2 years, for example: *P. salignus* (3.2 years), *Saxegothaea conspicua* (4.2 years) and *P. nubigenus* (7.3 years) (Lusk 2001). The prolonged leaf lifespan is a thrifty way of retaining nutrients though increased nutrient use efficiency in nutrient-poor conditions (Enright and Ogden 1987; Becker 2000).

1.2.4.2. Drought adaptations and stress tolerance

Brodribb (2011) argued that drought could be a significant agent preventing Southern Hemisphere podocarp success at higher latitudes. During the late Tertiary (5.33 – 2.58 MYA) the climate became drier in the temperate regions of the Southern Hemisphere, this period coincides with significant podocarp contraction and extinction of some taxa in Southern landmasses (Kershaw *et al.* 1994). Recently, diminishing rainfall has resulted in podocarps from mid-latitudes being confined to rainforests or montane forests.

Temperate podocarps seldom grow in dry regions; their drought intolerance is mainly due to their vascular system (Brodribb 2011). Exceptions include *Halocarpus bidwillii, Podocarpus hallii* and *Phyllocladus alpinus* in the dry lowland forests of New Zealand (Bergin 2000; McGlone 2001), *Podocarpus drouynianus* in Western Australia (Chalwell and Ladd 2005; Ladd and Enright 2011), and *Afrocarpus falcatus* drier regions of Southern Africa (Adie and Lawes 2011). Drought damages plants if the water potential gets so low that conduits implode, or cavitation occurs (Tyree and Sperry 1989). Conifers that occur in dry regions, such as Pinaceae and Cupressaceae, can withstand higher tension within water columns of their vascular systems by having thick tracheid walls (prevent implosion), and torus margo within pit membranes (prevent air bubbles from moving through the vascular system) (Pittermann *et al.* 2006b). Despite having thick tracheid walls and relatively high hydraulic resistance across pit membranes, podocarps are susceptible to embolism at relatively low water tensions (Pittermann *et al.* 2006a, 2006b). It may also be the case that the leaf sclereids that allow podocarps to produce broad leaves are vulnerable to implosion under tension (Brodribb 2011). Brodribb and Holbrook (2005) reported that *Podocarpus grayi* leaf sclereids collapsed at moderate tension resulting in a decline in hydraulic conductivity and a loss in photosynthetic function in the leaf. Unlike many drought-deciduous angiosperms, podocarps have long-lived scleophyllous leaves (Kershaw and McGlone 1995) which they are unable to drop during dry spells. For example, *Podocarpus totara* was found to be a drought avoider where it maintained high internal water potential, lost many leaves, and produced shorted leaves during a year drought (Innes and Kelly 1992). Drought sensitivity in podocarps seems to be linked to the trade-off between the ability to resist water stress and wood construction costs.

1.2.4.3. Fire adaptations and stress tolerance

Fire has been reported as an important disturbance controlling species composition and structure as well as distribution of Afromontane and Afrotemperate forests (Geldenhuys 1994; Coomes and Bellingham 2011; Adie *et al.* 2017). Afromontane forests establish and persist in fire refugia (fire-protected moist gullies) where they escape the prevailing grassland fires (Geldenhuys 1994). In tropical environments, fires are more likely during drought years when fragmented forests in a grassy matrix are more vulnerable (Adie *et al.* 2017). South African forests are similar in that they occur within a fire-prone grassland matrix; however, fire has been largely suppressed in attempts to drive forest change (Mucina and Rutherford 2006).

A great majority of podocarps are fire intolerant (Coomes and Bellingham 2011). However, African podocarps persist having come through a 5 – 7-million-year filter that has been punctuated by fire and drought. Like most conifers they do not resprout after fire, except for *P. elongatus* (Midgley *et al.* 1995a; Keeley *et al.* 1998; Bond and Midgley 2003). *Podocarpus elongatus*, endemic to the fynbos biome, can coppice from epicormic buds post-fire (Midgley *et al.* 1995a). *Afrocarpus falcatus* and *P. latifolius* have been observed to resprout post a fire event, also through epicormic buds (T. Twala, personal observation). *Podocarpus drouynianus* and *P. spinulosus* are fire-tolerant species which resprout from below-ground reserves post-fire (Chalwell and Ladd 2005; Ladd and Enright 2011).

Podocarps lack the traits found in fire-adapted confers such as the ability to resprout from the roots, thick bark, highly flammable leaves, serotinous, cones and fire-tolerant seeds.

1.3. Aim and objectives

The aim of this thesis was to examine and understand the distribution and dispersal of podocarps in South Africa, and their responses to climate change.

This study had the following objectives:

1. Develop correlative models to predict which abiotic factors have an influence on the current and future distribution of *A. falcatus, P. henkelii* and *P. latifolius* in South Africa, and to characterise their current and future distribution.
2. Investigate inter-specific variation of morphological and physiological traits of two Podocarpaceae species in response to heat and drought stress and how these may affects the current and/or future distribution of podocarps.
3. Evaluate the dispersal of focal Podocarpaceae populations in Mpumalanga, KwaZulu-Natal, Eastern Cape, and Western Cape provinces, South Africa, using a population genetics approach.

1.4. Scientific contribution of the research

This research uses correlative species distribution modelling to identify climatic variables shaping the current distribution of podocarps in South Africa and predict how they may respond to climate change. In addition, we predict the current and future distribution and environmental niches for South African podocarps using species distribution and niche modelling techniques. The distribution of podocarps is well known, however, there is limited information on the environmental variables driving their distributions and how podocarp distributions will be impacted by climate change. Species distribution and environmental niche modelling techniques have significantly contributed to our knowledge of the environmental variables driving species distributions, has helped us predict species distributions and have informed us of how species may respond to climate change (Phillips *et al.* 2004; Elith and Leathwick 2009; Peterson and Soberón 2012; Baker *et al.* 2022; Brode *et al.* 2022). Recently, species distribution models have been used to predict past, current, and future podocarp distribution with research focussing on the Americas (Quiroga *et al.* 2012; Ornelas *et al.* 2019; Bernadi *et al.* 2020). However, few studies have attempted to predict the past, current and/or future distribution of podocarps in Africa (Ivory *et al.* 2019; Tesfamariam *et al.* 2022), with no known research in southern Africa. In addition, there are no known studies that have attempted to map the environmental niches of podocarps and how their environmental niche and its availability might change under climate change.

To understand how future species distributions may change under climate change I investigate the possible interactive effects of heat and drought stress on the morphology and physiology of seedlings and whether these environmental factors, as well as their physiological thresholds, influence the current and future distribution of podocarps. There is limited information on the effects of heat and drought stress on the persistence and distribution of podocarps (Brodribb and Hill 1997; Spiers *et al.* 2022). Most research on podocarps has focused on neotropical podocarps, focussing on how mature trees respond to light and/or nutrient availability (Ebbett and Ogden 1998; Adie and Lawes 2009; Xu *et al.* 2019; Luo *et al.* 2020; Song *et al.* 2022). It is important to understand how light, nutrient and water availability as well as temperature influences tree growth, biomass allocation patterns, leaf traits and physiology. This will allow us to understand how tolerant these species are to these environmental stresses, to evaluate how changes in the availability of resources in an ecosystem may impact the plants' ability to restore depleted resources, and determine how they are able to persist in their distribution. Studies that can be referred to in tropical forests have focussed more on the seed anatomy, germination requirements, propagation, life history, seedling recruitment, growth rates, angiosperm-gymnosperm competition and physiology (Osborn 1960; Lübbe and Geldenhuys 1991; Geldenhuys 1993; Brodribb and

Hill 1997, 1998, 2004; Kowalski and van Staden 1998, 2001; Hill and Brodribb 1999; Negash 2003a, 2003b; Brodribb *et al.* 2005; Aidie and Lawes 2009, 2011; Krepkowski *et al.* 2012). This study sets out to contribute towards this lack of information about the response of podocarps to environmental stress through experimental manipulations by exposing seedlings to different temperature and water conditions to inform us about the persistence of podocarps in their current and future distribution under climate change. In addition, genetical analyses were used to explain whether these populations are connected or have become isolated overtime which insights into podocarp dispersal patterns and distributions. It is important to understand whether populations that are connected tend to have more genetic diversity which will be vital during stressful times – such as climate change, competition and/or other natural disasters – as genetic diversity will enable plants to tolerate, adapt and/or persist under different stresses.

1.5. Thesis structure

All three major chapters in this thesis are either published, accepted for publication, or written to be submitted for publication, with the exception of the introduction (chapter 1) and synthesis (chapter 5). The first chapter of this thesis consists of the rationale, general introduction, and scientific contribution of this research. Chapter 2 identifies which environmental factors influence the distribution of *A. falcatus, P. latifolius, P. henkelii* and *P. elongatus* and the effects of climate change on the distribution of these podocarps. Chapter 3 is an ecophysiology chapter which aims to investigate the effects of heat and drought stress on the physiology and morphology of *A. falcatus* and *P. henkelii,* which could ultimately affect the current and future distribution and persistence of these species in South Africa. Chapter 4 is a population genetics chapter which seeks to determine whether there is dispersal (gene flow) between different populations of *A. falcatus, P. latifolius* and *P. henkelii.* Chapter 5 is the synthesis chapter which will discuss and highlight the important findings of the study's results describing the mechanisms driving the current and future distribution of podocarps under climate change.

1.6. Author contributions

Due to the collaborative nature of this study, multiple authors have contributed to the manuscripts in chapter two, three and four. The authors have contributed differently to the different manuscripts. The following list contains a description of the authors contribution towards each manuscript:

- T. C. Twala: Primary author, formulated research questions, collected samples, conducted laboratory work, analysed the data, and wrote the manuscript.
- J. T. Fisher: PhD supervisor, provided guidance and input regarding theoretical ideas for the manuscript, provided funding and resources for data collection, and commented on various drafts of all chapters.
- K. L. Glennon: PhD co-supervisor, provided guidance and input regarding theoretical and statistical ideas for the manuscript, provided funding and resources for data collection, and commented on various drafts for chapter 1, 2, 4 and 5.
- E. T. F. Witkowski: Co-author, provided guidance and input regarding theoretical and statistical ideas for the manuscript and commented on various drafts for chapter 3.
- O. Hardy: Université Libre de Bruxelles (ULB) Evolutionary Biology and Ecology lab collaborator, provided guidance and input regarding theoretical and statistical ideas for the manuscript, provided funding and resources for data collection and assisted with data analysis for chapter 4.
- S. Sergeant: ULB Evolutionary Biology and Ecology lab collaborator lab technician and collaborator, assisted with laboratory work for chapter 4.

Study species identity

Afrocarpus falcatus

Afrocapus falcatus is a long-lived dioecious tree species that is distributed in Eastern (Ethiopia, Kenya, Malawi) and Southern Africa (South Africa, Lesotho and Eswatini). It is located in Afromontane forests and rainforests but has been found to descend to the subtropics moist coastal forests. *Afrocarpus falcatus* produces grey-brown to reddish thick bark that is smooth and ridged when the tree is young but becomes flaky when the trunk is older (Perrie 2010). It produces evergreen, hairless, leathery, and waxy leaves that are arranged opposite or in spirals. It is vulnerable to logging but is globally of least concern but protected in South Africa. The wood is used for furniture and as firewood, and its seeds are edible along with its extracted oils (Abdillahi *et al.* 2011). Both the bark and seeds are used for to treat ailments.

Figure 3. *Afrocarpus falcatus* (**A**) adult tree, (**B**) leaf and (**C**) seeds.

Podocarpus elongatus

Podocarpus elongatus is a long-lived evergreen dioecious tree distributed in the Western Cape province from the Van Rhynsdorp area through the Cedarberg and Bokkeveld mountains to

Swellendam but does not occur on the Cape Peninsula. It occurs predominantly on deep sandy soil near rivers and streams (Prins *et al.* 2004; Mucina *et al.* 2022). *Podocarpus elongatus* can resprout from epicormic buds (Midgley 1996) which allows it to survive after being uprooted during floods. It was used to as firewood and charcoal as the tree seldom produces straight rods. Stem extracts are used for traditional medicine purposes (Abdillahi *et al.* 2008, 2011).

Podocarpus henkelii

Podocarpus henkelii is a long-lived dioecious tree distributed in the montane forests of the Eastern Cape to KwaZulu-Natal. It produces evergreen leathery revolute margin leaves in a spiral and pale grey-brown fissured bark. The species is protected by the South African Forestry Legislation and Nature Conservation Legislation but is considered of least concern by the IUCN. *Podocarpus henkelii* is used for medicinal purposes (Abdillahi *et al.* 2008, 2011; Balga *et al.* 2014).

Figure 4. *Podocarpus henkelii* (**A**) adult tree, **(B, C)** leaves, (**D**) whole seeds, (**E**) seeds cut transversely and (**F**) cross section.

Podocarpus latifolius

Podocarpus latifolius is a long-lived diecious tree that occurs in southern, eastern and southern parts of South Africa, extending further north along the coast. *Podocarpus latifolius* occurs in montane forests with rocky hillsides and mountain slopes. It has a greyish and smooth bark when young but has longitudinal fissures when it matures. It produces leathery long and narrow evergreen leaves in a spiral or opposite. *Podocarpus latifolius* is considered of least concern by the IUCN but is protected in South Africa. It has been used as timber, firewood and medicinal purposes (Abdillahi *et al.* 2011).

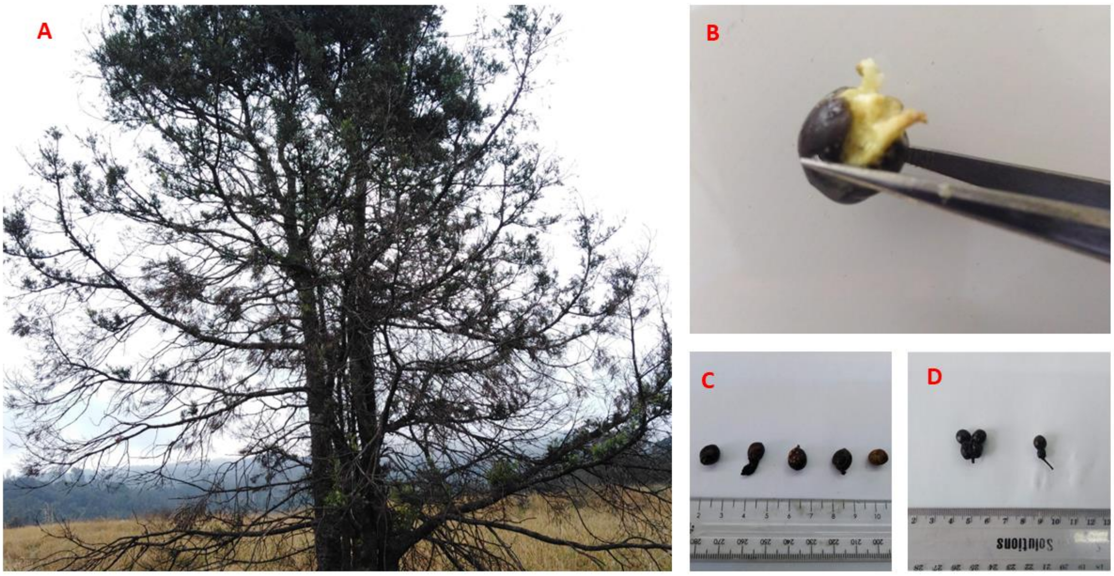

Figure 5. *Podocarpus latifolius* (**A**) adult tree post-fire, (**B**) imbibed seed after being soaked in water for 12 hours, (**C, D**) different forms of seeds.

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Chapter 2: Projecting Podocarpaceae response to climate change: we are not out of the woods yet

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Abstract

Under the changing climate, the persistence of Afrotemperate taxa may be threatened as suitable habitat availability decreases. The unique disjunct ranges of podocarps in southern Africa raise questions about the persistence of these species under climate change. Here, we identified likely environmental drivers of these distributions, characterised the current and future (2070) environmental niches, and projected distributions of four podocarp species in South Africa. Species distribution models were conducted using species locality data for *Afrocarpus falcatus, Podocarpus latifolius, P. elongatus* and *P. henkelii* and both historical climate data (1970 – 2000) and future climate scenarios (RCP 4.5 and 8.5, 2061 – 2080) to estimate the current and future distributions. We also used this opportunity to identify the most important climatic variables that likely govern each species' distribution. Using niche overlap estimates, a similarity test, and indices of niche expansion, stability and unfilling, we explored how niches change under different climate scenarios. The distribution of the study species was governed by max temperature of the warmest month, temperature annual range, mean temperature of the wettest quarter, and precipitation of the wettest, driest, and warmest quarters. The current distribution of *A. falcatus* was predicted to expand to higher elevations under RCP 4.5 and RCP 8.5. *Podocarpus henkelii* was predicted to lose most of its suitable habitat under RCP 4.5 and expand under RCP 8.5, however this was the opposite for *P. elongatus* and *P. latifolius*. Interestingly, *P. elongatus,* which had the smallest geographic distribution, showed the most vulnerability to climate change in comparison to the other podocarps**.** Mapping the distribution of podocarps and understanding the differences in their current and future climate niches provides insight into potential climate drivers of podocarp persistence and the potential for adaptation of these species. Overall, these results suggest that *P. elongatus* and *P. henkelii* may expand to novel environmental niches.

Keywords: Climate change, environmental niche, persistence, Podocarpaceae, species distribution.

2.1. Introduction

Conifers are a crucial component of global forests with both economic and ecological importance (Farjon 2018). Podocarpaceae (podocarp) is the second most species rich conifer family and the largest clade in southern conifers with 19 genera and 187 species (Christenhusz and Byng 2016) and are one of the few gymnosperms that inhabit tropical forests in the Southern Hemisphere. Species in the Podocarpaceae were historically centered in Gondwana, subsequently expanding to Australasia, southernmost Africa and currently also occur in Malaysia (Leslie *et al.* 2012; Quiroga *et al.* 2016). Podocarps were prominent in Gondwana during the Cretaceous period (Krassilov 1974) diversifying by the early Triassic period into the podocarps we know today (White 1981; Mill 1999). This diversification was possibly due to the onset of warmer and wetter climates because of the opening of the South Ocean (Atlantic and Indian Ocean; Morley 2011). During the mid-Cretaceous, podocarps went extinct in West Africa during the flattening of the uplands, reduced in geographic extent in India during the late Cretaceous when India drifted northwards (Morley 2000), and subsequently dispersed into its current Southeast Asian distribution following global cooling. In Africa, global cooling may have enabled the dispersal of podocarps from East Africa to the highlands of West Africa through a northern and southern pathway (Migliore *et al.* 2022). Presently, podocarps have a largely pantropical distribution. Some taxa extend into subtropical and temperate latitudes, where they mainly occur in Australasia, central and South America, and tropical montane Africa (Farjon 2010; Klaus *et al.* 2020). In Afrotemperate forests, podocarps persist within fynbos, heathland, and grassland matrices where fire and grass competition are the predominant factors that influence forest distribution (Adie and Lawes 2009a; Adie and Lawes 2011; Adie *et al.* 2017).

Climate change and angiosperm competition have been some of the most important factors influencing podocarp distribution and persistence (Quiroga and Premoli 2007; Migliore *et al.* 2022). Podocarps appeared to respond to these threats through persistence in refugia (Quiroga *et al.* 2018; Tesfamariam *et al.* 2022). As a result, podocarps show remarkable adaptability in habitat, growth form and physiological traits. For instance, some members of the family are semiaquatic (*Retrophyllum minor* (Carriére) C. N. Page), parasitic (*Parasitaxus usta* (Vieill.) de Laub.), exhibit resprouting and fire and drought tolerance (*Podocarpus drouynianus* F.Mueller. and *P. spinulosus* R.Br. ex Mirb.), shade tolerance (*P. latifolius* (Thunb) R.Br. ex Mirb.), tolerance to soil anoxia (*Manoao colensoi* (Hook.) Molloy), diverse fleshy seed cones (*P. elongatus* E. Mey. ex Endl*,* *Afrocarpus falcatus* (Thunb.) C. N. Page), diverse leaf morphology (*P. nagi* (Thunb.) Pilg., *P. henkelii* Stapf ex Dallim. & Jackson*, P. macrophyllus* (Thunb.) Sweet), leaf and individual longevity, and have conspicuous root nodules which house arbuscular mycorrhiza fungi (Molloy 1995; Hill and Brodribb 1999; Biffin *et al.* 2012, Contreras *et al.* 2017). Remarkably, most podocarps are restricted to humid, mountainous environments, including angiosperm-dominated forests (Kitayama *et al.* 2011). Many extant podocarps are slow growing, fire and drought intolerant, and these characteristics put them at a competitive disadvantage when co-occurring with angiosperms (Bond 1989; Brodribb and Hill 1998), which restricts them to these 'steppingstone' refugia.

Developing management strategies and practical measures to conserve podocarps is difficult without identifying key environmental variables to each species' climatic niche and predicted geographical distribution, particularly under future climate conditions. Moreover, climate change affects habitat requirements for many species (Bakkenes *et al.* 2002; Aitken *et al.* 2007; Essl *et al.* 2011; Moran 2020; Fricke *et al.* 2022). Consequently, determining whether climate change will affect the geographical distribution and environmental niches of podocarps presents another critical challenge linked to their ecological value and significance. The current distributions of *Afrocarpus falcatus, P. elongatus, P. henkelii* and *P. latifolius* are relatively well known; however, little is known about the environmental drivers of podocarp distribution, their dispersal patterns and how climate change will affect their distribution and environmental niches.

Niche conservatism is used to test how species shift (expand and/or contract) their ranges in response to climate change (Wiens 2004; Wiens and Graham 2005; Wiens *et al.* 2010). This ecological and evolutionary process is an issue of concern particularly due to the expected adverse effects of climate change on species distributions and diversity (Araújo *et al.* 2013). Therefore, modelling the environmental niche and projecting it into geographic space allows us to test whether species exhibit environmental niche conservatism as ranges shift under climate change. Testing environmental niche conservatism is necessary to enable us to develop strategies to mitigate the negative effects of climate change and provide important ecological insights. For the purpose of this study, "niche conservatism" is defined as when the predicted environmental niche tends to remain similar to the current environmental niche (Warren *et al.* 2008). Eeley *et al.* (1999) and Colyn *et al.* (2020) suggested that the ability of forest species to track suitable environmental conditions under climate change could be significantly limited by anthropogenic landscape change. Therefore, in this study, the ability of podocarps to persist under climate change will be determined by the ability of the species to track favourable niches and expand their geographic range.

Species distribution models (SDMs) are mathematical algorithms that estimate a species' climatic niche by characterising a species' occurrence in relation to environmental factors (Elith *et al.* 2006). SDMs can be used to predict and analyse patterns of distribution and could estimate the risk these environmental drivers pose to species (Guisan and Thuiller 2005; Elith and Leathwich 2009; Guisan *et al.* 2013). SDMs quantify the relationship between plant distributions and environmental factors (Guisan and Thuiller 2005; Elith and Leathwich 2009; Wiens *et al.* 2010; Guisan *et al.* 2013); thus, demonstrating their vulnerability more accurately. SDMs often assume niche conservatism, which suggests that species can maintain their environmental niches (Peterson *et al.* 1999; Wiens 2004; Wiens *et al.* 2009; Peterson 2011). It is still controversial whether species' environmental niches are preserved in space and time (Peterson *et al.* 1999; Broennimann *et al.* 2007). As a result of the ongoing theoretical development and quantification of a species' environmental niche, researchers have advanced our understanding of how species fluctuate in their requirements for and tolerance of various factors (Soberón 2007). In this study, we used a combined environmental (niche overlap indices) and geographical approach (temporal transferability of SDMs) to characterise the distribution of *A. falcatus, P. elongatus, P. henkelii* and *P. latifolius*. The objectives of this study were to (1) identify the environmental variables that shape the distribution of *A. falcatus, P. elongatus, P. henkelii* and *P. latifolius*; (2) model the current distribution of South African podocarps; (3) project the current models onto two future climate emissions scenarios (RCP 4.5 and RCP 8.5); and (4) compare the future geographic distribution of podocarp environmental niches to their current environmental niche geographic distribution. Such comparisons will enable us to infer the vulnerability of these South African podocarps to the changing environment.

2.2. Methods

2.2.1. Study species

South Africa consists of four podocarp species from sister genera: *Afrocarpus* (*A. falcatus*) and *Podocarpus* (*P. elongatus, P. henkelii* and *P. latifolius;* Farjon 2001). In Africa, podocarps are restricted to highland archipelagos in montane forests along the Afromontane Forest belt, where they persist in small forest patches within a grassland, fynbos, or heathland matrix. The Afromontane Forest belt extends from Ethiopia along the eastern mountain range, all the way to the southern Cape in South Africa. *Afrocarpus falcatus* is present throughout Afromontane forests and is absent in West Africa. *Afrocarpus falcatus* and *P. latifolius* are widely distributed geographically across South Africa but are restricted to Afromontane Forest habitats. In South Africa, *A. falcatus* and *P. latifolius* occur along the southwest of South Africa and extend to the south and east coast of the country up the Drakensberg mountains in the eastern parts of the country and extend northwards in temperate midland regions towards Zimbabwe. *Podocarpus henkelii* occurs in forest habitats and is restricted to summer rainfall areas in the mesic east of the country. *Podocarpus elongatus* only occurs in the extreme western and southern parts of the country (winter rainfall) in the forest and fynbos biome.

Generally, our study included three major steps, with relevant details reported below: (i) data were downloaded, compiled and variables were selected, (ii) *A. falcatus, P. elongatus, P henkelii* and *P. latifolius* occurrence and climate data were used to characterise niche and environmental spaces, and (iii) a variety of species distribution modelling algorithms were used to project the potential distribution of *A. falcatus, P. elongatus, P henkelii* and *P. latifolius* in South Africa.

2.2.2. Species occurrences

Afrocarpus falcatus, *P. elongatus, P. henkelii* and *P. latifolius* occurrence data were downloaded from the Global Biodiversity Information Facility online repository (GBIF; https://www.gbif.org/). Where necessary, location information was georeferenced and duplicate localities were removed. Records were validated using Google Earth v7.1.2 and outliers associated with non-natural locations were removed. After filtering, 562 localities were retained in the final analysis for all four species: *A. falcatus* (n = 246), *P. latifolius* (n = 242), *P. elongatus* (n = 49) and *P. henkelii* (n = 25; Figure 1).

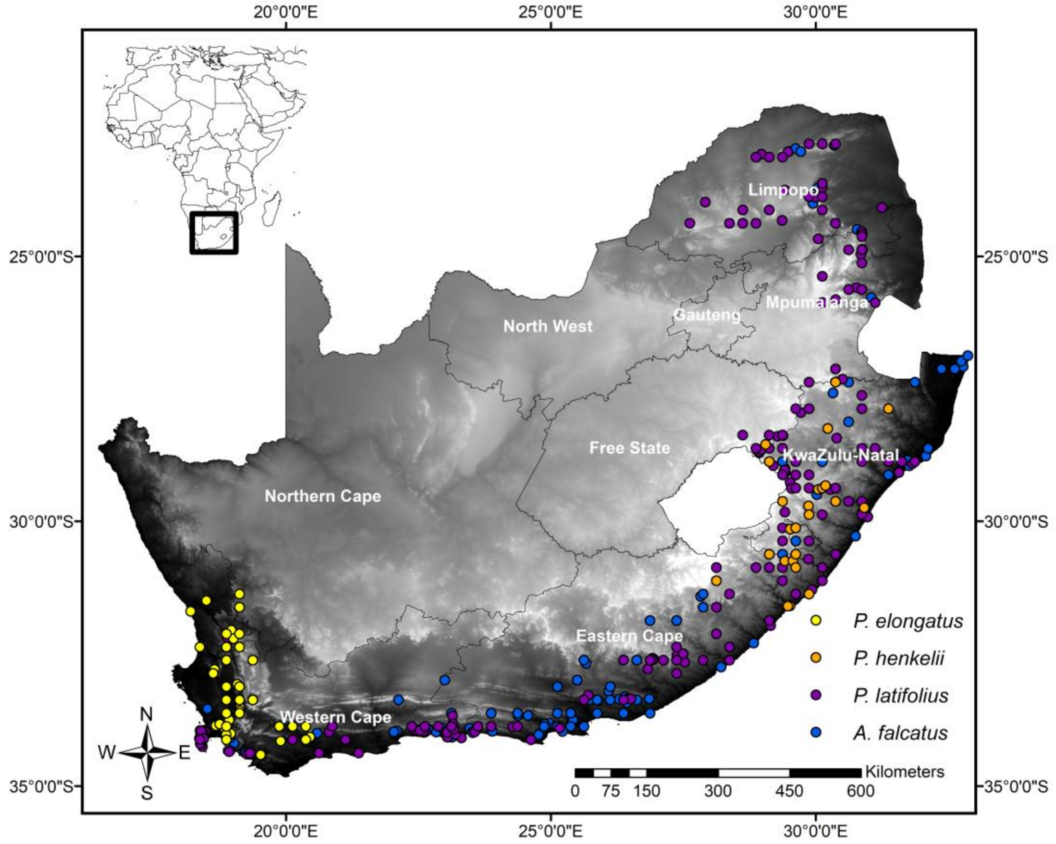

Figure 1. South African occurrences of *Afrocarpus falcatus, Podocarpus latifolius, P. henkelii* and *P. elongatus* that were downloaded from the Global Biodiversity Information Facility (http://gbif.org). The map is shaded according to elevation where darker grey indicates low elevation and light grey/white areas indicate high elevation (> 1800m.a.s.l).

2.2.3. Climate variables

Current climate data were downloaded from Chelsa version 2.1 (historical (1970 – 2000) and future (2061 – 2080) climate data were downloaded under Representative Concentration Pathways (RCP) 4.5 and 8.5 emission scenarios from the Hadley Global Environment Model 2-Atmosphere Ocean (HADGEM2-AO) circulation model which provides adequate coverage for Africa (Martin *et al.* 2011). These data were downloaded using "Chelsa.CMIP_5.download" function from the *ClimDatDownloadR* R package (Karger *et al.* 2017; Jentsch *et al.* 2021). All environmental variables had a spatial resolution of 30 arc seconds (~1km^2 pixel area at the equator). To avoid obtaining spurious results and overparameterization of the models due to variable multicollinearity (Elith *et al.* 2010; Synes and Bsborne 2011; Boria *et al.* 2014), variable correlations were minimized using dimension reduction techniques. A Pearson's correlation test based on all 19 climate variables was conducted for all species presence points and used to exclude highly correlated variables ($r > 0.65$) from our models. To further avoid overparameterization of the model and obtaining spurious results due to variable multicollinearity, we used the variance inflation factor (VIF) on the remaining variables to remove significantly correlated variables. Seven predictor variables were used in subsequent processing: Max Temperature of the Warmest Month (BIO5), Temperature Annual Range (BIO7), Mean Temperature of Wettest Quarter (BIO8), Mean Temperature of Coldest Quarter (BIO11), Precipitation of Wettest Quarter (BIO16), Precipitation of Driest Quarter (BIO17), and Precipitation of Warmest Quarter (BIO18). These seven predictor variables were used to model the species distribution for all the species to allow for comparisons between species and are variables used by other studies predicting the species distribution of podocarps (e.g., Bernardi *et al.* 2020; Tesfamariam *et al.* 2022).

2.2.4. Species distribution modelling (SDM)

Species distribution modelling uses occurrence data and environmental variables to simulate suitable/habitable environmental conditions for focus species. Here, we used ten different algorithms available within the *biomod2* package (Thuiller *et al.* 2021) in R version 4.1.1 to obtain an ensemble of predicted distributions. An ensemble approach was used because numerous studies have shown that ensemble SDMs improve the predictive performance of SDMs in comparison to using one algorithm (Grenouillet *et al.* 2011; Guo *et al.* 2015; Bernardi *et al.* 2020; Zurell *et al.* 2020). The following algorithms were used to develop the ensemble SDMs: generalised linear model (GLM), generalised additive models (GAM), generalised boosting model/boosted regression trees (GBM), surface range envelop/BIOCLIM (SRE), classification tree analysis (CTA), artificial neural network (ANN), flexible discriminant analysis (FDA), multiple adaptive regression splines (MARS), random forest (RF) and maximum entropy (MAXENT). The following parameters were set for the models: one run of five hundred pseudo-absence points was used on one model evaluation, split into 80% for training and 20% for testing for each SDM, and variable importance was determined using three permutations for the full extent of South Africa. The remaining model values were set to default values. Area under the receiver operating curve (AUC), true skills statistics (TSS) and the continuous Boyce index (CBI) were used to assess model predictive efficiency and performance (Hanley and McNeil, 1982; Allouche *et al.* 2006). The Boyce index uses presence data only to measure how different the model predictions are from a random distribution of observed presence across the prediction gradient. Boyce index values range between -1 and +1, where positive values indicate that the model predictions are consistent with the presence data, values close to zero suggest that the model is no different from a random model, and negative values indicate no occurrence when there is. All statistical analyses were performed in R using scripts from Broennimann *et al.* (2012), which are now available in the *ecospat* R package (Di Cola *et al.* 2017; Broennimann *et al.* 2020). The potential distribution for each species was projected under the Representative Concentration Pathway (RCP) 4.5 and RCP 8.5 future climate emission scenarios for the year 2070 (average for 2061 – 2080). Representative Concentration Pathways (RCP) are models of greenhouse emissions scenarios which predict future climate under different greenhouse emissions scenarios. RCPs 4.5 and 8.5 were chosen because RCP 4.5 greenhouse gas emissions are projected to peak around 2040 and then decline, while in RCP 8.5 greenhouse gas emissions are projected to continue to increase throughout the 21st century, respectively a “best-case” and “worst-case” scenario (Meinshausen *et al.* 2011). At the current rate of emissions and mitigation processes these are the most likely scenarios. RCP 2.6 was not included because it was unrealistic, as it assumed peak emissions by 2020 and subsequent decline requiring negative emissions (Meinshausen *et al.* 2011; Van Vuuren 2011).

In order to identify change in geographic area of *A. falcatus, P. latifolius, P. henkelii* and *P. elongatus* binary maps were generated from SDMs, and the current and future binary models were used to calculate the change in geographic area between the current and future climate emissions scenarios. Changes in geographic area were classified as ‘loss’, ‘gain,’ and ‘stable’. Loss referred to the area the species originally occupied, but no longer occupies after climate change. Gain referred to occupied areas that the species did not originally occupy and then occupy after climate change. Stable referred to areas that persisted after climate change, or also referred to as climatic microrefugia (Pang *et al.* 2021). All geographic information system analyses were done in ArcGIS v 10.8 (ESRI).

2.2.5. Calculating niche overlap in environmental space

Assessing climatic niche characteristics is a powerful approach for studying niche divergence and conservatism. Broennimann et al. (2012) presented a principal component analysis (PCA) method which places environmental variables into two-dimensional space identified by their first and second principal components. When testing niche divergence and niche conservatism hypotheses, niche overlap can provide a relatively reliable measurement of overlap. We quantified the degree of shared environmental niche space between the current and the future climate emissions scenarios of *A. falcatus, P. elongatus, P. henkelii* and *P. latifolius*. In this study, niche overlap between current and future climate emissions scenarios for each podocarp species was computed using the Schoener's *D* statistic (Schoener 1968; Warrren *et al.* 2008). Schoener's *D* ranges from 0 (no overlap) to 1 (complete overlap). Results were then interpreted using the classification scheme suggested by Rödder and Engler (2011) where *D* ranges indicated potential interpretations: 0.0 – 0.2 = no or very limited overlap, 0.2 – 0.4 = low overlap, 0.4 – 0.6 = moderate overlap, 0.6 – 0.8 = high overlap, and 0.8 – 1.0 = very high overlap/identical niche. Subsequently, niche overlap was interpreted in the context of three general niche categories: niche expansion, stability/stasis and unfilling (Petitpierre *et al.* 2012; Glennon *et al.* 2014; Guisan *et al.* 2014; Di Cola *et al.* 2017). Niche stability (i.e., the proportion of the current range niche overlapping with the future range niche), niche expansion (i.e., the proportion of the future range niche not overlapping with the current range niche) and niche unfilling (i.e., the proportion of the current range niche not occupied in the future range niche) enables the ability to generate hypotheses about the potential drivers of podocarp niche dynamics between current and predicted climatic emission scenarios. The niche stability and expansion values always sum to 100%. The niche unfilling value corresponds to the expansion value when shifting current and future ranges. Niche conservatism is defined as the tendency for species to retain their environmental niche in space and time and is represented by "niche stability".

We used an ordination technique that applies kernel density smoothers to species presences in environmental space (Broennimann *et al.* 2012) to determine the environmental niche occupied by each species under current and future climate emissions scenarios. The kernel density function is applied for smoothing the density of occurrences for each of the generated grid cells, which correspond to the vector of the available environmental conditions in the study area under each climate emission scenario. This smoothing approach removes any biases to obtain a better representation of the environmental conditions suitable for each species under current and future climate emissions scenarios. This approach was implemented using a principal component analysis which is an ordination technique that is calibrated on the entire environmental space based on the focal variables of both current and future climate emission scenarios (hereafter referred to as PCA-env). Further details can be found in Broennimann *et al.* (2012).

We also performed the niche similarity test, which assesses if the environmental niches of each species are more similar or more dissimilar than expected by chance in comparison to each other under current and future climate scenarios, accounting for differences in the surrounding areas of the localities (background space) under current and future climate emissions scenarios (Warren *et al.* 2008; Warren *et al.* 2010; Warren *et al.* 2018). The niche similarity test compares the niche overlap of one species range randomly distributed over its background, while keeping the other unchanged (e.g., Current: *A. falcatus* → *P. elongatus*), and then carries out the reciprocal comparison (e.g., Current: *P. elongatus* → *A. falcatus*). For the niche similarity test, an alpha value of 0.05 was considered to indicate that niches were no more similar than expected by chance.

2.3. Results

2.3.1. Model evaluation indices

We calculated the commonly used (AUC) and the less commonly used (TSS and CBI) model evaluation indices. Model performance was good for all the emission scenarios (Table 1). AUC varied from 0.990 to 0.992, TSS varied from 0.681 to 0.978. The CBI suggested that all the model predictions were consistent with the distribution of the actual data as they all had positive CBI values. However, CBI values for *P. elongatus* under the current emission scenario was low.

Table 1. Area under the ROC curve (AUC) and true skills statistics (TSS) values showing species distribution model performance and continuous Boyce index (CBI) showing how well the models fit the presence data of *Afrocarpus falcatus, Podocarpus latifolius, P. elongatus* and *P. henkelii* current and future climate emissions scenarios (RCP 4.5 and 8.5) in their South African distribution.

Species	Emission scenario	AUC	TSS	CBI
A. falcatus	Current	0.961	0.834	0.818
	RCP 4.5	0.958	0.814	0.806
	RCP 8.5	0.955	0.795	0.943
P. elongatus	Current	0.992	0.978	0.250
	RCP 4.5	0.990	0.940	0.889
	RCP 8.5	0.992	0.957	0.911
P. henkelii	Current	0.978	0.954	0.529
	RCP 4.5	0.945	0.858	0.732
	RCP 8.5	0.967	0.909	0.754
P. latifolius	Current	0.942	0.738	0.854
	RCP 4.5	0.918	0.681	0.894
	RCP 8.5	0.920	0.690	0.868

2.3.2. Importance of environmental variables

Precipitation of the driest quarter (BIO17) and precipitation of the wettest quarter (BIO16) were the most important variables shaping the current distribution of *A. falcatus* and *P. latifolius*, respectively (Table 2). Temperature annual range (BIO07) was the most important variable constraining the future distribution of *A. falcatus* and *P. latifolius*. The current and future distribution of *P. elongatus* was predicted to be constrained by precipitation of the warmest quarter (BIO18). The most influential variable shaping the current distribution of *P. henkelii* was predicted to be BIO18. Interestingly, max temperature of the warmest month (BIO5) was the most important variable constraining the distribution of *P. henkelii* under RCP 4.5, while BIO18 was the most important predictor of *P. henkelii* under RCP 8.5.

Table 2. Environmental variable importance scores (mean and standard deviation is shown for the three iterations) for *Afrocarpus falcatus, Podocarpus elongatus, P. henkelii* and *P. latifolius* species distribution models under current and future climate emissions scenarios. Variable importance scores range between 0 (low importance) and 1 (high importance). The variables represented in bold were the most important variable for each taxon under current and future conditions. BIO5 – Max Temperature of Warmest Month; BIO7 – Temperature Annual Range; BIO8 - Mean Temperature of Wettest Quarter; BIO11 – Mean Temperature of Coldest Quarter; BIO16 – Precipitation of Wettest Quarter; BIO17 – Precipitation of Driest Quarter; BIO18 – Precipitation of Warmest Quarter.

Species	**Emission Scenario**	**BIO5**	**BIO7**	**BIO8**	**BIO11**	**BIO16**	**BIO17**	**BIO18**
A. falcatus	Current	0.084 (0.001)	0.188 (0.002)	0.024 (0.001)	0.157 (0.002)	0.046 (0.001)	**0.455 (0.007)**	0.116 (0.001)
	RCP 4.5	0.056 (0.001)	**0.607 (0.001)**	0.029 (0.001)	0.079 (0.001)	0.037 (0.001)	0.126 (0.002)	0.066 (0.000)
	RCP 8.5	0.029 (0.000)	**0.554 (0.008)**	0.037 (0.001)	0.059 (0.000)	0.122 (0.001)	0.084 (0.003)	0.115 (0.002)
P. elongatus	Current	0.035 (0.001)	0.045 (0.002)	0.168 (0.006)	0.029 (0.002)	0.121 (0.003)	0.262 (0.015)	**0.340 (0.005)**
	RCP 4.5	0.051 (0.001)	0.100 (0.002)	0.168 (0.003)	0.046 (0.002)	0.178 (0.006)	0.123 (0.005)	**0.335 (0.005)**
	RCP 8.5	0.094 (0.004)	0.154 (0.005)	**0.492 (0.011)**	0.051 (0.001)	0.104 (0.004)	0.043 (0.002)	0.063 (0.003)
P. henkelii	Current	0.095 (0.001)	0.010 (0.001)	0.011 (0.000)	0.040 (0.002)	0.068 (0.001)	0.222 (0.017)	**0.553 (0.017)**
	RCP 4.5	**0.414 (0.008)**	0.184 (0.003	0.122 (0.001)	0.083 (0.002)	0.100 (0.002)	0.059 (0.001)	0.040 (0.001)
	RCP 8.5	0.284 (0.003)	0.080 (0.000)	0.035 (0.001)	0.045 (0.002)	0.057 (0.001)	0.056 (0.006)	**0.544 (0.003)**
P. latifolius	Current	0.254 (0.009)	0.197 (0.004)	0.037 (0.001)	0.054 (0.001)	**0.281 (0.002)**	0.081 (0.001)	0.097 (0.002)
	RCP 4.5	0.078 (0.002)	**0.542 (0.001)**	0.037 (0.001)	0.099 (0.001)	0.173 (0.008)	0.028 (0.000)	0.043 (0.001)
	RCP 8.5	0.052 (0.001)	**0.585 (0.007)**	0.035 (0.001)	0.079 (0.002)	0.191 (0.003)	0.020 (0.000)	0.039 (0.001)

2.3.3. South African podocarp distribution under climate change

The ensemble SDM predicted high habitat suitability for *A. falcatus, P. elongatus, P. henkelii* and *P. latifolius* in the exterior part of the country, near the coastal limits and are excluded on the interior of the country (Figure 2). *Podocarpus latifolius* occupied the largest current and future geographic area followed by *A. falcatus*. *Afrocarpus falcatus* and *P. latifolius* were distributed in the Limpopo, Mpumalanga, KwaZulu-Natal, Eastern Cape, and Western Cape provinces, which span the summer and winter rainfall regions of the country. *Podocarpus elongatus* was restricted to the southern parts of the Western Cape, which is associated with winter rainfall. *Podocarpus henkelii* was constrained to the summer rainfall provinces of Mpumalanga, the midlands of KwaZulu-Natal, and the Eastern Cape, with moderately suitable habitat in the Limpopo province. Although *P. elongatus* and *P. henkelii* had the most restricted distribution, *P. elongatus* occupied the smallest geographic area.

Under climate emission scenarios, *A. falcatus* was predicted to expand to higher altitudes into the interior of the country, this was the case for under RCP 4.5 and RCP 8.5 (Figure 3). The southwestern part of the distribution of *P. elongatus* was predicted to expand to higher elevations to track favourable conditions; however, the south-eastern and the northern part of the distribution was predicted to become less suitable under both RCPs. Interestingly, the suitable habitat of *P. henkelii* under current climate was predicted to lose most of its suitable habitat under RCP 4.5 and expanding its habitat to higher altitude in its northern distribution. This was the opposite for *P. latifolius,* where it gained suitable habitat under RCP 4.5 and lost suitable habitat under RCP 8.5. A common finding was that all four podocarp species expanded their habitat by moving to higher altitudes.

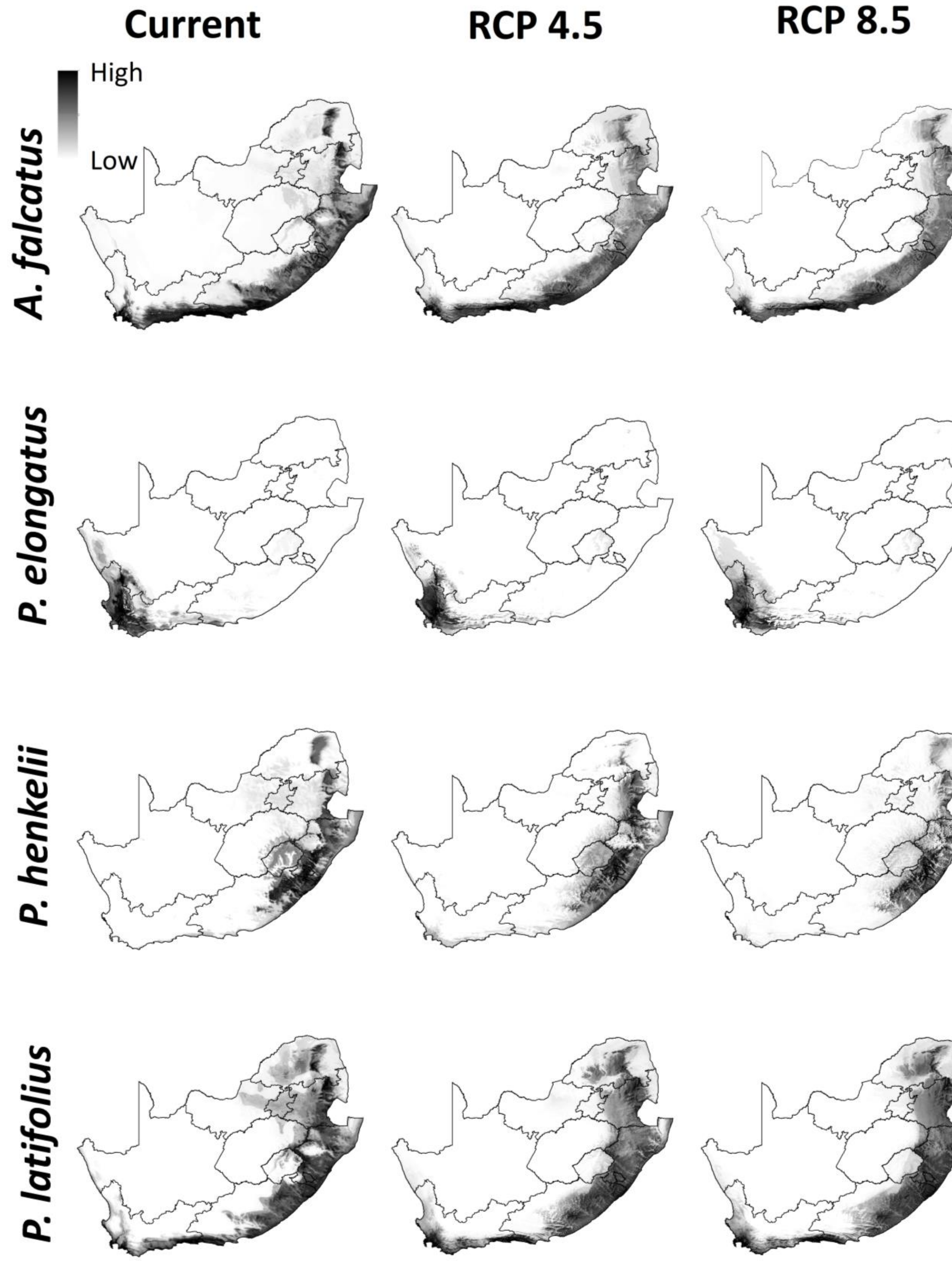

Figure 2. Distribution for *Afrocarpus falcatus*, *Podocarpus elongatus, P. henkelii,* and *P. latifolius* in South Africa predicted by using ensemble models based on climatic environmental variables under the current climate and mild (RCP 4.5) and severe (RCP 8.5) climate emission scenarios predicted for 2070. Habitat suitability ranges from high (black), moderate (grey) to low (white) suitability.

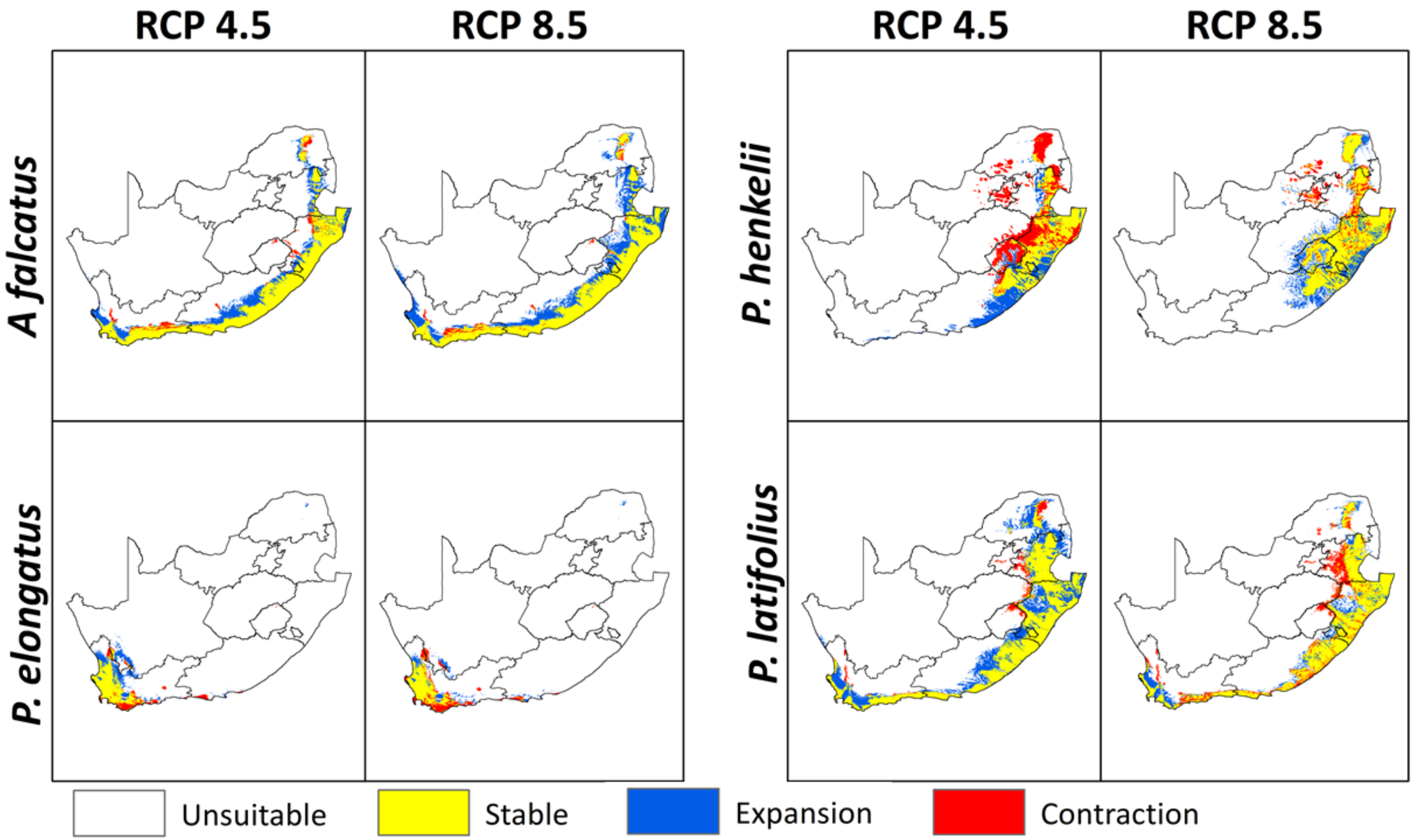

Figure 3. The projected impact of climate change (2070) on the habitat suitability of *Afrocarpus falcatus, Podocarpus latifolius, Podocarpus elongatus* and *Podocarpus henkelii* in South Africa under RCP 4.5 and 8.5 climate emissions scenarios. Yellow represents stability of geographic space, blue represents areas of habitat expansion, and red represents areas of habitat contraction/loss.

2.3.4. Environmental niche overlap and similarity

Podocarpus elongatus occurs over the largest environmental niche, while *P. henkelii* occurs over the smallest environmental niche (Figure 4). The current environmental niches of *A. falcatus, P. elongatus* and *P. henkelii* are predicted to contract in 2070, however the environmental niche of *P. latifolius* is predicted to remain stable under RCP 4.5 and 8.5.

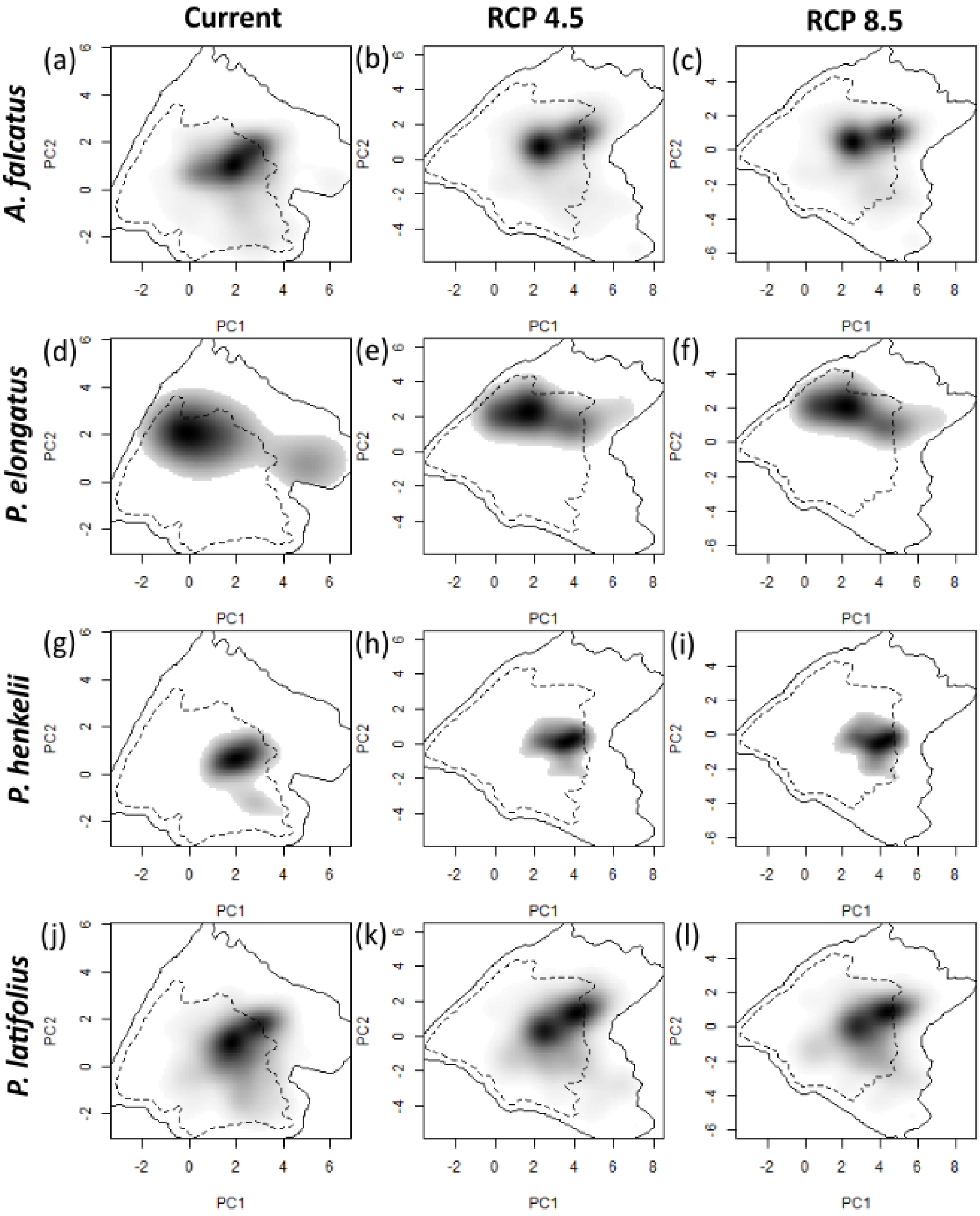

Figure 4. Current and future (RCP 4.5 & RCP 8.5) environmental niches of *Afrocarpus falcatus* (a – c), *Podocarpus elongatus* (d – f), *P. henkelii* (g – i) and *P. latifolius* (j – l). Darker shading indicates higher densities. The solid line indicates 100% of the available environmental space and the dashed contour lines represent 50% of the most common background space.

2.3.4.1. Podocarp environmental niche similarity

The comparison of the current and future environmental niches of *A. falcatus, P. latifolius* and *P. henkelii* similar than expected at random (Table 3; Figure 4). Interestingly, the comparison of the current and RCP 4.5 environmental niche for *P. elongatus* was similar, however the other niche comparisons between periods show niche divergence.

Table 3. Niche similarity *Afrocarpus falcatus, Podocarpus latifolius, P. henkelii* and *P. elongatus* under current and future climate emissions scenarios (RCP 4.5 and RCP 8.5). Significant similarity test *P*-values are in bold.

Species	Current → RCP 4.5	RCP 4.5 → Current	Current → RCP 8.5	RCP 8.5 → Current
A. falcatus	**0.0099**	**0.0099**	**0.0099**	**0.0198**
P. latifolius	**0.0198**	**0.0198**	**0.0099**	**0.0099**
P. henkelii	**0.0396**	**0.0198**	**0.0099**	**0.0198**
P. elongatus	**0.0495**	0.1089	0.1089	0.0693

2.3.4.2. Niche overlap between the current and future environmental niches

The current and future environmental niches of *A. falcatus, P. elongatus* and *P. latifolius* showed very limited overlap (Table 4; Figure 4). Interestingly, *P. henkelii* had the highest environmental niche overlap of the four podocarps with 59.17% and 55.43% overlap between the current–RCP 4.5 and current–RCP 8.5 environmental niches, respectively.

When the overlap between the current and future environmental niches were further broken down, we observed that the current and future environmental niches of all four podocarps was stable (Table 4). *Afrocarpus falcatus* showed greater niche expansion compared to niche unfilling. A greater extent of niche unfilling was observed for *P. elongatus* and *P. henkelii* in comparison to niche expansion for both niche comparisons (Table 4). Low niche unfilling and expansion was observed for *P. latifolius* between the current and future environmental niches.

Table 4. Niche overlap and dynamics between the current and future projected environmental niches of *Afrocarpus falcatus, Podocarpus elongatus, P. henkelii* and *P. latifolius*. Each metric ranges from zero (low) to one (high).

Species	Pairwise comparisons	Niche overlap (*D*)	Expansion	Stability	Unfilling
A. falcatus	Current → RCP 4.5	0.1212	0.3543	0.6456	0.1309
	Current → RCP 8.5	0.1633	0.3869	0.6131	0.1348
P. elongatus	Current → RCP 4.5	0.0759	0.2137	0.7863	0.4936
	Current → RCP 8.5	0.0428	0.3007	0.6993	0.6733
P. henkelii	Current → RCP 4.5	0.5917	0.0240	0.9760	0.3716
	Current → RCP 8.5	0.5543	0.0501	0.9499	0.4335
P. latifolius	Current → RCP 4.5	0.1076	0.3005	0.6995	0.0755
	Current → RCP 8.5	0.2068	0.2884	0.7116	0.0753

2.4. Discussion

This study characterised environmental niches of South African podocarps using ordination techniques to better understand how these species may respond to the changing climate. In addition, we used ensemble SDMs to identify the environmental constraints that shape the current and future geographic distributions of *A. falcatus, P. elongatus, P. henkelii* and *P. latifolius.* We found that the ensemble SDMs accurately predicted the known geographic distributions of each podocarp species, that podocarps were generally limited by rainfall, particularly around the seed germination timeframe (Baskin and Baskin 1998), and that there are likely reproductive and dispersal patterns as well as physiological traits that potentially prevent species from dispersing outside their current distributions.

Identifying environmental variables that shape and maintain species geographic distributions is an essential component of evolution and ecology. Among the seven environmental variables used for these models, total rainfall during the summer and winter months (irrespective of summer/winter rainfall region) was predicted to shape the current and future distribution of podocarps in South Africa. These findings were expected as numerous studies have identified drought stress as a factor constraining podocarp distribution and persistence (Brodribb and Hill 1998; Tesfamariam *et al.* 2022; Twala *et al.* 2022). Consequently, the distribution of podocarps in high rainfall areas may be due to traits that reduces their ability to survive in low rainfall regions. For instance, podocarps have small single-veined leaves that limit hydraulic conductivity (Brodribb *et al.* 2007) and recalcitrant seeds that become less viable as they lose moisture (Noel and Staden 1976; Dodd, Staden and Smith 1989a; 1989b; Negash 2003). Nearly all extant podocarps exhibit low to no drought tolerance (Brodribb and Hill 2004; Twala *et al.* 2022) except for the New Zealand species *P.*

totara, which uses drought-avoidance strategies (Innes and Kelly 1992) and *P. macrophyllus* found across China and Japan (He et al. 2020). In addition, *P. latifolius* recruitment is significantly reduced under drought and deep shade where seedlings are outcompeted by grasses in the landscape (Adie and Lawes 2009b). Drought and shade are antagonistic selection pressures and together with rainfall appear to constrain podocarp distribution (Chiariello 1984; Givnish 1987; Brodribb and Hill 2000; Fiorucci and Fankhauser 2017; Lusk *et al.* 2019). Furthermore, the models indicated that South African podocarps tend to persist in moist forest patches where fires are an important driver of forest distribution yet fires hardly occur in the patches themselves (Geldenhuys 1994; Kellman and Meave 1997).

In addition to rainfall, the future distribution of *A. falcatus* and *P. latifolius* was predicted to be limited by temperature annual range (BIO7). This finding suggests that in the future there will be variation in seasonal temperatures where *A. falcatus* and *P. latifolius* occur across the highlands and midlands in the northeastern parts of South Africa compared to the southwestern parts of the country. In the northeastern parts of South Africa, the models predict that the future daily temperatures fluctuations will be smaller in comparison to present higher daily temperature fluctuations in the southwestern parts of the country. Notably, the future distribution of *P. elongatus* was also constrained by mean temperature during the wettest quarter, which coincides with the winter months for this species as it is currently restricted to winter rainfall areas of the country. Winter rainfall and temperature are particularly important for *P. elongatus* because it produces recalcitrant seeds during the winter months and minimum rainfall and temperature requirements need to be met for recruitment and persistence. *Podocarpus henkelii* may be limited to warm high rainfall areas because it has foliar sclereids and broad leaves (Brodribb and Hill 1998; Adie and Lawes 2011; Brodribb 2011). Furthermore, *P. henkelii* produces seeds that are more drought-sensitive than *A. falcatus* seeds (Dodd *et al.* 1989a; Negash 1992).

The current and future potential distributions of podocarps obtained from our ensemble SDMs coincide with known geographic ranges (White 1981; Farjon 2001). The broad geographically distributed *P. latifolius* and *A. falcatus* co-occur with *P. elongatus* and *P. henkelii* in their respective geographic ranges, however *P. elongatus* and *P. henkelii* do not co-occur. *Podocarpus latifolius* had the broadest geographic distribution followed by *A. falcatus*. Both *A. falcatus* and *P. latifolius* were predicted to co-occur in the north-eastern part of South Africa, along the southern coast, and through to the southwest of South Africa. This finding coincides with the actual distribution of each species. The geographic range extends across the savanna, grassland, forest, fynbos, and Albany thicket biomes and extends to the Indian Ocean coastal belt. *Afrocarpus falcatus* is the only one of these four podocarps that persists in the dry forest of the coastal lowlands (Adie and Lawes 2011; Coomes and Bellingham 2011) and this occurrence is likely as a relic from population expansion during the cool and moist conditions of the last glaciation (Finch and Hill 2008; Neumann *et al.* 2008). *Podocarpus elongatus* was predicted to occupy the smallest geographic area of all the examined podocarp species under current and future climate emissions scenarios. This taxon is presently distributed in the south and southwestern parts of South Africa within the fynbos and succulent karoo biomes. *Podocarpus elongatus* can persist in this fire prone habitat due to its ability to resprout after fire (Midgley *et al.* 1995). Unlike the other podocarps, *P. elongatus* produces epicormic sprouts that enable tolerance of fire and persistence in the fynbos biome that is prone to fire (Midgley *et al.* 1995), which makes it better adapted for Mediterranean habitat. *Podocarpus elongatus* may be restricted to its Gondwanan origin and its disjunct distribution a result of retreating to higher elevations to track favourable environmental conditions as rising sea levels during the Last Glacial Maximum that lead to loss of coastal habitat (Geldenhuys 1992). *Podocarpus henkelii* is predicted to have a current distribution in summer rainfall regions with high rainfall in eastern Limpopo province, midlands of KwaZulu-Natal, and northern Eastern Cape province of South Africa.

The effects of climate change on the geographic distribution of a wide range of tree communities have been reported across the world (Bakkenes *et al.* 2002; Aitken *et al.* 2008; Essl *et al.* 2011; Aguileé *et al.* 2019; Burley *et al.* 2019; Kambach *et al.* 2019; Pavlović *et al.* 2019; Sedmáková et al. 2019; Fricke *et al.* 2022). *Podocarpus henkelii* was predicted to occupy the third largest geographic range under future climate emission scenarios (Figure 2). Under future scenarios, *P. henkelii* may experience a contraction of its geographic distribution under relatively ‘mild’ climate change scenarios (e.g., RCP 4.5) yet will potentially expand its geographic distribution as temperatures rise (e.g., under RCP 8.5, Figure 2). Its geographic range was predicted to contract in higher elevations and expand to lower elevations for both climate change emission scenarios. The southern expansion of its geographic area under both emission scenarios may suggest that the southern parts of South Africa will become hotter under climate change (Bomhard et al., 2005; Collier *et al.* 2008). Therefore, it is plausible that *P. henkelii* is a thermophilic species and can expand its distribution with increasing temperatures under climate change. This is also evident through the reduced geographic expansion of *P. henkelii* under RCP 8.5 in comparison to the cooler RCP 4.5 (Figure 3). Twala et al. (2022) reported that *P. henkelii* was more heat tolerant than *A. falcatus.* Therefore, the ability of *P. henkelii* seeds to disperse across the grass matrix and/or establish in newly colonised areas could limit species expansion. In contrast, under both emission scenarios, *A. falcatus* and *P. latifolius* were predicted to expand to higher altitudes and lower longitudes to track favourable climatic conditions. The dispersal of *A. falcatus* and *P. latifolius* seeds by birds, bats, baboons, and monkeys (Geldenhuys 1993; Negash 2003) will likely enable them to disperse to climatically favourable areas faster relative to *P. elongatus* and *P. henkelii.* The past responses of *P. elongatus* and *P. henkelii* to climate change indicate a tight synchronisation with climate fluctuations, particularly in response to water availability. For *P. latifolius,* recruitment is less episodic than the other species thus making it less vulnerable to climatic instability. *Podocarpus elongatus* was predicted to lose suitable habitat in its southern distribution under both RCPs and expand northwards, particularly under RCP 4.5. However, shifting to higher elevations and longitudinal shifts seem to be the most common responses to climate change (Pavlović *et al.* 2019; Sedmáková *et al.* 2019).

Numerous studies have reported that plant species that occupy a large geographic range also occupy different environmental niches (Cardillo *et al.* 2018; Early *et al.* 2019; Kambach *et al.* 2019; Quiroga and Souto 2022). We found that niche conservatism was evident for between current and future RCP's for *A. falcatus, P. latifolius* and *P. henkelii* (Table 3). The current and future environmental niche under RCP 4.5 for *P. elongatus* also showed niche similarity, however, the RCP 4.5 → current, RCP 8.5 → Current and Current → RCP 8.5 comparisons showed niche divergence. The tendencies toward divergent environmental niches in podocarps cannot be explained by differences in the climate suitability alone. However, this divergence could be explained by differences in seed dispersal, establishment requirements, habitat use, competition and ecophysiological limits. There was no consistent trend in niche conservatism or divergence among the comparisons of current environmental niches and predicted future niches across the podocarp species examined. For example, *P. henkelii* may shift to new geographic areas under climate change/predicted emission scenarios, per niche conservatism, given the high niche overlap value and unfilling value (i.e., the future environmental niche space is available but currently unoccupied). Notably, there may be elevated propagule pressures that ultimately limit the dispersal of *P. henkelii* to sites with suitable environmental conditions, which will result in a reduced realised niche. It is also possible that *P. henkelii* may continue to spread in its geographic range even though the breadth of its environmental niche is predicted to decrease. Relative to the other three podocarps examined, *P. henkelii* had the lowest niche expansion value, which suggests that it will not expand into novel environmental niches when moving southwards geographically under climate change. On the other hand, the current environmental niche of *P. elongatus* diverged slightly from its future environment niche due to low niche overlap as a result of high niche expansion and unfilling (Table 4). This divergence may be attributed to differences in microhabitats and competition. In addition, the current and future environmental niches of *P. latifolius* showed low niche overlap, which was associated with low niche unfilling values. *Podocarpus latifolius* can occur within areas with variable gradients of soil moisture, temperature, rainfall, and altitude along different latitudes and it produces small seeds annually which are dispersed by birds (Geldenhuys 1993; Adie and Lawes 2009b). Moreover, frequent recruitment occurs due to lower seed susceptibility to desiccation and shade tolerant seeds, which facilitates germination and reduces susceptibility to climate change. These characteristics indicate that *P. latifolius* is a vagile species. Despite this*,* results showed low niche expansion. This may be because *P. latifolius* currently occupies most of the suitable environmental niche available already, and there is no additional niche to expand into.

The ability of a species to occupy different environmental niches may have important implications for the understanding of the vulnerability of the species under climate change (Wiens, & Graham, 2005). Previous studies recognised niche shifts in a variety of plant taxa such as *Picea sitchensis* (Aguilée *et al.* 2016), *Agave* spp. (Gómez-Ruiz and Lacher 2019) and *Betula utilis* (Hamid et al. 2019) under climate change, but the causes of these shifts are not well known. However, several studies have shown that some species will not be able to track favourable climate fast enough to escape climate change (Zhu *et al.* 2012; Cang *et al.* 2016; Burley *et al.* 2019). The combination of some niche expansion and unfilling for *A. falcatus* under both RCPs resulted in moderate niche stability under global change. The inability of *A. falcatus* to occupy environmental niches different from those of its current environmental niche may be due to a combination of post dispersal mortality and loss in seed viability after harvesting, coupled with infrequent fruit production (Geldenhuys 1993; Negash 2003). Consequently, dispersal may be the main factor that governs whether *A. falcatus* reaches suitable habitats under global change.

Overall, we found that podocarp distribution is determined primarily by seasonal drought, likely due to the recalcitrant seeds of podocarps which are produced generally when rainfall is limited. The difference in the distribution of these podocarps underscores the variation of environmental conditions that they are adapted to and may also be a reflection on the differences in their physiology. This is particularly important as the physiological traits of podocarps may limit their distribution across environmental gradients (Brodribb and Hill, 1998, 1999; Brodribb 2011, Twala *et al.* 2022). Physiologically oriented distribution models should be useful to predict whether the physiological thresholds of podocarps are the mechanism responsible for podocarp distribution and not their reproductive means. Insights from this study are useful for on-going conservation actions for South African podocarps by directing the re-introduction of these species in suitable habitats and guiding the establishment of effective networks of protected areas for future dispersal events by selecting suitable habitats that are not expansive and will not have negative social and socio-economic consequences while maximising on conservation.

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Statements & Declarations

Conflict of Interest

The corresponding author confirms on behalf of all authors that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

Author Contribution Statement

TCT – Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, validation, visualization, writing – original draft, writing – review & editing.

JTF – Conceptualization, funding acquisition, resources, software, supervision, validation, writing – reviewing & editing.

KLG – Conceptualization, funding acquisition, methodology, resources, supervision, validation, writing – reviewing & editing.

Data Availability

The datasets generated during and/or analysed during the current study are available in the Open Society Foundation repository, DOI 10.17605/OSF.IO/RNG5K (https://osf.io/rng5k/).

Chapter 3: The effects of heat and drought stress on the ecophysiological responses and growth of *Afrocarpus falcatus* and *Podocarpus henkelii* seedlings

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Abstract

Drought and heat are major abiotic stresses that affect recruitment and growth which in turn affects the distribution of species. Plant distribution and forest composition may be determined by species abilities to tolerate and/or avoid drought and heat stress, particularly at the vulnerable seedling stage. Here we investigate the physiological and morphological responses of *Afrocarpus falcatus* (needle-like leaf) and *Podocarpus henkelii* (broadleaf) to heat and drought stress. To measure these responses, *A. falcatus* and *P. henkelii* seedlings were exposed to heat (ambient, heat wave and heat stress) and drought stress (well-watered and water-stressed) over a 4-week period. Both species showed a significant reduction in maximum quantum yield efficiency of photosystem II (PSII) and chlorophyll content under stressful conditions. Under both stresses *P. henkelii* was able to maintain minimal fluorescence yield, and maximum activity of PSII. Water use efficiency (WUE) of *A. falcatus* was significantly reduced under drought stress, while *P. henkelii* seedlings exposed to drought stress had greater WUE than well-watered seedlings across all heat treatments. Morphologically, leaf senescence and loss were accelerated by the drought and heat treatments in both podocarps. However, the drought and heat treatments did not have a significant effect on biomass allocation pattern, root:shoot ratio and leaf relative water content of *A. falcatus* and *P. henkelii*, except for leaf mass fraction in *A. falcatus* which was significantly reduced by drought and heat stress combined. Overall, the morphological and physiological responses of *A. falcatus* and *P. henkelii* to drought and heat stress are indicative of a heat and drought avoidance strategy, allowing both podocarps to persist during heat stress and under limited water availability. This research informs us of the responses of podocarp species to environmental stress, and how this may influence the distribution and potential persistence of these species under current and future environmental conditions.

Keywords: Allocation pattern, drought stress, ecophysiology, growth, heat stress, water use efficiency

3.1. Introduction

Plants can persist in an environment that is within their dispersal ability and where biotic and abiotic conditions permit establishment (Thompson and Fronhofer, 2019). Temporal variation in conditions and metapopulation dynamics may result in individuals persisting regardless of adverse conditions, thus making range margins dynamic. In the context of large scale geographical and climatic changes, Podocarpaceae (podocarps) are excellent species to study how organisms can persist over a variety of environmental conditions. Most podocarp species occur in wet tropical and subtropical forests dominated by angiosperms (Biffin et al., 2011). This patten is interesting considering that podocarps are less competitive than angiosperms in productive environment and thus thrive stressed environments such as high altitudes, arid regions with low temperatures, and poorly drained and/or nutrient poor soils globally (Bond 1989; Enright & Ogden 1995; Coomes et al., 2005). Podocarps are restricted to stressful environmental conditions because they are slow growing, have a lower photosynthetic capacity and a more efficient vascular system in comparison to angiosperms (Bond, 1989; Lusk et al., 2003), however, *A. falcatus* is the fastest growing of the South African podocarps. In productive environmental conditions, podocarp seedling establishment and growth is hindered by insufficient light transmission due to shading by angiosperms (Coomes et al., 2005). Nonetheless, leaf flattening, and the physiology of podocarps have enabled them to persist in the face of angiosperm competition (Brodribb and Hill, 1997; Brodribb and Feild, 2008).

Podocarps are widely distributed throughout the southern hemisphere due to a wide range of ecophysiological tolerances to extreme drought, high and low temperatures, and waterlogged habitats (Hill & Brodribb 1999). Podocarps in South Africa occur across different elevations including coastal lowlands and temperate highlands, rainfall, and temperature gradients (Midgley et al., 1995; Adie and Lawes, 2011). This distribution pattern has been attributed to ecological limitations and competition with angiosperms.

The physiological and morphological determinants of podocarp distribution may be related to their leaf morphology. Podocarps display a wide variety of leaf morphologies from imbricate shoots (needle-like) to bilaterally flattened leaves similar in appearance to broad-leaved angiosperm species (Brodribb and Hill, 1997). In the Northern Hemisphere, “conservative” traits such as needle-like leaves enable podocarps to persist in nutrient poor, water limited and cold environments (Lusk et al., 2003; Coomes et al., 2005). Podocarps with flattened leaves have been shown to persist in productive environments such as tropical habitats (Brodribb et al., 2007), and can compete with angiosperms within these productive environments, especially in the Southern Hemisphere (Hill and Brodribb, 1999). Thus, the adaptation of flattened leaves of Southern Hemisphere podocarps favours successful competition in productive environments, whereas needle-like leaves favour competitive success in stressful environments.

In South Africa, there are four Podocarpaceae (podocarp) species: *Afrocarpus falcatus Podocarpus latifolius*, *P. elongatus* and *P. henkelii*. *Afrocarpus falcatus* and *P. latifolius* are widespread and *P. elongatus* and *P. henkelii* have restricted distributions (Farjon, 2001). In this study we focussed on *A. falcatus* and *P. henkelii* both of which have different ecophysiological properties. *Afrocarpus falcatus* has needle-like leaves while *P. henkelii* has bilaterally flattened leaves which are similar to broad-leaved angiosperms (Brodribb & Hill 1997). *Afrocarpus falcatus* occurs in the highlands of East Africa, southern coast of Mozambique, northern South Africa as well as along the east and southern coastal areas of the country. This patchy distribution spans across both winter and summer rainfall regimes. *Podocarpus henkelii* is restricted to the Southern Mistbelt Forest of KwaZulu-Natal province and eastern South Africa, which is characterised by summer rainfall on loamy, nutrient-rich soils (Mucina and Rutherford, 2006).

To understand how environmental factors affect distribution, abundance, and coexistence of tree species, it is necessary to understand how individual species respond to resource availability in forest communities. Rainfall has been shown to influence species distribution at a large scale (Toledo et al., 2012) and composition (Amissah et al., 2014), whereas irradiance, topography and soil fertility affect species distribution at a smaller scale (Opedal et al., 2014; Wang et al., 2015). Most subtropical forests show variation in rainfall, and accordingly, the distribution of subtropical species is determined by species drought performance and physiological drought tolerance (Kuang et al., 2017). It has long been recognised that plant photosynthetic capacity, growth, and physiological stress responses can be influenced by drought. The response of podocarp species to drought stress has been extensively studied (Brodribb and Hill, 1998; Brodribb and Hill, 1999; Brodribb and Holbrook, 2005), however far less attention has been paid to how this may affect podocarp distribution as well as the role of temperature on the distribution of podocarps. Podocarps are long-lived, and show sporadic germination, their seeds have short-term viability and have varied dispersal distances from seeds clustered close to the parents to some seeds dispersed further away (Geldenhuys, 1993; Veblen et al., 1995). Unlike fast growing species, most conifers are unable to move in and out of marginal environments in response to short-term fluctuations in the environment and persist at the extremes of their distributions by the long-term survival of adult plants.

This study aimed to assess the morphological and ecophysiological responses of *A. falcatus* (fine leafed) and *P. henkelii* (broadleaf) seedlings to heat and drought stress. We predicted that *A. falcatus* would be more heat and drought tolerant due to its fine leaves, compared with the broadleafed *P. henkelii*.

3.2. Methods

Afrocarpus falcatus (13.36 ± 2.18 cm) and *P. henkelii* (8.34 ± 2.11 cm) 6-month-old seedlings were purchased from Willow Feather Farm, Centurion, South Africa in February 2020, which were 11 months old at the time of the experiment. Sixty seedlings of each species were selected based on similar heights. These were transplanted into 1.5L pots with Culterra professional potting mix and left to acclimate for five weeks at the Oppenheimer Life Sciences rooftop greenhouse, University of the Witwatersrand, Johannesburg, South Africa.

To examine the effects of drought, heat stress, and drought and heat stress on podocarp species, we used a split design which included a drought treatment with two levels and a heat treatment with three levels. After acclimation, 36 seedlings of similar height were selected for each species. Twelve seedlings of each species were then randomly assigned to each of the three illuminated walk-in growth chambers (Conviron, Winnipeg, Canada), with a 15 h/9 h day/night photoperiod, 60% relative humidity, and 750 μmol^{-1} photosynthetic photon flux density (PPFD). Seedlings were rotated within and between growth chambers every week to minimise the effects of the growth chambers on plant performance. The annual mean temperature of karkloof forest was selected to be the ambient/baseline temperature to test podocarp responses to heat and drought stress. Karkloof forest is a large mistbelt forest in KwaZulu-Natal province in South Africa containing *Afrocarpus falcatus, Podocarpus latifolius* and *P. henkelii* occur in this mixed angiosperm-podocarpus forest. The growth chamber conditions were as follows: (1) ambient: the chamber was maintained at the average growing season temperature for karkloof forest throughout the growing season (27 ˚C/ 14 ˚C), (2) heat wave: on day 14 the temperature in the growth chamber was increased by 15 ˚C from the ambient temperature in 4 steps, i.e. 30 ˚C, 34 ˚C, 38 ˚C and 42 ˚C and was maintained at each step for four hours to allow the plants to acclimate to the temperature changes (as per Duan et al. 2017), where the temperatures were maintained at 42 ˚C/27 ˚C day/night temperature cycle for 7 days then returned to ambient temperature on day 21, and (3) high: average summer temperature at karkloof + 10 °C (37 ˚C/20 ˚C). Each of the 12 seedlings in each growth chamber were randomly assigned to two drought treatments (i.e., well-watered and water-stressed), respectively. The six treatments in this experiment were thus:

i. ambient temperature + well-watered;
ii. ambient temperature + water-stressed;
iii. heat wave temperature + well-watered;
iv. heat wave temperature + water-stressed;
v. High temperature + well-watered;
vi. High temperature + water-stressed.

All pots were fully saturated with water and excess water was allowed to drain overnight and weighed the next morning to determine the pot weight at field capacity. Foil and brown paper bags were placed over the top of the pot to minimise evaporation from the soil. Thereafter, seedlings in the well-watered treatments were watered twice a week with 200 ml of water. Seedlings in the water-stressed treatments were not watered. Pots were weighed twice a week to estimate water loss. Both podocarp species varied widely in their water use. To standardise the drought treatment, we terminated the experiment when the soil water content reached 0%. The 0% soil moisture value was selected to ensure that both species were exposed to significant water limitation. The experiment was conducted over four weeks; numerous studies which have also conducted short-term drought experiments on slow growing trees (Amissah et al., 2015; Feichtinger et al., 2015; Marchin et al., 2020).

3.2.1. Seedling growth measurements

Stem diameter (*D*, cm) was measured at 1 cm from the ground using digital Vernier callipers. Height (H, cm) was measured from the collar (which was marked) to the terminal apical bud. Stem volume was calculated as D^2H (cm^3) (Kubiske et al., 2006). Measurements were done at the beginning of the experiment and then weekly thereafter. The number of new leaves and leaves lost were counted each week to record the leaf demography on all plants in each treatment per week. These measurements were recorded weekly for five weeks.

3.2.2. Relative water content

At the end of the experiment, all leaves were picked from all the seedlings, weighed to quantify fresh weight (FW), water saturated for 24 h at 4 °C and subsequently the turgid fresh weight (TW) measured to determine the leaf relative water content. Leaves were then placed into brown paper bags and oven dried at 65 °C for three days. Leaf relative water content (RWC) was calculated as:
$RWC = 100 \times (FW - DW)/(TW - DW)$ for all individuals of each species (Saura-Mas and Lloret, 2007) (1).

3.2.3. Seedling biomass allocations

To test the effect of heat and drought stress on seedling biomass allocation patterns, all the seedlings of each species from each treatment were harvested at the end of the experiment and separated into three parts: leaves (including the petiole), stems, and the roots (cut at the root collar). The roots were separated from the soil and washed with deionised water under a fine sieve to capture the fine roots. After harvesting, the leaves, stems and roots were weighed to determine their fresh mass. Thereafter, the plant parts of each individual were placed in labelled brown paper bags and oven dried at 65 °C for three days. The dry mass of leaves, stems and roots were weighed for each individual seedling. Leaf, stem, and root mass fractions (LMF, SMF and RMF) were calculated as follows:

Leaf Mass Fraction (%) = $\frac{\text{Leaf Mass}}{\text{Leaf Mass+Stem Mass+Root Mass}} \times 100$ (2)

Stem Mass Fraction (%) = $\frac{\text{Stem Mass}}{\text{Leaf Mass+Stem Mass+Root Mass}} \times 100$ (3)

Root Mass Fraction (%) = $\frac{\text{Root Mass}}{\text{Leaf Mass+Stem Mass+Root Mass}} \times 100$ (4)

3.2.4. Chlorophyll content and fluorescence

The chlorophyll content of the three most recently developed fully expanded leaves from each seedling was measured every week using a single-photon avalanche diode (SPAD) 502-Plus chlorophyll content meter (Minolta, Osaka 542, Japan). After the chlorophyll content was measured, the same leaves were dark adapted for 30 minutes using leaf clips. Minimal fluorescence yield (F_o), maximum activity of photosystem II (PSII) (F_v/F_o) and maximum quantum yield efficiency of PSII ($F_V/F_m = F_m – F_o/F_m$) was measured using a OS1p Modulated Chlorophyll Fluorescence Meter (Opti-Science, Inc. NH 03051, USA), where F_m is maximum initial fluorescence yield, respectively. Chlorophyll fluorescence and is a good indicator of photosynthetic rate (Havaux et al., 1991; Barua et al., 2003) and organismal thermotolerance has been extensively used to determine plant tolerance to extreme temperatures (Cunningham and Read, 2006; Zhang et al., 2012; O'sullivan et al., 2017).

3.2.5. Water use efficiency

To calculate water use efficiency (WUE) initial weight of each pot + plant was recorded, and the amount of water added was noted. After 24 hours of watering, the pot + plants were weighed again, this was repeated twice a week for five weeks. Water use efficiency was calculated according to Ryan et al. (2016) using the following formula:

Water use efficiency = above ground dry weight (g)/water used (L) (4)

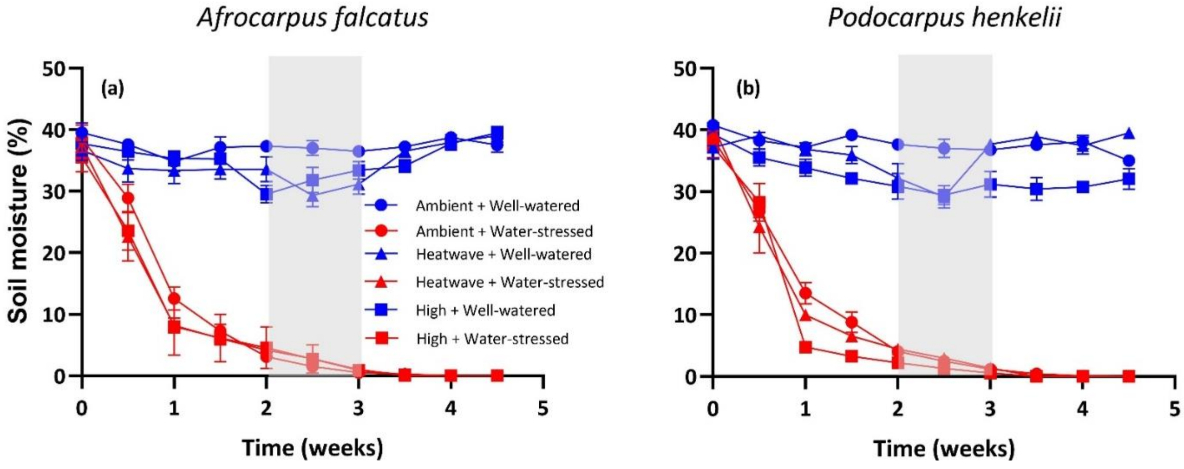
3.2.6. Statistical analyses

Statistical analyses were performed with R 4.1.0 (R Core Team, 2020). The homoscedasticity and normality were checked prior to all statistical analyses. Linear mixed-effects models were used to assess the effect of drought, heat, and the combined effects of drought and heat stress on morphology and physiology (from the *nlme* package in R; Pinheiro et al., 2020) with a Tukey HSD multiple comparisons test (from the emmeans package in R; Lenth, 2020), to determine the differences among the treatments. The linear mixed-effects models included drought treatment (well-watered and water-stressed), heat treatment (ambient, heat wave, and high), and time throughout the experiment as categorical fixed effects and seedling number was set as a random effect for each species. We analysed soil moisture with drought, heat treatment, and time as fixed effects and seedling number as a random effect for each species. In all cases, the results were considered significant if $P \leq 0.05$.

3.3. Results

3.3.1. Soil moisture

Afrocarpus falcatus and *P. henkelii* seedlings within the water-stressed treatment experienced a gradual decrease in soil moisture during the experiment ($F_{9,269} = 120.6$, $P < 0.0001$; Figure 1A-B, Table 1). Soil moisture was lower in the heatwave (22.03 ± 1.06%) and high heat treatments (20.89 ± 1.05%) compared to the ambient heat treatment (23.49 ± 1.09%).



Afrocarpus falcatus

Podocarpus henkelii

Figure 1. Soil moisture for well-watered (blue) and water-stressed (red) (a) *Afrocarpus falcatus* and (b) *Podocarpus henkelii* seedlings exposed to ambient, heatwave and high temperature stresses over a 4-week period. Values are mean ± SE (n = 36). The grey area represents the 7-day period during which a 42˚C heatwave was applied to the heatwave treatment plants.

3.3.2. Growth

Stem diameter of *A. falcatus* and *P. henkelii* seedlings was reduced by the drought treatment (Figure 2A-B). Heat stress significantly reduced stem diameter in *A. falcatus* and *P. henkelii*. In contrast, the effects of heat did not influence stem diameter in either podocarp species over time (Table 1). The combination of heat and drought stress reduced the stem diameter of *A. falcatus,* but not *P. henkelii* (Figure 2A-B). Stem volume of *A. falcatus* and *P. henkelii* seedlings were substantially reduced by drought stress during the experiment (Figure 2C-D; Table 1). The effects of heat stress on stem growth were more severe for *P. henkelii* than for *A. falcatus* (Table 1). The combination of heat and drought stress reduced stem volume in *A. falcatus* seedlings but did not influence stem volume in *P. henkelii* seedlings.

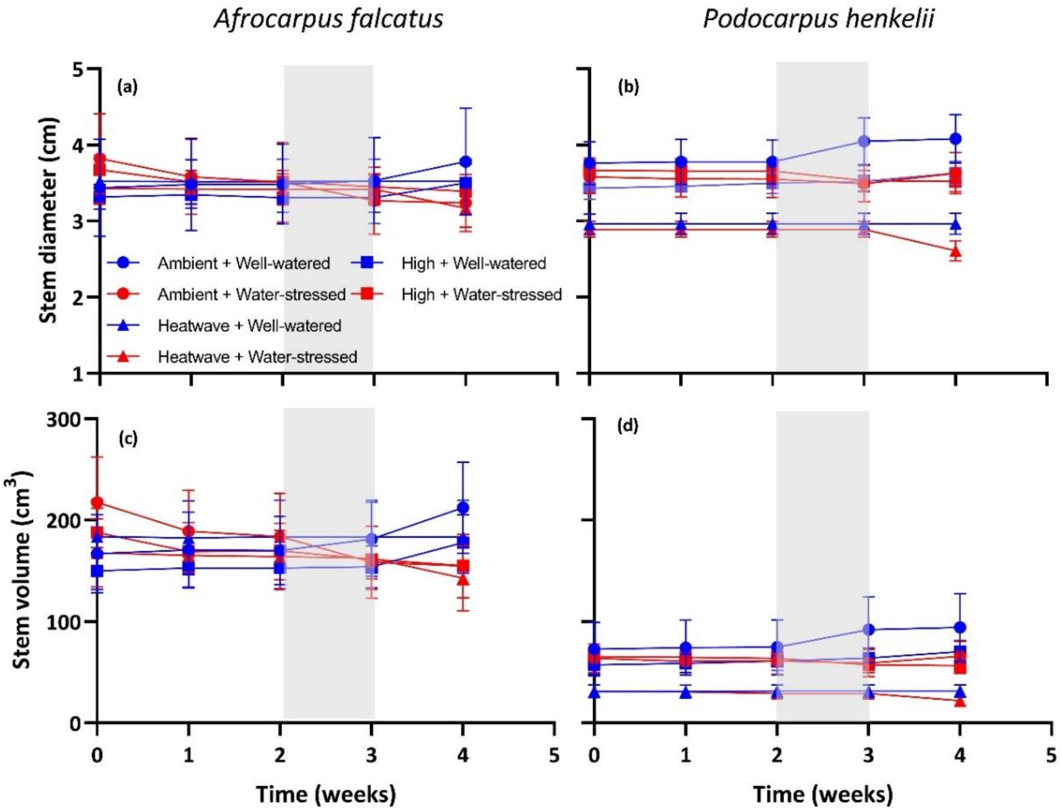


Figure 2. Stem diameter (a – b) and stem volume (c – d) for well-watered (blue) and water-stressed (red) (a, c) *Afrocarpus falcatus* and (b, d) *Podocarpus henkelii* seedlings exposed to ambient, heatwave and high temperature stresses over a 4-week period. Values are mean + SE (n = 36). The grey area represents the 7-day period during which a 42˚C heatwave was applied to the heatwave treatment plants.

The drought treatment did not influence new leaf production in *A. falcatus* (well-watered = 2.25 + 1.07%, water-stressed = 1.56 + 0.63%) nor *P. henkelii* (well-watered = 1.89 + 0.79%, water-stressed = 1.06 + 0.42%; Figure 3A-B, Table 1). The heat treatment did not have a significant effect on new leaf production in *P. henkelii*, however, plants grown at ambient temperature produced more leaves (3.75 + 1.08%), than plants under high temperatures (1.97 + 1.47%) or heatwave (0 + 0%), which produced no leaves. Water-stressed plants shed more leaves than well-watered plants for both podocarps (Figure 3C-D, Table 1). *Afrocarpus falcatus* lost more leaves when exposed to the heatwave (9.30 + 2.65%), than under ambient (4.47 + 1.68%) and high temperatures (3.87 + 1.40%). Leaf shedding and senescence significantly increased as the experiment proceeded (Figure 3C-F, Table 1). Both heat and drought stress increased leaf senescence in *P. henkelii* seedlings (Figure 3F). Water-stressed *A. falcatus* seedlings had more senescent leaves than well-watered seedlings (Figure 3E), however it was not influenced by heat stress.

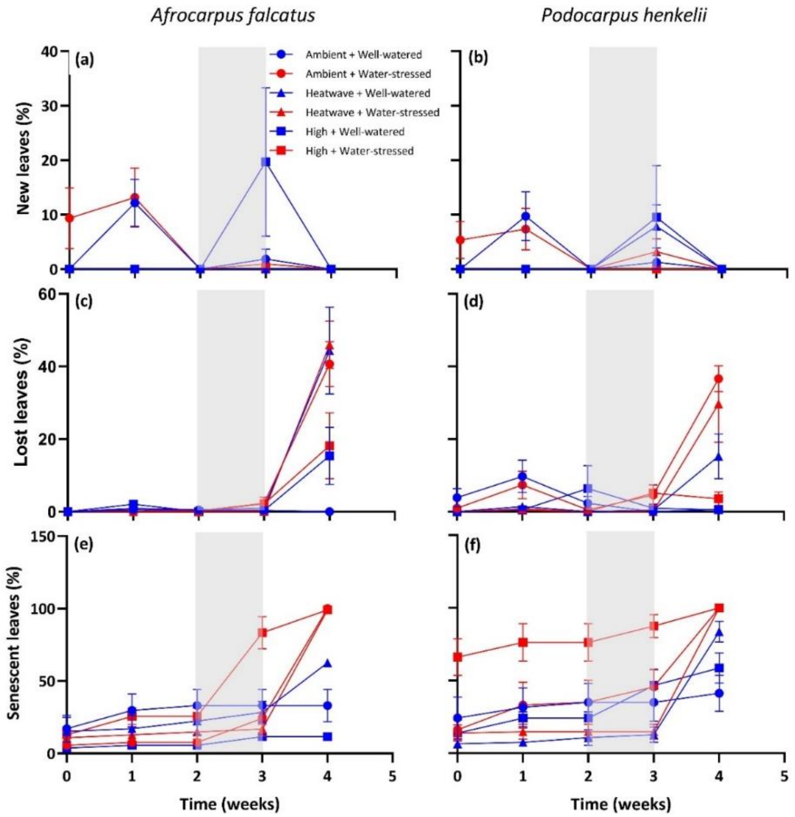


Figure 3. Percentage of new (a – b), lost (c – d) and senescent (e – f) *Afrocarpus falcatus* (a, c, e) and *Podocarpus henkelii* (b, d, f) leaves for well-watered (blue) and water-stressed (red) seedlings exposed to ambient, heatwave and high temperature stresses over a 4-week period. Values are mean + SE (n = 36). The grey area represents the 7-day period during which a 42˚C heatwave was applied to the heatwave treatment plants.

Table 1. Summary of linear mixed effect model analysis of drought and heat stress over time on measured parameters of *Afrocarpus falcatus* and *Podocarpus henkelii* seedlings. Significant values are shown in bold ($P < 0.05$). Treatments: D – Drought (water-stress) treatment; T – heat treatment; W – Week (Time).

Variables		D	D*W	T	T*W	W	D*T	D*T*W
Afrocarpus falcatus								
Stem diameter	numDF	1	4	2	8	4	2	8
	denDF	177	177	177	177	177	177	177
	P	<0.0001	0.0202	0.0003	0.9824	0.6604	<0.0001	0.9035
Stem volume	numDF	1	4	2	8	4	2	8
	denDF	177	177	177	177	177	177	177
	P	<0.0001	0.0081	<0.0001	0.9922	0.7551	<0.0001	0.8823
New leaves	numDF	1	4	2	8	4	2	8
	denDF	119	119	119	119	119	119	119
	P	0.5146	0.0606	0.0257	0.0006	0.0315	0.1039	0.1494
Lost leaves	numDF	1	4	2	8	4	2	8
	denDF	119	119	119	119	119	119	119
	P	0.0270	0.0003	0.0029	<0.0001	<0.0001	0.0218	0.0004
Senescent leaves	numDF	1	4	2	8	4	2	8
	denDF	119	119	119	119	119	119	119
	P	<0.0001	<0.0001	0.6994	<0.0001	<0.0001	0.0006	<0.0001
F_o	numDF	1	4	2	8	4	2	8
	denDF	475	475	475	475	475	475	475
	P	0.0034	0.0890	0.0632	0.0002	0.0002	0.2996	0.0279
F_v/F_o	numDF	1	4	2	8	4	2	8
	denDF	475	475	475	475	475	475	475
	P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
F_v/F_m	numDF	1	4	2	8	4	2	8
	denDF	475	475	475	475	475	475	475
	P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Chlorophyll value (SPAD)	numDF	1	4	2	8	4	2	8
	denDF	475	475	475	475	475	475	475
	P	<0.0001	<0.0001	0.0007	0.4502	0.0081	0.0074	0.0720
Soil moisture	numDF	1	9	2	18	9	2	18
	denDF	269	269	269	269	269	269	269
	P	<0.0001	<0.0001	0.0014	0.4196	<0.0001	0.3537	0.0187
Podocarpus henkelii								
Stem diameter	numDF	1	4	2	8	4	2	8
	denDF	177	177	177	177	177	177	177
	P	<0.0001	0.0091	<0.0001	0.3566	0.9921	0.0997	0.9508
Stem volume (D^2H)	numDF	1	4	2	8	4	2	8
	denDF	177	177	177	177	177	177	177
	P	0.0014	0.0202	<0.0001	0.6474	0.8837	0.0574	0.9687
New leaves	numDF	1	4	2	8	4	2	8
	denDF	119	119	119	119	119	119	119
	P	0.3469	0.1016	0.3890	0.0039	0.0126	0.6559	0.8149
Lost leaves	numDF	1	4	2	8	4	2	8
	denDF	119	119	119	119	119	119	119
	P	0.0041	<0.0001	0.0013	<0.0001	<0.0001	0.0479	0.0010
Senescent leaves	numDF	1	4	2	8	4	2	8
	denDF	119	119	119	119	119	119	119
	P	<0.0001	0.0031	0.0002	<0.0001	<0.0001	0.0727	0.0001
Fo	numDF	1	4	2	8	4	2	8
	denDF	470	470	470	470	470	470	470

	P	0.8269	0.0079	0.0123	0.0001	<0.0001	0.6974	0.0155
F_vF_O	numDF	1	4	2	8	4	2	8
	denDF	470	470	470	470	470	470	470
	P	0.7306	0.4641	0.1105	0.2227	0.2731	0.2137	0.3953
F_v/F_m	numDF	1	4	2	8	4	2	8
	denDF	470	470	470	470	470	470	470
	P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0931	<0.0001
Chlorophyll (SPAD)	numDF	1	4	2	8	4	2	8
	denDF	470	470	470	470	470	470	470
	P	<0.0001	0.0059	<0.0001	<0.0001	<0.0001	0.0018	0.2887
Soil moisture	numDF	1	9	2	18	9	2	18
	denDF	269	269	269	269	269	269	269
	P	<0.0001	<0.0001	<0.0001	0.0026	<0.0001	0.0257	<0.0001

numDF and denDF are the numerator and denominator degrees of freedom for the F-test.

3.3.3. Photosynthetic characteristics

Minimal fluorescence yield (F_o) was significantly reduced in water-stressed *A. falcatus* seedlings in comparison to well-watered seedlings (Figure 4A, Table 1). Interestingly, the heat treatment did not have an effect on F_o in *A. falcatus* seedlings. The drought treatment did not influence F_o of *P. henkelii* seedlings (Figure 4B, Table 1). Minimal fluorescence yield was higher in heatwave-exposed *P. henkelii* seedlings (187.22 ± 6.22) compared to the high (175.57 ± 7.79) and ambient temperature (151.20 ± 12.28) treatments. However, the combination of heat + drought stress did not influence F_o in either podocarp species. Maximum activity of PSII of *A. falcatus* seedlings was significant reduced by drought and heat treatments as well as the combination of heat and drought stress, however this was quite the contrary for *P. henkelii* seedlings (Figure 4C-D, Table 1). In *A. falcatus*, F_V/F_m was reduced in heat stress (0.40 ± 0.22) seedlings in comparison to ambient (0.58 ± 0.22) and heatwave (0.52 ± 0.24) seedlings (Figure 4E, Table 1). Maximum quantum yield of PSII was significantly reduced in heat stress (0.31 ± 0.22) *P. henkelii* seedlings in comparison to ambient (0.51 ± 0.22) and heatwave (0.49 ± 0.23) treatment plants (Figure 4F, Table 1). Water-stressed plants (*A. falcatus*: 0.43 ± 0.02; *P. henkelii*: 0.38 ± 0.02) had significant lower F_V/F_m in comparison to well-watered (*A. falcatus*: 0.67 ± 0.01; *P. henkelii*: 0.49 ± 0.01) seedlings in both podocarp species. The combined effects of heat and drought significantly reduced F_v/F_m in *A. falcatus*, however the interaction of heat and drought stress on F_v/F_m in *P. henkelii* seedlings was not significant.

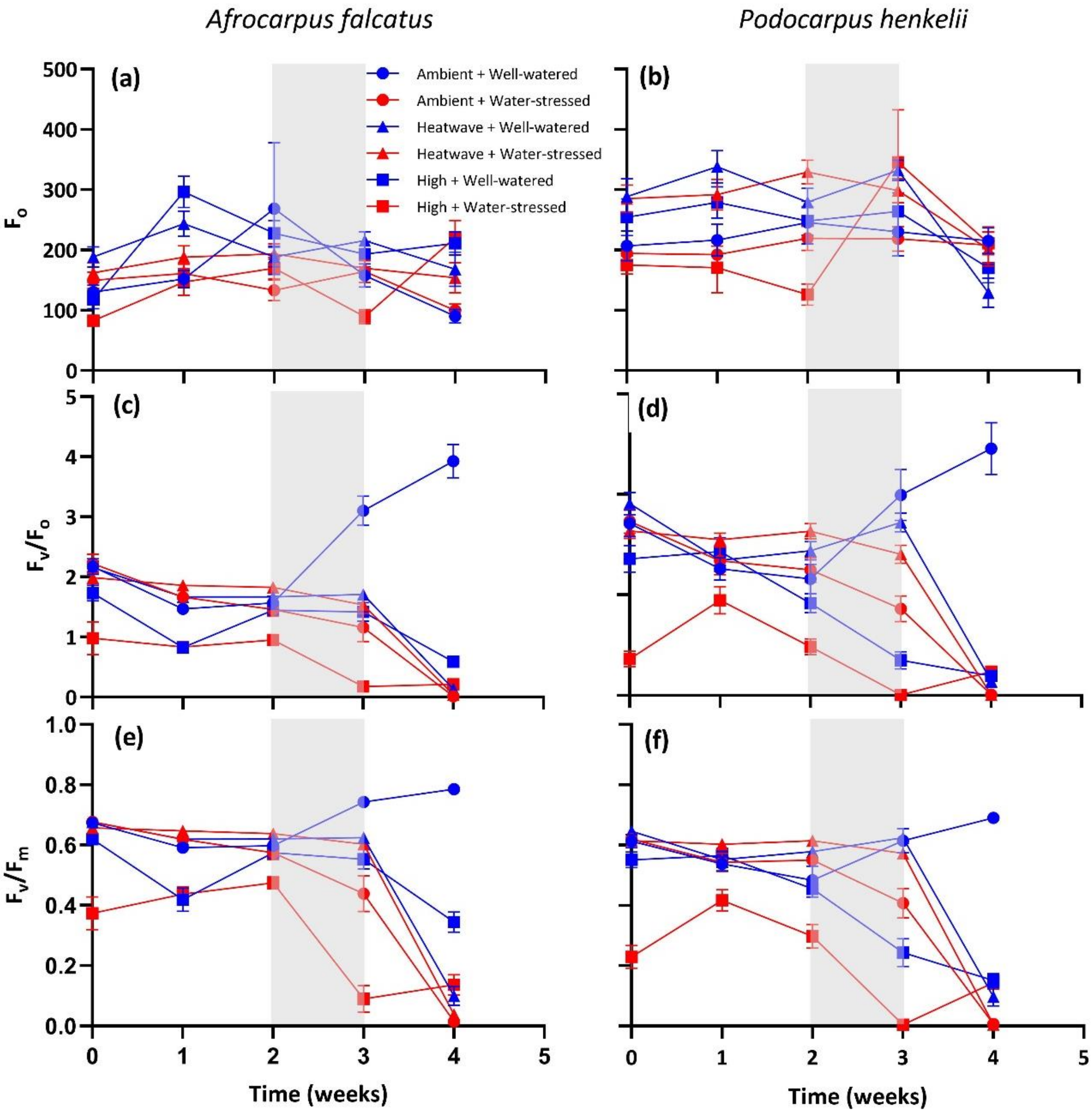


Figure 4. Minimal fluorescence yield (F_o; a - b), potential activity of photosystem II (F_v/F_o; c - d) and maximum quantum yield efficiency of photosystem II (Fv/Fm; e – f) well-watered (blue) and water-stressed (red) (a, c, e) *Afrocarpus falcatus* and (b, d, f) *Podocarpus henkelii* seedlings exposed to ambient temperatures, heatwave, and high temperature stress over a 4-week period. Values are mean + SE (n = 36). The grey area represents the 7-day period during which a 42˚C heatwave was applied to the heatwave treatment plants.

Leaf chlorophyll content (SPAD) was higher in seedlings exposed to the heatwave (*A. falcatus*: 34.65 + 9.90; *P. henkelii*: 33.62 + 0.81), then seedlings grown at ambient (*A. falcatus*: 29.86 + 0.75; *P. henkelii*: 32.72 + 0.83) or elevated temperature (*A. falcatus*: 27.25 + 12.51; *P. henkelii*: 22.24 + 0.95; Figure 5A-B, Table 1). Chlorophyll content in water-stressed plants was 22.65% lower than in well-watered plants for *A. falcatus* and 16.99% lower for *P. henkelii*. Heat and drought stress combined had a significant effect on the chlorophyll content of each species, where ambient + well-watered *A. falcatus* seedlings had greater chlorophyll content than the other treatments. In *P. henkelii*, heat stress and drought (14.82 ± 1.10) seedlings had the lowest chlorophyll content, compared with well-watered heatwave (33.64 ± 1.01), water-stressed heatwave (33.59 ± 1.10) and well-watered ambient (33.58 ± 1.13) seedlings.

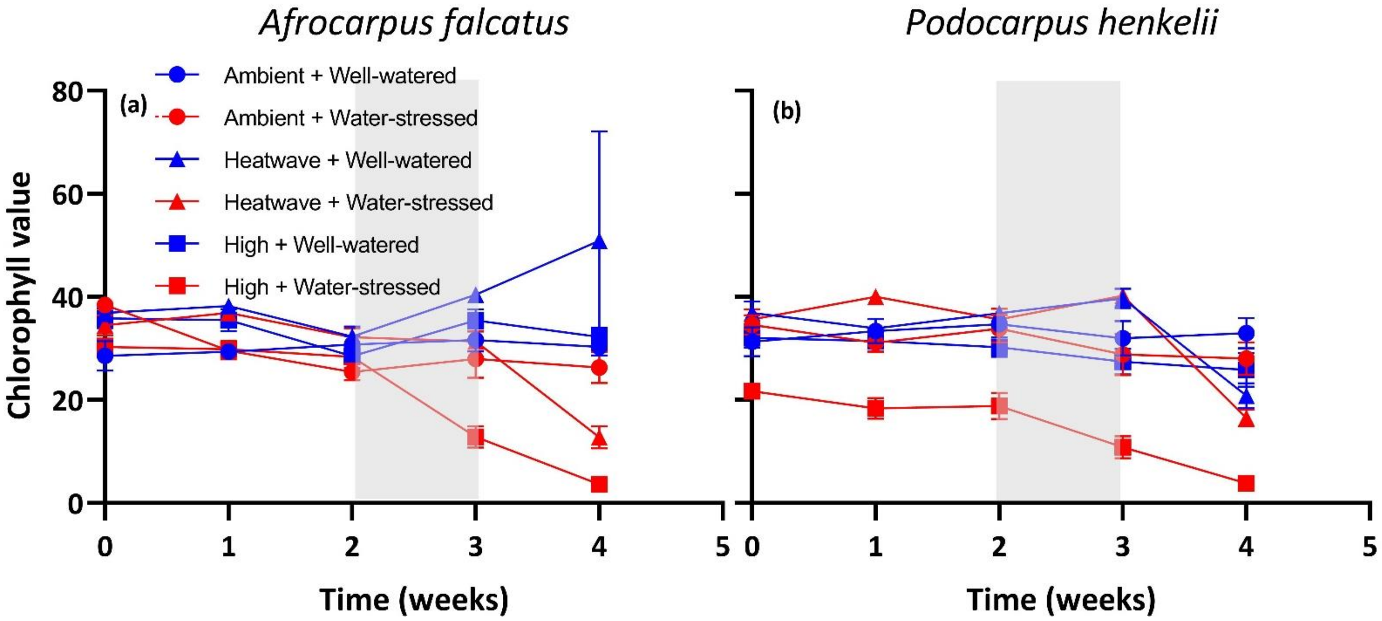


Figure 5. Chlorophyll value (using the SPAD meter) for well-watered and water-stressed *Afrocarpus falcatus* (a) and *Podocarpus henkelii* (b) seedlings exposed to ambient, heatwave and high temperature stresses over a 4-week period. Values are mean ± SE (n = 36). The grey area represents the 7-day period during which a 42˚C heatwave was applied to the heatwave treatment plants.

Neither heat nor drought stress had a significant effect on RWC of *A. falcatus* and *P. henkelii* (Table 2-3). However, the combination of heat + water-stressed resulted in a reduction in RWC of *A. falcatus* (numDF = 2, denDF = 29, $P < 0.0001$) and *P. henkelii* (numDF = 2, denDF = 29, $P = 0.0005$). *Afrocarpus falcatus* seedlings that were water-stressed (7.78 ± 0.58 g.L^{-1}) were more water use efficient than well-watered (1.02 ± 0.12 g.L^{1}) seedlings. Both the drought (numDF = 1, denDF = 1, $P = 0.0659$) and heat stress (numDF = 2, denDF = 1, $P = 0.3120$) treatments did not have a significant effect on the WUE of *P. henkelii* seedlings.

Table 2. Dry biomass allocation for well-watered and water-stressed *Afrocarpus falcatus* and *Podocarpus henkelii* seedlings. Values are mean ± SE (n = 36). Different lowercase letters indicate significant differences between treatments (Tukey HSD multiple comparisons test).

Afrocarpus falcatus

	Ambient		Heatwave		High	
Variables	Well-watered	Water-stressed	Well-watered	Water-stressed	Well-watered	Water-stressed
Leaf dry mass (g)	1.716 ± 0.324^{a}	0.788 ± 0.201^{a}	1.242 ± 0.139^{a}	1.122 ± 0.318^{a}	1.527 ± 0.223^{a}	0.999 ± 0.096^{a}
Stem dry mass (g)	0.715 ± 0.215^{a}	0.589 ± 0.077^{a}	0.823 ± 0.150^{a}	0.639 ± 0.073^{a}	0.627 ± 0.097^{a}	0.517 ± 0.025^{a}
Root dry mass (g)	0.861 ± 0.207^{a}	0.621 ± 0.169^{a}	2.328 ± 0.571^{a}	1.827 ± 0.290^{a}	1.351 ± 0.191^{a}	1.193 ± 0.085^{a}
Total dry mass (g)	2.752 ± 0.683^{ab}	1.997 ± 0.432^{a}	4.393 ± 1.028^{b}	3.588 ± 0.426^{ab}	3.505 ± 0.495^{ab}	2.709 ± 0.495^{ab}
Root shoot ratio (g.g^{-1})	1.366 ± 0.592^{a}	1.005 ± 0.136^{a}	2.722 ± 0.178^{a}	2.929 ± 0.486^{a}	2.207 ± 0.138^{a}	2.336 ± 0.212^{a}
Leaf mass fraction (%)	41.452 ± 1.339^{ab}	37.715 ± 2.902^{ab}	31.769 ± 2.020^{ab}	27.873 ± 1.462^{a}	43.403 ± 1.618^{b}	36.598 ± 1.681^{ab}
Stem mass fraction (%)	26.165 ± 2.798^{a}	31.790 ± 2.626^{a}	19.660 ± 1.228^{a}	18.216 ± 1.496^{a}	17.710 ± 0.342^{a}	19.404 ± 1.325^{a}
Root mass fraction (%)	32.383 ± 3.165^{ab}	30.495 ± 1.927^{a}	52.468 ± 0.660^{c}	50.015 ± 2.905^{c}	38.887 ± 1.847^{ab}	43.997 ± 1.465^{bc}
Relative water content (%)	83.339 ± 2.561^{a}	22.900 ± 6.967^{b}	24.953 ± 1.616^{bc}	40.763 ± 1.426^{ac}	22.768 ± 1.673^{bc}	55.040 ± 5.176^{abc}
Water use efficiency (g.L^{-1})	6.882 ± 1.366^{ab}	0.946 ± 0.261^{c}	1.033 ± 0.229^{c}	8.804 ± 0.976^{b}	1.077 ± 0.160^{c}	7.580 ± 0.494^{ab}

Podocarpus henkelii

	Ambient		Heatwave		High	
Variables	Well-watered	Water-stressed	Well-watered	Water-stressed	Well-watered	Water-stressed
Leaf dry mass (g)	0.436 ± 0.077^{a}	0.256 ± 0.159^{a}	0.789 ± 0.131^{a}	0.607 ± 0.163^{a}	0.631 ± 0.090^{a}	0.755 ± 0.123^{a}
Stem dry mass (g)	0.381 ± 0.074^{a}	0.338 ± 0.072^{a}	0.397 ± 0.078^{a}	0.291 ± 0.038^{a}	0.363 ± 0.049^{a}	0.436 ± 0.026^{a}
Root dry mass (g)	0.476 ± 0.134^{a}	0.450 ± 0.159^{a}	0.705 ± 0.132^{a}	0.596 ± 0.079^{a}	1.116 ± 0.222^{a}	0.141 ± 0.324^{a}
Total dry mass (g)	1.293 ± 0.357^{a}	1.044 ± 0.291^{ab}	1.891 ± 0.350^{ab}	1.494 ± 0.216^{ab}	22.110 ± 0.366^{b}	2.603 ± 0.334^{b}
Root shoot ratio (g.g^{-1})	1.196 ± 0.211^{a}	1.192 ± 0.253^{a}	1.848 ± 0.306^{a}	2.158 ± 0.368^{a}	3.271 ± 0.718^{a}	3.402 ± 0.958^{a}
Leaf mass fraction (%)	30.286 ± 5.358^{a}	26.353 ± 6.644^{a}	38.710 ± 4.545^{a}	41.608 ± 1.926^{a}	30.691 ± 3.778^{a}	29.864 ± 1.755^{a}
Stem mass fraction (%)	32.544 ± 2.463^{a}	34.541 ± 3.096^{a}	21.479 ± 19.989^{b}	19.989 ± 1.543^{b}	18.617 ± 2.950^{b}	18.423 ± 2.684^{b}
Root mass fraction (%)	37.169 ± 5.573^{a}	39.106 ± 6.248^{a}	36.913 ± 3.436^{a}	41.301 ± 4.829^{a}	51.519 ± 4.261^{b}	50.887 ± 6.126^{b}
Relative water content (%)	73.367 ± 5.923^{a}	32.825 ± 17.960^{b}	18.784 ± 0.445^{b}	40.797 ± 1.815^{c}	19.635 ± 1.192^{b}	48.074 ± 2.203^{c}
Water use efficiency (g.L^{-1})	0.408 ± 0.116^{a}	2.970 ± 0.695^{b}	0.593 ± 0.119^{a}	4.488 ± 0.839^{c}	0.497 ± 0.082^{a}	5.952 ± 0.558^{c}

Table 3. Summary of linear mixed effects model analysis of the effects of temperature and drought treatment on measured parameters of *Afrocarpus falcatus* and *Podocarpus henkelii* seedlings at the end of the experiment. Values are mean + SE (n = 36). Significant values are shown in bold ($P < 0.05$). Treatments: D – Drought treatment; T – Heat treatment.

Afrocarpus falcatus					*Podocarpus henkelii*				
Variables		D	T	D*T	Variables		D	T	D*T
Leaf dry mass	numDF	1	2	2	Leaf dry mass	numDF	1	2	2
	denDF	1	1	29		denDF	1	1	29
	P	0.3243	0.6169	0.6892		*P*	0.5871	0.3027	0.4022
Stem dry mass	numDF	1	2	2	Stem dry mass	numDF	1	2	2
	denDF	1	1	29		denDF	1	1	29
	P	0.4449	0.7179	0.8159		*P*	0.6923	0.7200	0.3263
Root dry mass	numDF	1	2	2	Root dry mass	numDF	1	2	2
	denDF	1	1	29		denDF	1	1	29
	P	0.4317	0.2147	0.8339		*P*	0.7893	0.2235	0.5433
Total biomass	numDF	1	2	2	Total biomass	numDF	1	2	2
	denDF	1	1	29		denDF	1	1	29
	P	0.3567	0.3482	0.9991		*P*	0.8787	0.2619	0.3500
Root shoot ratio	numDF	1	2	2	Root shoot ratio	numDF	1	2	2
	denDF	1	1	29		denDF	1	1	29
	P	0.9759	0.1545	0.5064		*P*	0.7978	0.2435	0.9591
Leaf mass fraction	numDF	1	2	2	Leaf mass fraction	numDF	1	2	2
	denDF	1	1	29		denDF	1	1	29
	P	0.3665	0.1641	0.0254		*P*	0.4610	0.3316	0.9339
Stem mass fraction	numDF	1	2	2	Stem mass fraction	numDF	1	2	2
	denDF	1	1	29		denDF	1	1	29
	P	0.4404	0.1529	0.1750		*P*	0.9675	0.1522	0.7813
Root mass fraction	numDF	1	2	2	Root mass fraction	numDF	1	2	2
	denDF	1	1	29		denDF	1	1	29
	P	0.9082	0.1088	0.1691		*P*	0.7284	0.3347	0.8905
Relative water content	numDF	1	2	2	Relative water content	numDF	1	2	2
	denDF	1	1	29		denDF	1	1	29
	P	0.4157	0.1824	<0.0001		*P*	0.4800	0.2666	0.0005
Water use efficiency	numDF	1	2	2	Water use efficiency	numDF	1	2	2
	denDF	1	1	29		denDF	1	1	29
	P	0.0314	0.6174	0.4664		*P*	0.0659	0.3120	0.0269

numDF and denDF are the numerator and denominator degrees of freedom for the F-test.

3.3.4. Biomass allocation

The interaction of heat and drought stress reduced RWC and WUE in *P. henkelii* seedlings, however these traits were not influenced by heat and drought stress individually (Table 2 & 3). Leaf dry mass, stem dry mass, root dry mass, total biomass, root:shoot, LMF, SMF and RMF were not influenced by the heat, drought and the combination of heat and drought stress in *P. henkelii* seedlings. In *A. falcatus* seedlings, LMF and RWC were significantly reduced by the combination of heat and drought stress, however the individual stresses did not influence these traits (Table 2 & 3). Water-stressed *A. falcatus* seedlings were more WUE then well-watered seedlings, however heat stress and the combination of heat and drought stress did influence WUE. The remaining traits were not influenced by heat, drought and the interaction of heat and drought stress in *A. falcatus* seedlings.

3.4. Discussion

To understand what constrains the distribution of extant southern conifer species, it is important to be able to describe known distributions in terms of the most likely physiological constraints. We analysed the physiological, morphological and growth responses of *A. falcatus* and *P. henkelii* seedlings to drought and thermal stress and how this affects their distribution. *Afrocarpus falcatus* and *P. henkelii* showed differential responses to the treatments. We found that temperature and drought stress and the combination of heat and drought stress had significant negative effects on the physiology and morphology of *A. falcatus*. However, the physiology and morphology of *P. henkelii* seedlings was significantly affected by only the heat and drought treatments individually, and not the combination of the two.

Our study showed that both podocarp species used drought tolerance and avoidance strategies to persist under drought conditions. Water-stressed *A. falcatus* seedlings became more water use efficient than seedlings that were well-watered, however, the drought treatment did not affect WUE in *P. henkelii* seedlings. This is an indication that *A. falcatus* can persist in water limited environments such as the coastal forests by increasing WUE to conserve water, however, even though *P. henkelii* is confined or restricted to high rainfall areas it uses other mechanisms to mitigate the negative effects of drought. Both species had significantly reduced chlorophyll content in water-stressed seedlings due to the breakdown of different enzymes involved in the synthesis of chlorophyll and the heightened production of enzymes that degrade chlorophyll (Hussain et al., 2018). These water-stressed leaves quickly senesced and were shed. Leaf abscission (loss) is a drought avoidance mechanism that can reduce the surface area of transpiration thus reducing water loss. However, substantial leaf loss decreases the ability of plants to assimilate carbon (Blackman et al., 2019; Ruehr et al., 2016). This is caused by the quick closure of stomata during a drought event to maintain a consistent minimum leaf water potential and prevent disruptions of water balance through high water losses (Brodribb et al., 2014; Tombesi et al., 2015; Blackman et al., 2019). This stomatal response strategy can reduce carbon dioxide uptake which leads to carbon starvation. Other conifers such as spruce can maintain their photosynthetic rate under low water conditions through stem shrinkage (Rötzer et al., 2017). Although leaf shedding certainly reduces evaporative surface area, the growth patterns of *A. falcatus* and *P. henkelii* showed that leaf loss may not stabilise plant water potential during drier seasons and under water stressed environments because leaf shedding did not protect stems from hydraulic stress. Similarly, numerous studies have shown that several tree species in dry forests shed all their leaves under drier conditions, however they experienced stem diameter contraction which is associated with water loss (Reich and Borchert, 1984; Wolfe et al., 2016), as shown in this study. This is interesting since *A. falcatus* occurs in both winter and summer rainfall areas and is the only temperate podocarp species that persists in the drier regions of southern African forests, while *P. henkelii* is restricted to warm-temperate forests that experience summer rainfall (Adie and Lawes, 2011). This suggests that *P. henkelii* is not restricted to warm-temperate forests by its morphological and physiological thresholds to drought and/or temperature but rather by other factors such as competition and/or its reproductive biology. Considering the demonstrated potential of podocarps for long-distance dispersal, *P. henkelii* is gravity dispersed because the seed is too large to be dispersed by birds and the epimatium is reduced in comparison to *A. falcatus* (Dodd et al., 1989; Geldenhuys, 1993). Alternatively, it produces fleshy fruit which may suggest that *P. henkelii* seeds may have been previously dispersed by an animal which may no longer be present in its current distribution such as elephants. Both *A. falcatus* and *P. henkelii* produce recalcitrant seeds, *P. henkelii* seeds are highly sensitive to desiccation while those of *A. falcatus* are only moderately sensitive to desiccation (Woodenberg et al., 2018), which may limit the establishment of *P. henkelii* in drier environments. *Afrocarpus falcatus* may be widely distributed in comparison to *P. henkelii* because its seeds are dispersed by birds and bats enabling it to be dispersed over greater distances.

Our results also indicate that plant performance is dependent on the duration of the heat stress, however there was variation in the response of both podocarp species to elevated temperatures. Under elevated temperatures *P. henkelii* showed signs of chlorosis (senescence) where leaf chlorophyll content was significantly reduced. Reduced chlorophyll content can be due to the thylakoids in the heat stressed leaves being damaged, thus resulting in reduced chlorophyll levels (Guo et al., 2006, Ristic et al., 2007; Hüeve et al., 2011; Teskey et al., 2015) and can be an indication of nitrogen deficiency (Boussadia et al., 2015; Huang et al., 2020). The decreased chlorophyll build-up in plants under heat stress could also be due to dieback or decreased biosynthesis of chlorophyll in plants, as well as the combined effects of both drought and heat stress. In addition, we also found that both *A. falcatus* and *P. henkelii* accelerated leaf loss in heat stressed seedlings which is an effective heat avoidance strategy that reduces water loss through transpiration.

The negative impacts of drought on plant ecophysiology are often exacerbated by heat stress during times of drought. Drought stress in the present study appeared to have substantial negative impacts on plant growth and the ecophysiology of *A. falcatus,* while *P. henkelii* ecophysiology was significantly affected by heat stress. Interestingly, *Podocarpus henkelii* seedlings were more tolerant to a combination of heat and drought stress in comparison to *A. falcatus. Podocarpus henkelii* was able to maintain stem diameter, and the functioning of *PSII* while continuously turning over new leaves under heat and drought stress. It is predicted by boundary layer theory that due to the tight coupling between leaves and their environment, plants with smaller leaves such as *A. falcatus* will dissipate heat via convective cooling when stomata are closed thus making them less susceptible to heat stress. Convective cooling decreases leaf temperature by keeping water loss to a minimum, thus preventing a leaf from reaching lethal limits. If having smaller leaves is a survival strategy to maintain leaf temperature in water stressed environments, then this explains why *A. falcatus* can persist in hot and arid regions in comparison to other South African podocarp species which have larger leaves. Due to their wide size, broad leaves are more susceptible to microclimatic changes resulting from changes in absorbed radiation and transpiration per unit leaf area, so they may reach higher leaf temperatures than the air temperature (Hardwick et al., 2015; Leigh et al., 2017; Wang et al., 2019), thus making them more susceptible to heat stress as seen in *P. henkelii*. As a result of less effective convection cooling, broad-leaved plants such as *P. henkelii* are restricted to areas with high water availability where evaporative cooling is more likely. However, it is important to add that broad-leaved species do occur in hot environments but use a combination of leaf traits and strategies to dissipate heat such as leaf rolling and changing the angle of the leaf (Brochert et al. 2005). The synergistic effects of drought + heat stress on *A. falcatus* and *P. henkelii* seedlings resulted in reduced chlorophyll content, WUE and accelerated leaf shedding. Heat and drought stress combined have more of a severe effect than the individual stresses and often act together in the field. The interaction of heat and drought stress triggers protective responses in both species resulting in the reduction of chlorophyll content and F_v/F_m resulting in leaf abscission is an indication of the mobilisation of nutrients because of chlorophyll degradation during leaf senescence (reviewed by Feller and Fischer, 1994; Hörtensteiner and Feller, 2002; Munné-Bosch and Alegre, 2004; Havé et al., 2017). Tamary et al. (2019) observed that leaf senescence in *Arabidopsis thaliana* is preceded by chlorophyll breakdown which happens faster than the decrease in the efficiency of PSII. The reduction in fluorescence values (F_v/F_o and F_v/F_m) in *A. falcatus* seedlings in comparison to *P. henkelii* under drought and heat stress may be an indication that the functioning of PSII in *A. falcatus* is compromised and in turn may have reduced the photosynthetic capacity of the leaves. This suggest that *P. henkelii* has higher thresholds to photoinhibition under heat and drought stress than *A. falcatus*. Photoinhibition of PSII is a mechanism which protects PSII resulting in the reduction of photosynthetic processes (Valladares and Pearcy, 1997; Guidi et al., 2019). However, photoinhibition of PSII may be reversed under low light intensity once plants are hydrated and temperatures are no longer excessively high (Valladares and Pearcy, 1997; Zhang and Scheller, 2004).

In conclusion, both heat and drought stress trigger similar physiological, morphological, and biochemical responses in these plant species, and the combination of both stresses results in more severe damage than the individual stresses (Lamaoui et al., 2018). *Podocarpus henkelii* was more tolerant to heat, drought, and the combination of heat and drought stress than *A. falcatus.* This is contrary to expectation as *P. henkelii* is restricted to moist forests while *A. falcatus* is the most drought tolerant of the four podocarps that occur in South Africa as it is the only podocarp species that occurs in drier regions of South Africa (Lawes and Adie, 2011). In addition, *P. henkelii* is a broadleaf species and *A. falcatus* is a fine-leaved species, as such *A. falcatus* should be able to reduce water loss and dissipate heat better than *P. henkelii*. This also suggests that the distribution of *P. henkelii* is not limited by its ecophysiological thresholds but rather because of other mechanisms such as seed dispersal and germination as well as intra-specific competition due to the seeds being gravity dispersed.

Author Statement

Thando Twala: Conceptualization, Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Validation; Visualization; Writing – original draft

Jolene Fisher: Conceptualization; Funding acquisition; Resources; Supervision; Validation; Writing – original draft

Ed Witkowski: Formal analysis; Validation; Writing – original draft

No conflict Statement

All authors have read and approved this manuscript titled “The effects of heat and drought stress on the ecophysiological responses and growth of *Afrocarpus falcatus* and *Podocarpus henkelii* seedlings” and report no conflicts of interest.

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Chapter 4: Population structure and gene flow of three Podocarpaceae species in South African forests.

Abstract

Afrocarpus falcatus (Thunb.) R. Br. Ex Mirb, *Podocarpus latifolius* (Thunb.) R.Br. ex Mirb. and *P. henkelii* Stapf ex Dallim. & Jacks. are three of four Podocarpaceae species in South Africa (SA) that have a disjunct distribution. *Afrocarpus falcatus* and *P. latifolius* are widespread species and occur in west, central, and east Africa and extend to South Africa. However, *P. henkelii* has a South African distribution and is restricted to the KwaZulu-Natal and Eastern Cape provinces. In this study, the genetic diversity and differentiation between podocarp populations in SA was examined to explain podocarp dispersal patterns and connectivity. We collected fresh leaves from four *A. falcatus*, sixteen *P. latifolius* and three *P. henkelii* populations across four South African provinces. Six microsatellites developed for *A. falcatus* and ten microsatellites developed for *P. latifolius*, six of which were used in *P. henkelii*. Genetic structure was analysed using Bayesian and cluster analyses and genetic diversity estimated using standard diversity estimates. Genetic diversity was high in all populations of the focal species, however genetic diversity was lowest in *P. henkelii* populations due to the microsatellites being developed for *P. latifolius*. *Podocarpus latifolius* kinship deteriorated with geographic distance in the KwaZulu-Natal populations. There was no genetic structure between *A. falcatus* and *P. henkelii* populations. However, *P. latifolius* populations shared genetic structure with Mpumalanga, KwaZulu-Natal, northern Western Cape and southern Western Cape populations forming separate clusters. The overall higher genetic diversity observed in this study in comparison to other podocarp species across Africa suggest that the studied populations have been refugia allowing these species to evolve and persist through phases of forest contraction and expansion since at least the beginning of the Miocene. Naturally disjunct populations of *A. falcatus, P. latifolius* and *P. henkelii* were not genetically divergent from one another indicating that the populations were connected. However, geographically distant populations showed low gene flow indicating geographical isolation. Overall, podocarps are connected which may suggest that the current disjunct populations were once one large population. In addition, it is likely that podocarps can disperse across the savanna-grassland matrix, however, long-distance dispersal declines over large geographic distances and will affect podocarps ability to track suitable habitats under climate change.

Keywords: Connectivity, differentiation, diversity, microsatellites, podocarp, population genetic structure

4.1. Introduction

Genetic variation increases a species' ability to adapt to environmental changes and improves resilience [1]. Several factors affect genetic variation including species range, population size, life cycle, demographic history, mating system, and evolutionary processes like gene flow, mutation, and natural selection [2]. Species' habitats have been separated and fragmented in recent years because of anthropogenic activities, which affect genetic diversity within and between populations. The capacity for evolution, adaptation, and the viability of a species is reduced by habitat fragmentation [3,4]. Fragmentation of forests and the accompanying habitat disturbance and isolation could have a detrimental effect on the persistence of tree species in small forest fragments [5]. Increased anthropogenic fragmentation of forested landscapes has drawn attention to the effects of genetic connectivity of forest tree communities [6-9]. Forest fragmentation can affect gene flow within and between populations by limiting both pollen and seed dispersal [10-12]. Forest fragmentation is of concern because the lack of gene flow causes interpopulation genetic divergence through genetic drift, reduced population variability through inbreeding depression, loss of adaptive potential, and ultimately increased risk of local extinction [13,14].

African Podocarpaceae (podocarps) comprises two genera; *Podocarpus* and *Afrocarpus* which are common in montane forests that span from Ethiopia to South Africa. Podocarpaceae persist in archipelagos within a grassland or fynbos/heathland matrix in areas protected from fire. These archipelagos in highlands act as refugia under extreme paleoclimatic conditions [15] and correspond with locations of high species diversity and endemism. South Africa is home to four podocarp species: *Afrocarpus falcatus* (Thunb.) R. Br. Ex Mirb, *Podocarpus latifolius* (Thunb.) R.Br. ex Mirb., *P. henkelii* Stapf ex Dallim. & Jacks. And *P. elongatus* L'Herit. (Podocarpaceae). *Afrocarpus falcatus* occurs in the Limpopo, Mpumalanga, KwaZulu-Natal, Eastern Cape, and Western Cape provinces, where trees primarily grow in coastal and montane forests, and the distribution of *P. latifolius* is similar [15,16]. *Podocarpus henkelii* has a restricted distribution from the Mpumalanga province south into the Eastern Cape [16-19]. *Podocarpus elongatus* is restricted to the Western Cape province which experiences winter rainfall [16,20]. *Afrocarpus falcatus, P. latifolius, P. henkelii* and *P. elongatus* are long-lived dioecious conifers that produce wind-dispersed pollen and fleshy fruits [21-23]. *Afrocarpus falcatus, P. latifolius* and *P. elongatus* seeds are dispersed by birds, monkeys, and bats [22].

Forest researchers and conservation biologists alike have found that a population genetics approach is useful to evaluate the connectivity of forest fragments [6]. These data provide some guidance around mitigating potential detrimental impacts of genetic bottlenecks. By understanding the genetic structure of podocarp populations, we can better predict the ability of populations to adapt to changing environmental conditions. Here we describe potential patterns of expansion and contraction and identify extinction-recolonisation mechanisms in populations. Genetic diversity, population structure, and gene flow has been extensively studied in natural podocarp populations around the world [9,24-30]. For example, genetic patterns of *P. parlatorei* in the southern Yungas in Bolivia showed that southern populations acted as long-term refugia during climate oscillations [26]. However, there is limited research on genetic population structure of South African podocarps.

Here, we aim to use genetic data to better understand population connectivity and the species population changes in South African podocarps. We focus on three species to test whether widespread populations are genetically connected, and whether there is evidence of a bottleneck/population expansion for the spatially restricted podocarp, *P. henkelii*. Using *A. falcatus, P. latifolius* and *P. henkelii*, we test the following specific hypothesis that widespread species will have greater population differentiation and greater genetic diversity than restricted taxa because their populations are isolated. We were unable to sample *P. elongatus* leaf material, due to limited sampling abilities, therefore we could not include this species in our study.

4.2. Methods

4.2.1. Population sampling, DNA extraction and microsatellite amplification

A total of seventeen populations were sampled across the South African distribution of *Afrocarpus falcatus, P. latifolius* and *P. henkelii* (Figure 1). Leaf samples were collected from trees between one to three meters (as leaf samples were easily accessible at this height). *Afrocarpus falcatus* leaf samples were collected in two provinces; KwaZulu-Natal (Karkloof Nature Reserve [KrK] and Umgano Forest [Umg]) and Eastern Cape (Storms River [Sri]) and Western Cape (Circles in the Forest [CiF]; Table 1; Figure 1). Leaf tissue was collected across 16 populations for *P. latifolius*, these included: Mpumalanga (God's Window [GoW], Mac Mac Pools [Mac]), KwaZulu-Natal (Royal Natal Nature Reserve [RNa], Mnweni Forest [Mnw], Monks Crowl [MoC], KrK, Mbona Nature Reserve [Mbo], Lotheni Nature Reserve [Lot], Swartkop [Swk], Marutswa Forest [Mts], Umg) and Western Cape (SRi, Groenkop Forest [Grk], Grootvadersbosch Nature Reserve [Gvb], Marloth Nature Reserve [Mot] and Wilderness National Park [WNP]; Table 1; Figure 1). *Podocarpus henkelii* leaf samples were collected in KrK, Mbo, and Umg. Between eight and thirty individuals were sampled for each species in each population depending on accessibility, with five leaves collected for each tree. Leaf tissue was then placed in filter paper labelled with the species name, collection date and global position system (GPS) coordinates of the individual. The tissue was subsequently dried in the field using silica gel and stored at room temperature until the extraction of genomic DNA.

Table 1. Sampling populations' abbreviations and locations within South Africa. N.R.: Nature Reserve; N.P.: National Park. **Podocarpus latifolius;* º*Afrocarpus falcatus;* ∞*Podocarpus henkelii;* †all three focal species (*P. latifolius, A. falcatus* and *P. henkelii*); ®two focal species (*P. latifolius* and *A. falcatus*); øtwo focal species (*P. latifolius* and *P. henkelii*).

Abbreviation	Population	Province	N individuals
GoW	God's Window*	Mpumalanga	16
Mac	Mac Mac Pools*	Mpumalanga	13
RNa	Royal Natal N.R.*	KwaZulu-Natal	31
Mnw	Mnweni Forest*	KwaZulu-Natal	4
MoC	Monks Cowl*	KwaZulu-Natal	26
KrK	Karkloof Forest†	KwaZulu-Natal	12*, 22º, 30∞
Mbo	Mbona N.R.ø	KwaZulu-Natal	29*, 22∞
Lot	Lotheni N.R.*	KwaZulu-Natal	30
Swk	Swartkop Forest*	KwaZulu-Natal	21
Mts	Marutswa Forest*	KwaZulu-Natal	29
Umg	Umgano Forest†	KwaZulu-Natal	33*, 18º, 21∞
SRi	Storms River*	Eastern Cape	2*, 9º
CiF	Circle in the Forestº	Western Cape	12
Grk	Groenkop Forest*	Western Cape	20
Gvb	Grootvadersbosch N.R.*	Western Cape	23
Mot	Marloth N.R.*	Western Cape	15
WNP	Wilderness N.P.*	Western Cape	8

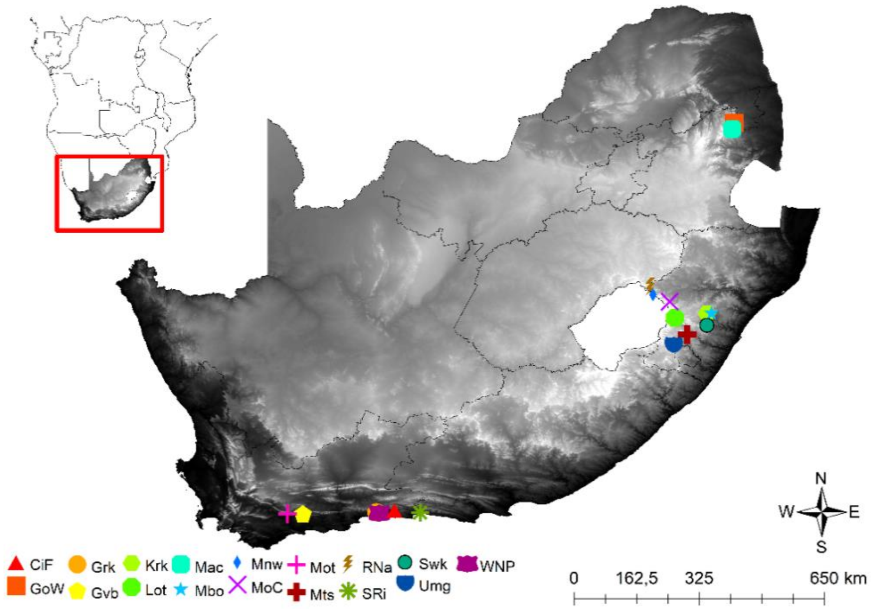

Figure 1. (a) *Afrocarpus falcatus, Podocarpus latifolius* and *P. henkelii* populations sampled for genetic analyses in South Africa (names in Table 1). Insert A and B provide a closer look into the distribution of the study populations (Appendix 1). Grey scale colour on the map indicates elevation above sea level (m).

Leaf samples were ground into powder using liquid nitrogen and a pestle and mortar. Total genomic DNA was extracted from 15 – 25 mg of dried leaf material using a modified CTAB-based protocol [31]. The protocol was modified by 1) adding 700 ul of the CTAB solution, 2) putting the isopropanol at -40 ˚C for 10 minutes before adding it to the solution, and 3) washing the pallet at least four times with 70% ethanol before leaving the pellet in the tube at room temperature to dry. Genomic DNA quality was verified and quantified after extraction using a NanoDrop 2000C spectrophotometer (Thermofisher Scientific Inc., Waltham, MA, USA).

A total of 73 *A. falcatus,* 312 *P. latifolius* and 74 *P. henkelii* samples were genotyped. Forty-seven primers were developed for *A. falcatus*, of those primers only six nSSR primers amplified and were used to genotype the *A. falcatus* samples [developed by 9]. Of the 46 nSSR primers developed for genotyping *P. latifolius*, ten amplified for *P. latifolius* and only six amplified for *P. henkelii* (developed by [9]). *Afrocarpus falcatus, P. latifolius* and *P. henkelii* PCR reactions comprised 0.15 µL (0.2 µM) reverse primer and 0.1 µL (0.2 µM) forward primer, 0.15 µL (0.2 µM) of relevant labelled primers Q1 (FAM), Q2 (NED), Q3 (VIC) and/or Q4 (PET), 7.5 µL of the Qiagen Type-it Microsatellite PCR Kit (Qiagen, Venlo, Netherlands), 3.6 µL of distilled water, and 1.5 µL of DNA extract (c. 10–50 ng/µL). The thermocycler program was as follows: 3 min initial denaturation at 94 ˚C, followed by 25 cycles of 94 ˚C for 30 s, 55 ˚C for 45 s, and 72 ˚C for 1 min, 10 cycles of 94 ˚C for 30 s, 53 ˚C for 45 s, and 72 ˚C for 1 min; and a final elongation step at 72 ˚C for 30 mins. All individuals were genotyped using an ABI3730 sequencer at the Evolutionary Biology and Ecology Unit, Université Libre de Bruxelles (Belgium) using 0.8 µL of each PCR product, 12 µL of Hi-Di formamide (Life Technologies, Carlsbad, CA, USA), and 0.3 µL of Map-Mapper 500 labelled with DY-632 (Eurogentec, Seraing, Belgium). The individual generated data were scored using the microsatellite plugin for GENEIOUS R7 7.1.3 [32].

4.2.2. Genetic Diversity, Differentiation and Structure

For each population of each species, multilocus genetic diversity parameters were estimated using POLYGENE version 1.5 [33]. The diversity parameters include allelic richness (*A_R*), number of private alleles (*A_P*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and individual inbreeding coefficient (*F*). F_{ST} was used to calculate genetic differentiation between pairs of populations, and R_{ST} was used to estimate the phylogeographic signal between populations. The R_{ST} matrix takes allelic sizes into account and is generally larger than F_{ST} if mutations have contributed to the differentiation. An analysis of molecular variance (AMOVA) was computed using the stepwise mutation model (SMM) based on 10 000 permutations in POLYGENE.

Visualisation of the similarities between the four *A. falcatus,* three *P. henkelii,* and 16 *P. latifolius* populations was analysed using a discriminant analysis of principal components (DAPC). The DAPC method transforms data using a principal component analysis before a discriminant analysis is applied, so that genetic structure can be explored without explicit evolutionary models or assumptions [34,35]. In addition, POLYGENE was used to perform Bayesian clustering. For these cluster analyses, the admixture and independent allele frequencies models were used, and genetic clusters assessed ranged from *K* = 1 to 10 with a 5000 burn-in and 500000 MCMC chains with five iterations per *K*. The lowest LnP(d) was used to select the most likely K values per Larson [36].

4.3. Results

4.3.1. Genetic diversity

Allelic richness (A_R) was higher in all *P. latifolius* populations (A_R = 14.966 to 17.697) and lower in *P. henkelii* populations (A_R = 4.804 to 5.327; Table 2). The highest average number of private alleles was observed in KrK for *A. falcatus* (A_P = 1.333), Gvb for *P. latifolius* (A_P = 1.571) and Umg for *P. henkelii* (A_P = 0.833). There were no private alleles in the CiF population of *A. falcatus* nor in the Mac Mac Pools and SRi populations of *P. latifolius.* Observed heterozygosity (H_O) and expected heterozygosity (H_E) were lowest in *P. henkelii* populations, with the greatest H_O found in *P. latifolius* (H_o = 0.572 – 0.767; Total = 0.742) and greatest H_E in *A. falcatus* (H_E = 0.639 – 0.792; Total = 0.789) (Table 2). Only the Umg population of *A. falcatus* suggested low inbreeding (F_{IS} = 0.019, Table 2). In addition, the average F_{IS} for *P. latifolius* (F_{IS} = -0.021) and *P. henkelii* (F_{IS} = -0.147), which indicated outcrossing and for *A. falcatus* (F_{IS} = 0.051; Table 2), which suggested low instances of inbreeding, likely due to the Umg population.

Table 2. Genetic diversity estimates as measured across six microsatellite loci in *Afrocarpus falcatus* and *Podocarpus henkelii* and ten microsatellite loci in *P. latifolius*.

Population	***n***	A_R	A_P	H_O	H_E	F_{IS}
Afrocarpus falcatus						
KrK	24	9.721	1.333	0.826	0.792	-0.043
Umg	23	8.930	0.667	0.748	0.760	0.019
SRi	14	8.038	0.333	0.659	0.639	-0.045
CiF	12	3.938	0.000	0.712	0.701	-0.009
Total	73	10.833		0.751	0.789	0.051
Podocarpus latifolius						
GoW	16	15.772	0.100	0.739	0.652	-0.142
Mac	13	15.296	0.000	0.739	0.610	-0.223
RNa	31	15.637	0.500	0.746	0.654	-0.131
Mnw	4	16.925	0.100	0.767	0.639	-0.199
MoC	26	16.382	0.600	0.700	0.665	-0.054
KrK	12	17.281	0.222	0.701	0.624	-0.134
Mbo	29	15.489	0.300	0.721	0.635	-0.124
Lot	30	15.945	0.200	0.766	0.691	-0.106
Swk	21	15.935	0.100	0.709	0.616	-0.139
Mts	29	15.727	0.500	0.752	0.658	-0.148
Umg	33	15.956	0.667	0.732	0.614	-0.336
Grk	20	15.620	0.429	0.719	0.676	-0.100
Gvb	23	15.304	1.571	0.717	0.661	-0.103
SRi	2	17.697	0.000	0.572	0.411	-0.343
Mot	15	14.966	0.100	0.737	0.592	-0.312
WNP	8	16.055	0.300	0.767	0.614	-0.252
Total	312	18.800		0.742	0.717	-0.026
Podocarpus henkelii						
KrK	30	4.804	0.500	0.529	0.430	-0.186
Mbo	22	4.745	0.500	0.633	0.492	-0.276
Umg	22	5.327	0.833	0.587	0.492	-0.214
Total	74	5.833		0.577	0.499	-0.147

n: sample size. A_R: allelic richness. A_P: average number of private alleles present only in that population. H_O: observed heterozygosity. H_E: expected heterozygosity. F_{IS}: individual inbreeding coefficient.

4.3.2. Genetic Structure

Wright's [37] criterion for genetic differentiation was used to demarcate genetic differentiation into four classes: low ($F_{ST} < 0.05$), moderate ($0.05 < F_{ST} < 0.15$), high ($0.15 < F_{ST} < 0.25$) and very high ($F_{ST} > 0.25$; [38]). As per Wright's [37] criterion, there was low genetic differentiation between KrK – Umg ($F_{ST} = 0.041$) and SRi – CiF ($F_{ST} = 0.045$), and moderate differentiation between KrK – SRi ($F_{ST} = 0.064$), KrK – CiF ($F_{ST} = 0.058$), Umg – SRi ($F_{ST} = 0.072$) and Umg – CiF ($F_{ST} = 0.063$) for *A. falcatus* populations (Table 3). In *P. latifolius,* there was moderate genetic differentiation between the Mpumalanga (MP) and KwaZulu-Natal (KZN) populations, except for Umg where F_{ST} was high when compared to the MP populations (Table 4). Genetic differentiation was high between SRi and GoW and very high between SRi and Mac. F_{ST} was low among KZN populations for *P. latifolius*. The MP *P. latifolius* populations showed moderate differentiation in comparison to the Western Cape (WC) populations, except for Mot – Mac were the populations showed moderate genetic differentiation. SRi was highly differentiated from the *P. latifolius* KZN populations. KZN and WC *P. latifolius* showed moderate to high genetic differentiation. *Podocarpus henkelii* populations showed moderate differentiation, with Mbo and Umg ($F_{ST} = 0.053$) having the lowest F_{ST} and KrK and Mbo had the highest ($F_{ST} = 0.076$; Table 5).

The analysis of molecular variance (AMOVA) showed that 85.72% of genetic variation was within *A. falcatus* individuals and 6.77% was among populations ($F_{ST} = 0.068$, $P < 0.001$; Appendix 2). In *P. latifolius*, 76.10% of the genetic variation was within individuals, 12.30% was among individuals and the smallest variation was among populations ($F_{ST} = 0.116$, $P < 0.001$; Appendix 2). The highest genetic variation for *P. henkelii* was observed within individuals (76.31%) and the lowest among populations (7.28%), with 16.41% of the genetic variation observed among individuals ($F_{ST} = 0.073$, $P < 0.001$; Appendix 2).

Table 3. Pairwise R_{ST} values (R_{ST}, [39], above diagonal) between four *A. falcatus* populations and pairwise F_{ST} values (F_{ST}, [33], infinite allele model (IAM) , below diagonal).

Populations	**KrK**	**Umg**	**SRi**	**CiF**
KrK		0.006	0.050	-0.048
Umg	0.041		0.060	-0.081
SRi	0.064	0.072		0.117
CiF	0.058	0.063	0.045	
Average	R_{ST} = 0.017			F_{ST} = 0.057

.

Table 4. Pairwise R_{ST} values (R_{ST}, [39], above diagonal) between 16 *P. latifolius* populations and pairwise F_{ST} values (F_{ST}, [33], IAM, below diagonal).

Populations	**GoW**	**Mac**	**RNa**	**Mnw**	**MoC**	**KrK**	**Mbo**	**Lot**	**Swk**	**Mts**	**Umg**	**SRi**	**Grk**	**Gvb**	**Mot**	**WNP**
GoW		0.046	0.042	-0.001	0.161	0.058	0.103	0.130	0.049	0.134	0.190	0.078	0.059	0.049	0.114	0.011
Mac	0.055		0.065	0.194	0.278	0.211	0.153	0.196	0.110	0.189	0.201	0.079	0.078	0.000	0.270	0.157
RNa	0.041	0.080		-0.005	0.005	0.025	0.009	0.008	0.066	0.006	0.211	0.095	0.071	-0.037	0.154	0.033
Mnw	0.054	0.120	0.019		0.073	0.239	0.015	0.016	-0.003	0.025	0.113	0.213	0.087	-0.095	-0.016	0.128
MoC	0.052	0.102	0.014	0.018		0.129	-0.012	0.021	0.106	0.027	0.258	0.314	0.253	-0.059	0.306	0.257
KrK	0.063	0.113	0.042	0.056	0.018		0.046	0.093	0.003	0.107	-0.020	0.081	0.036	-0.108	0.231	0.255
Mbo	0.061	0.103	0.023	0.026	0.002	0.019		0.013	0.067	0.011	0.225	0.200	0.170	-0.042	0.222	0.111
Lot	0.058	0.093	0.025	0.015	0.026	0.043	0.028		0.107	0.006	0.238	0.210	0.146	-0.029	0.218	0.105
Swk	0.065	0.115	0.041	0.041	0.027	0.028	0.018	0.034		0.116	0.169	0.124	0.062	-0.067	0.151	0.041
Mts	0.050	0.087	0.027	0.028	0.016	0.033	0.019	0.027	0.027		0.242	0.228	0.160	-0.024	0.233	0.117
Umg	0.159	0.199	0.130	0.147	0.140	0.043	0.142	0.138	0.148	0.140		0.072	0.012	-0.013	0.229	0.129
SRi	0.117	0.140	0.153	0.132	0.157	0.120	0.158	0.141	0.150	0.150	0.098		0.042	0.015	0.351	0.126
Grk	0.115	0.136	0.140	0.107	0.160	0.127	0.164	0.124	0.154	0.143	0.077	0.036		-0.002	0.216	-0.006
Gvb	0.193	0.252	0.217	0.161	0.209	0.188	0.224	0.174	0.210	0.195	0.142	0.099	0.149		0.081	0.056
Mot	0.121	0.165	0.120	0.086	0.139	0.172	0.143	0.100	0.129	0.127	0.193	0.141	0.092	0.184		0.017
WNP	0.077	0.106	0.105	0.077	0.121	0.137	0.121	0.088	0.102	0.102	0.188	0.027	0.011	0.128	0.030	
Average	R_{ST} = 0.099															F_{ST} = 0.102

Table 5. Pairwise R_{ST} values (R_{ST}, [39], above diagonal) between three *P. henkelii* populations and pairwise F_{ST} values (F_{ST}, [33], IAM, below diagonal).

Populations	KrK	Mbo	Umg
KrK		0.024	0.047
Mbo	0.076		0.023
Umg	0.068	0.053	
Average	R_{ST} = 0.031 F_{ST} = 0.066		

4.3.3. Spatial genetic structure

Isolation by distance was detected within the Lot, MoC, RNa and Mbo *P. latifolius* populations represented by at least 30 samples, while isolation by distance was not detected in the Mts population (Figure 2).

DAPC with prior population information showed three genetic clusters for *A. falcatus*, and five genetic clusters for both *P. latifolius* and *P. henkelii* (Figure 3). For *A. falcatus,* all the individuals belonging to cluster one were from the KrK population (Figure 3a). Cluster two was composed of 16 Umg and two SRi individuals (Figure 3a). Interestingly, all the individuals from the CiF *A. falcatus* population were assigned to cluster three, while KrK (n = 7), Umg (n = 12) and Sri (n = 12) individuals were also assigned to cluster three. In *P. latifolius,* the southern populations were assigned to cluster four (Mot and WNP) and cluster five (Grk, Gvb and Sri; Figure 3b). The majority of the northern and eastern populations were assigned to cluster one, three, and five and the high overlap between the clusters is an indication of low genetic differentiation between populations (Figure 3b). All the KrK and Umg *P. latifolius* individuals were assigned to cluster two. *Podocarpus henkelii* individuals from the KrK population were assigned to clusters 1 – 4 (Figure 3c). Cluster three of the DAPC for *P. henkelii* was only composed of individuals from KrK population, while cluster five was only composed of individuals from Umg (Figure 3c). Individuals from the Mbo population were assigned to cluster one (n = 16) and cluster four (n = 6; Figure 3c).

The Bayesian cluster analyses showed a prominent peak at K = 3 for *A. falcatus*, K = 4 for *P. latifolius* and K = 2 for *P. henkelii* (Figure 4). For *A. falcatus*, the three genetic clusters were represented by KrK, Umg and Sri + CiF (Figure 4a). Interestingly, there was admixture between the some of the KwaZulu-Natal (KrK + Umg) and Western Cape (Grk, Gvb and SRi) *P. latifolius* populations (Figure 4b). There is gene flow between the northern Mpumalanga populations (GoW and Mac Mac Pools) and one WC population (WNP, Figure 4b). There is limited gene flow between KrK, Umg, Grk, Gvb and Sri and other populations (Figure 4b). *Podocarpus henkelii* populations formed two genetic groups, cluster one comprised KrK + Mbo and the second cluster was only represented by Umg individuals (Figure 4c). KrK and Mbo formed a separate genetic cluster to Umg, there was low admixture between the KrK + Mbo cluster and Umg and the genetic signature observed in the structure diagrams maybe due to the samples being from the same species and not necessarily due to gene flow between populations.

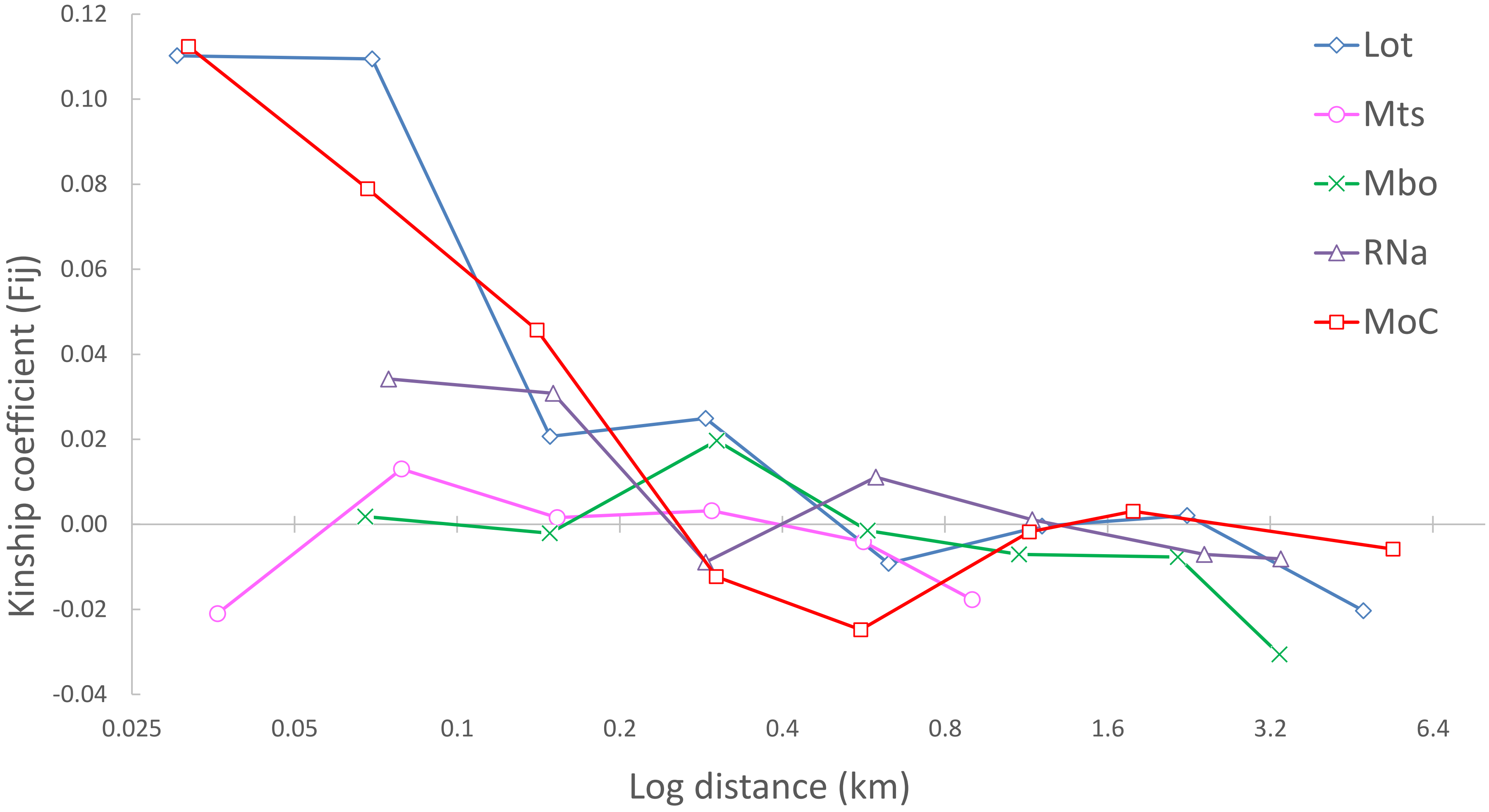

Figure 2. Spatial genetic structure between spatial distance and kinship coefficient (*Fij*) of *Podocarpus latifolius* populations within more than 30 individuals in the KwaZulu-Natal province, South Africa. Lot = Lotheni Nature Reserve; Mts = Marutswa Forest; Mbo = Mbona Nature Reserve; RNa = Royal Natal Nature Reserve; MoC = Monk's Cowl.

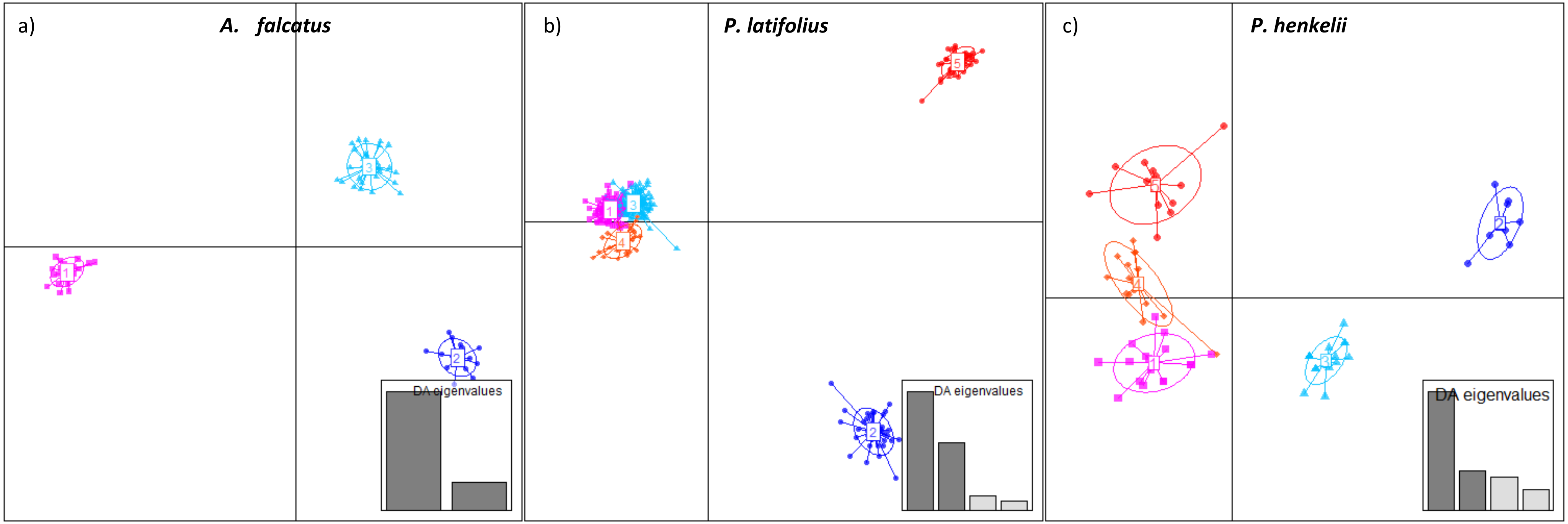

Figure 3. Discriminant Analysis of Principal Component (DAPC) including (a) 73 *Afrocarpus falcatus,* (b) 312 *Podocarpus latifolius* and (c) 74 *P. henkelii* samples from South Africa using SSR markers. X and Y axes of the DAPC scatterplots describe the first and second discriminant functions. Barplots of discriminant analysis eigenvalues (bottom right) show the proportion of genetic information comprised in each consecutive discriminant function. Clusters are distinguished by symbols and colours with 95% inertia ellipses.

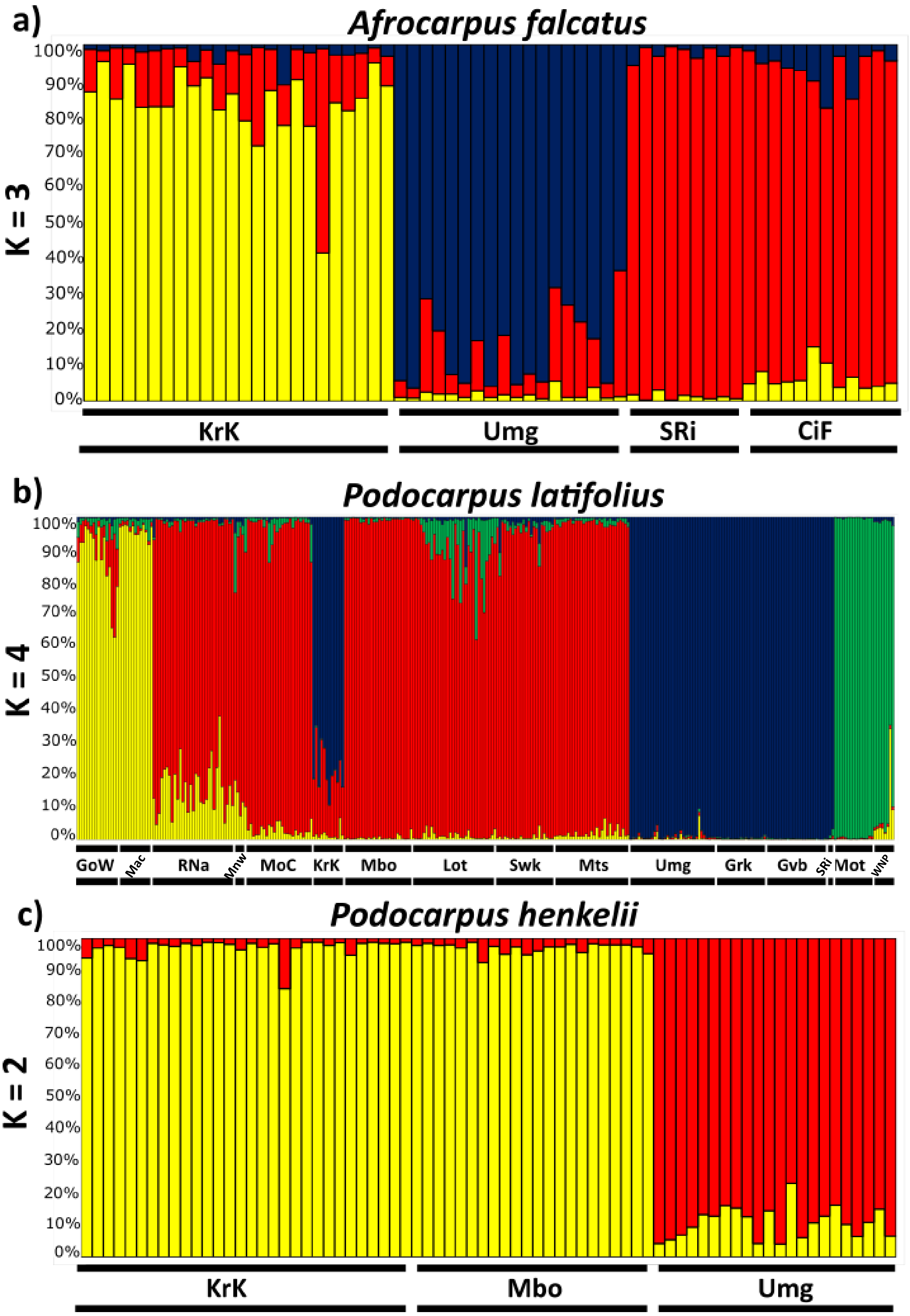

Figure 4. Population structure analysis from POLYGENE for (a) four *Afrocarpus falcatus,* (b) sixteen *P. latifolius* and (c) three *P. henkelii* populations. Where K = number of inferred genetic clusters/groups based on six nSSR for *A. falcatus,* ten nSRR for *P. latifolius* and six nSSR for *P. henkelii.* Each vertical bar represents an individual and individuals are grouped by sampled populations, with northern most populations on the left, and southern most populations on the right side. Different colours represent the proportion of each genotype which is determined by K.

4.4. Discussion

In the present study, genetic diversity, and population structure within and among *A. falcatus, P. latifolius* and *P. henkelii* populations was investigated in order to assess gene flow between podocarp populations. During the last glacial maximum, *A. falcatus* and *P. latifolius* underwent rapid demographic and range expansion resulting in the species occupying a large geographic range [30]. The extensive geographic range of *A. falcatus* and *P. latifolius* in southern Africa is an indication that these species have a high dispersal capacity. However, the rapid deterioration of kinship with geographic distance in four of the five *P. latifolius* populations implies that gene flow through pollen and/or seed dispersal are spatially restricted in these populations (Figure 2). However, podocarp distribution may not be limited by dispersal but rather by recruitment which is limited particularly in the hostile savanna-grassland matrix. Isolation by distance was observed in the Lot, Mbo, RNa and MoC populations but was absent in the Mts population. These findings are consistent with those in other studies which have shown that podocarp pollen dispersal deteriorates with distance from the parent plant [9,40-43], thus suggesting that pollen dispersal may not be sufficient at connecting populations. Enright et al. [43] and Migliore et al. [9] attributed this contradiction to asynchronous masting between forest patches resulting in lower fruit production. In addition, the absence of isolation by distance in the Mts population located in the Western Cape may imply that there is no limitation of pollen dispersal in this population, this may be due to the Mts population being close to other populations.

A high degree of gene flow is often represented by low genetic differentiation [44,45]. *Afrocarpus falcatus, P. latifolius* and *P. henkelii* populations generally showed F_{ST} values lower than 0.200 (Table 3 – 5). The *A. falcatus, P. latifolius* and *P. henkelii* KwaZulu-Natal populations have an *FST* value lower than 0.100 which suggests that although these populations are currently fragmented, they were connected. Interestingly, there is low genetic differentiation between some northern (e.g., GoW) and southern *P. latifolius* populations (e.g., WNP) indicating gene flow between populations. The higher differentiation between some *P. latifolius* populations in comparison to the other species populations is reflected in: (1) the presence of private alleles (particularly in Gvb), (2) high allelic richness (particularly in SRi and KrK), (3) and the DAPC and STRUCTURE results. Due to isolation by distance populations may become more isolated and less likely to exchange genetic material through pollen or seed dispersal. The considerable geographic distances, and tall mountain ranges and savanna-grassland matrices in between populations operate as physical barriers. However, considering that podocarp populations are connected, this may suggest sufficient occasional gene flow particularly between populations that are close to each other. *Podocarpus latifolius* populations in west, central and east Africa were reported by Migliore et al. [9] to be highly differentiated due to genetic drift and isolation by distance as observed in this study. Where the Dahomey Gap in north Africa has been an important dispersal barrier of podocarp dispersal in west, central and east Africa because it is dry and does not allow for podocarp dispersal [46]. Dantas et al. [28] attributed the high genetic differentiation between *P. sellowii* populations to the long-term isolation of populations. Like Migliore et al. [9]'s study, we also found that there were no shifts in microsatellite allele sizes with *A. falcatus, P. latifolius* and *P. henkelii* populations which is an indication of relatively recent divergence. Therefore, differentiation between these populations is driven by genetic drift as observed in *P. latifolius* populations in central, east and west Africa [9].

Outcrossing species with significant capacity for genetic migration typically tend to maintain greater genetic diversity, relative to other species [2,47]. *Afrocarpus falcatus* and *P. latifolius* seeds are dispersed by bats, birds, and monkeys [22], and their pollen is wind dispersed, allowing these two podocarps to disperse over greater distances leading to greater gene flow and thus greater genetic diversity. In contrast, *P. henkelii* populations showed relatively lower genetic diversity (H_O = 0.577, H_E = 0.499; Table 2) which we attribute to reduced population sizes, small sample size and the use of too few microsatellites which were developed for a sister taxa. Interestingly, the genetic diversity of these three species is higher than other podocarp species around the globe including *P. parlatorei:* H_O = 0.157, H_E = 0.160 [26]; *P. nubigena:* H_O = 0.279 [27]; *P. sellowii:* H_O = 0.393, H_E =

0.410 [28] in South America, *P. elatus* in Australia (H_O = 0.392, H_E = 0.528; [5]), *Pherophaera hookeriana* in Tasmania (H_O = 0.56, H_E = 0.56; [48]), and *Dacrydium elatum* in Vietnam (H_O = 0.555, H_E = 0.429; [49]). The higher genetic diversity found in this study may be due to the high heterozygosity within the sample populations and the use of more polymorphic genetic markers. All three focal species are dioecious and therefore outcrossing is necessary, leading to a heterozygous surplus resembling that of other podocarps [26,28]. High in heterozygosity may suggest that inbreeding is rare in large populations. Low inbreeding coefficients were observed in this study and were also indicated in other podocarp studies [26,28]. High genetic diversity, high heterozygosity, and low inbreeding in *A. falcatus, P. latifolius* and *P. henkelii* populations in South Africa are an indication that these podocarp populations are connected regardless of their disjunct distribution. Palaeoanthropology data has shown that South African forests were not one continuous belt of Afromontane forest but were always fragmented or archipelago-like, expanding and contracting during cold-dry and warm-wet cycles.

Fragmentation of these populations either naturally and/or due to climate change, habitat degradation and overexploitation [15,50,51] has not reduced genetic diversity in these populations. In contrast to this study, Migliore et al. [9] found that *P. latifolius* populations in eastern and central Africa had high levels of inbreeding and lower genetic diversity relative to *P. latifolius* populations in South Africa. This finding could be due to the large geographic extent covered by Migliore et al [9] relative to our sampling area. Alternatively, lower genetic diversity in eastern and central African populations of *P. latifolius* could have occurred during forest movements because of founder events during range expansion [52].

The genetic diversity and variation present in a population determines its ability to evolve, adapt, and persist over time. Higher allelic richness in *A. falcatus* and *P. latifolius* populations in comparison to *P. henkelii* populations suggests that these species have greater long-term evolutionary potential, adaptability, resilience, and persistence (e.g., [27]). The high allelic richness particularly in *A. falcatus* and *P. latifolius* populations may also suggest that the cutting/felling of podocarps in South African forests [53] did not cause genetic bottlenecks. However, it is worth noting that the CiF *A. falcatus* population had significantly lower allelic richness than the other populations. This suggests that the CiF *A. falcatus* population maybe be undergoing a genetic bottleneck.

When comparing populations of geographically localized plants to those of widely distributed congeners, there is no consistent pattern observed in terms of population genetic structure [54-56]. The Bayesian cluster analyses computed using POLYGENE identified three *A. falcatus* clusters, four *P. latifolius* clusters and two *P. henkelii* clusters for the sampled trees. The lack of genetic structure could be due to gene flow between populations, as populations within the same geographic region had low levels of differentiation and high gene flow. For instance, *A. falcatus* populations, SRi, and CiF (F_{ST} = 0.045) were assigned to one cluster (~120 km between populations) while KrK and Umg (F_{ST} = 0.043) were assigned to different clusters (~185 km apart). Similarly, *P. henkelii* sampled from KrK and Umg were each assigned to their own genetic cluster with individuals from Mbo assigned to the KrK cluster. Despite the F_{ST} estimates, there is little, if any, admixture among the genetic clusters within these two species.

The South African populations of *P. latifolius* were assigned to four clusters which included the populations of GoW + Mac in cluster one (Mpumalanga populations), RNa + Mnw + MoC + Mbo + Lot + Swk + Mts in cluster two (KwaZulu-Natal populations), KrK + Umg + SRi + Grk + Gvb in cluster three (Mpumalanga, Eastern Cape and Western Cape populations) and Mot + WNP in cluster four (Western populations; Figure 4b). The genetic structure for cluster one, two and five may be due to geographic distance due to habitat fragmentation, genetic divergence between the different clusters and/or insufficient sampling, particularly in the Eastern Cape province, may be the mostly likely scenario. Cluster three was particularly interesting because KrK + Umg are at least 930 km away from SRi which is closer to KrK and Umg than Grk and Gvb. The clustering of KrK and Umg (KwaZulu-Natal populations) with SRi, Grk and Gvb (Eastern and Western Cape populations) may be due to: (1) *P.*

latifolius populations in southern KwaZulu-Natal being connected with those in the Eastern Cape where we did not sample, (2) long distance dispersal events which is common in podocarps [57], (3) translocation of individuals (physical admixture) from the KwaZulu-Natal province to the Western Cape, and (4) wind patterns which allow for pollen to disperse over large distance. King and Ackerly [58] have shown that global wind patterns can shape gene flow, genetic differentiation, and genetic diversity in trees by isolating populations that are near each other and connecting geographically distant populations.

This study has allowed us to understand genetic diversity and connectivity among and within podocarp populations in South Africa. Here we show that there is intraspecific gene flow between populations of *A. falcatus, P. latifolius* and *P. henkelii*. Accordingly, *A. falcatus* and *P. henkelii* populations located near each other showed higher gene flow than those more distant, while *P. latifolius* showed an exchange of genetic material in geographically distant populations as well. We conclude that most of the variation we detected within and between populations reflects frequent outcrossing due to wind-dispersed pollen, seed dispersal, translocation and/or that the populations were one population. Translocation of podocarps may have been done by humans through the planting of seeds and/or seedlings due to the use of podocarp wood for furniture and ship building [59,60].

Based on trends observed in this study, we recommend that future studies: (1) expand sampling for (i) *A. falcatus* populations in the Limpopo Province, KwaZulu-Natal, Eastern Cape, and Western Cape, (ii) *P. latifolius* populations in the Eastern Cape to fill the gap in our sampling and (iii) *P. henkelii* populations in the north and south of its distribution to capture ghost populations, (2) develop and amplify microsatellites specifically for *P. henkelii* as this might have skewed the results, (3) collect leaf samples from seedlings to get an idea of current gene flow and (4) try to amplify as many microsatellites as possible to make the results more robust. This will allow us to establish whether the current geographic distribution of genetic variation corresponds to past changes in these focal species’ ranges and past connectivity events that may have affected their genetic differentiation or lack thereof. In addition, this will inform us of past and present gene flow in podocarp under climate change and other disturbances which can inform us in conservation decision making.

4.5. References

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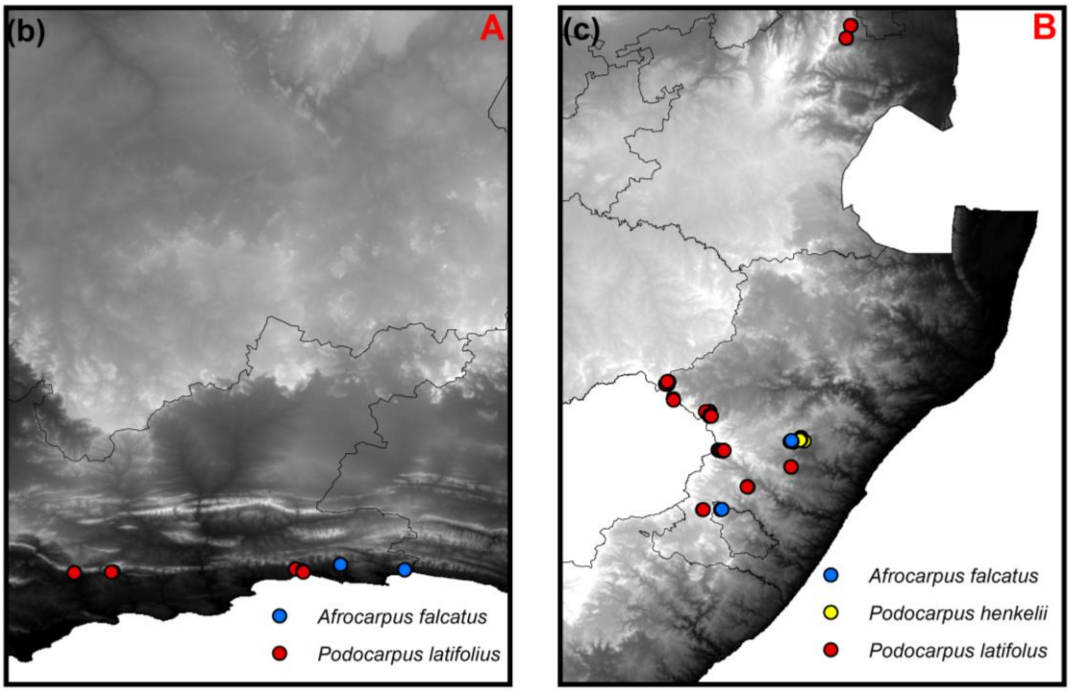
4.6. Supplementary Material



Appendix 1. (b) *Afrocarpus falcatus* and *P. latifolius* sampled localities and populations in Western Cape and Eastern Cape provinces (Inset A). (c) *Afrocarpus falcatus, P. latifolius* and *P. henkelii* sampled localities and populations in Mpumalanga and KwaZulu-Natal provinces (Inset B).

Appendix 2. Analysis of molecular variance for South African populations of *A. falcatus, P. latifolius* and *P. henkelii* from POLYGENE.

	df	Sum of Squares	Mean Squares	Total Variation (%)	F_{ST}	Fixation Indices
			A. falcatus			
Within individuals	416	144.302	0.347	85.723		
Among individuals	392	159.787	0.408	7.505		
Among populations	18	23.805	1.323	6.772		
Total	826	327.894	0.397	100	0.068	$P < 0.001$ ***
			P. latifolius			
Within individuals	2775	759.555	0.274	76.104		
Among individuals	2626	951.145	0.362	12.302		
Among populations	139	262.275	1.887	11.595		
Total	5540	1972.976	0.356	100	0.116	$P < 0.001$ ***
			P. henkelii			
Within individuals	389	75.609	0.194	76.308		
Among individuals	371	103.132	0.278	16.414		
Among populations	12	12.816	1.068	7.278		
Total	772	191.557	0.248	100	0.073	$P < 0.001$ ***

df, degree of freedom; *** $P < 0.001$.

Chapter 5: Synthesis

Gymnosperms and angiosperms are often pitted against each other due to palaeoecological reconstructions which suggest that angiosperms diversified at the expense of gymnosperms. Evolutionary ecologists have proposed theories to account for the radiation and dominance of angiosperms and gymnosperms in the competition for space in forest canopies. Conifers are currently the most prevalent and globally species rich gymnosperms and are the biggest contenders against angiosperms. In contrast to Northern Hemisphere conifer families, the Podocarpaceae (podocarps), which have a Southern Hemisphere distribution, exhibit incredible taxonomic, ecophysiological and morpho-anatomical variation (Biffin *et al.* 2012; Knopf *et al.* 2012; Khan *et al.* 2022; Leslie 2022) allowing them to inhabit various environments making them an ideal study system.

Bond (1989) proposed that gymnosperms were at a competitive disadvantage to angiosperms due to slow growth and photosynthetic limitations at the seedling stage. In this thesis, I investigate whether the ecology, physiology, morphology and dispersal of podocarps can predict their current and future distribution under a changing climate. Here, I use species distribution modelling techniques to predict the environmental variables that shape the current and future distribution and environmental niches of South African podocarps in Afromontane forests. While there is considerable research on South African and Australian podocarps, little is known about the physiology of South African podocarps due to challenges of collecting physiological and morphological data in remote locations and financial constraints. Numerous studies have attempted to understand how podocarps respond to shading (Geldenhuys and von dem Bussche 1997; Adie and Lawes 2009; Tadele and Fetene 2013) and drought stress (Brodribb and Hill 1998; Jooste 2015). Here I used experiments to understand how podocarp seedlings respond to environmental stresses linked to climate change to predict seedling establishment and persistence under climate change. Genetic data was also used to evaluate dispersal patterns (population genetics) in order to predict current and future podocarp distributions, particularly in South Africa. Ultimately, this thesis links podocarp biogeography, physiology, morphology, possible dispersal patterns based on genetic data, and their present and future distributions and provides an understanding of their future persistence in a changing climate.

5.1. Physio-morphological characteristic and limitations

5.1.1. Environmental variables determining podocarp distribution

Podocarps have a high foliar morphology which makes them well adapted to survive under different light conditions, therefore varying light conditions does not play an important role in podocarp distribution. To answer the question of why podocarps distributed where they are it is imperative to examine what constrains their distribution. The climatic variables shaping the current and future distribution of podocarps were predicted using species distribution modelling techniques (Twala *et al.* 2023 [Chapter 2]). Precipitation of the driest quarter (BIO17) and temperature annual range (BIO7) were the most important variables shaping the current and future distribution of *A. falcatus*. *Podocarpus elongatus* current distribution was predicted to be influenced by precipitation of the warmest quarter (BIO18) and BIO17. Under RCP4.5 *P. elongatus* distribution was predicted to be influenced by BIO18 and precipitation of the wettest quarter (BIO16). Temperature appeared to be the most important variable influencing *P. elongatus* distribution under RCP 8.5 where BIO7 and mean temperature of the wettest quarter (BIO8) were the most important variables. Like *P. elongatus*, BIO17 and BIO18 were predicted to influence the current distribution of *P. henkelii*. The future distribution of *P. henkelii* was predicted to be governed by max temperature of the warmest month (BIO5), BIO7 and BIO18. The current and future distribution of *P. latifolius* distribution was predicted to be influenced by BIO16. Temperature (BIO5 and BIO7) was also predicted to influence *P. latifolius* distribution. The effects of precipitation on podocarp distribution have been frequently demonstrated in literature (Truc *et al.* 2013; Bernardi *et al.* 2020; Tesfamariam *et al.* 2022; Xie *et al.* 2022). These results suggest that podocarp distribution in southern Africa is driven by seasonal drought which is exacerbated by temperature (hot or cold). Although Bond (1989) proposed that podocarp distribution may be limited by angiosperm competition these results along with those in Chapter 3 (Twala *et al.* 2022) suggest that podocarp distribution is determined by seasonal drought. In chapter 3 (Twala *et al.* 2022), drought and elevated temperatures reduced plant structures and physiological functions in *A. falcatus* and *P. henkelii,* except water use efficiency which increased under drought stress. A decrease in chlorophyll content and chlorophyll fluorescence can reduce plant physiological performance particularly stomatal conductance and photosynthetic rate resulting in reduced plant performance.

5.1.2. Current podocarp distribution

Conifer fossil records inform us about the factors (e.g., evolution of leaf shape and appearance of competitor clades) driving the evolution of conifers (Mellick 2012; Leslie *et al.* 2018; Condamine *et al.* 2020). Migration, range shifts and the speciation of conifers across the Southern Hemisphere have been affected by the interactions between climatic cycles and landmass shifts (Leslie *et al.* 2012). These processes are responsible for the present podocarp biogeographic distributions and their variety in form, function, and tolerance to different environmental conditions (Brodribb and Hill 1999, 2004; Brodribb and Field 2008; Adie and Lawes 2009; Khan and Hill 2021, 2022; Schmiege *et al.* 2021; Khan *et al.* 2023). In this study podocarps were predicted to be distributed in the Western Cape, Eastern Cape, KwaZulu-Natal coast and midlands, north-eastern Mpumalanga and eastern Limpopo Province (Twala *et al.* 2023 [Chapter 2]), which is similar to White (1981). Interestingly, the Great Escarpment in South Africa appears to be a geographic barrier preventing podocarp expansion to the interior of South Africa (Twala *et al.* 2023 [Chapter 2]). Although, yellowwoods occur along the escarpment they may be unable to expand past this range due to the climatic, edaphic, topographic, geological and environmental heterogeneity intervals along the escarpment which create dispersal corridors for some species but form barriers for others (Clark 2010).

5.1.3. Predicted podocarp distribution expansion under climate change

Plants may survive climate change by tracking favourable climates and/or adapting to the new climatic conditions (Kelly and Goulden 2008; Corlett and Westcott 2013). Podocarps are predicted to respond to climate change by expanding to higher altitudes where the climate is predicted to be cooler (Twala *et al.* 2023 [Chapter 2]); Casas-Gallego *et al.* (2023) observed similar patterns in Afromontane forests in the Horn of Africa during the last glacial maximum. The ability of plants to migrate from one area to another depends on dispersal events which are influenced by the time span between seed dispersal to first reproduction and the dispersal distance (Corlett and Westcott 2013). Podocarps produce animal-dispersed recalcitrant fleshy seeds (Geldenhuys 1993, Willson *et al.* 1996; von dem Bussche 1997; Negash 2003; Blendinger 2017). There is gene flow between proximal *A. falcatus* and *P. henkelii* populations, however gene flow deteriorates with distance; similar patterns are observed for *P. latifolius* in South African populations (Chapter 4), and *P. latifolius* African populations (Migliore *et al.* 2022). The results in Chapter 4 show that South African podocarp populations are/were connected regardless of them being disjunct. This may suggest that podocarps may be able to disperse and expand their current range as predicted by the future outcomes. However, if podocarps are unable to expand their distribution due to geographic barriers (e.g., escarpment and land use) or unforeseen dispersal limitations, then life history traits such as longevity (Lusk and Smith 1998; McDougall *et al.* 2012; Enright *et al.* 2022) will enable podocarps to persist in refugial patches until conditions improve. Podocarp longevity is an important trait that has contributed to podocarp survival when competing for resources with angiosperms and under climate oscillations as they can persist until conditions improve (Casas-Gallego *et al.* 2023).

The ability of plants to inhabit new environments is not only driven by dispersal but also by physiological thresholds (Eriksson 2000; Walther *et al.* 2002; Öster *et al.* 2009a, b). In forest habitats light plays an important role in species composition and dominance (Poorter 1999; Adie *et al.* 2009a, b; Adie and Lawes 2011). Superior drought resistance in *A. falcatus* relative to *P. latifolius* enables it to persist in coastal lowlands (Adie and Lawes 2011; Twala *et al.* 2022 [Chapter 3]). The reduced ability of *A. falcatus* seedlings to tolerant heat and drought stress suggests that recruitment will be limited in its current distribution during climate change (Twala *et al.* 2022 [Chapter 3]). Therefore, the ability of *A. falcatus* to expand to higher elevations is dependent on its ability to disperse over long distances. Unfortunately, the ability of *A. falcatus* to track favourable climates will be limited by it's ability to disperse over long distances (Chapter 4); thus, it's ability to expand to higher elevations as the climate changes will be slow (Twala *et al.* 2023 [Chapter 2]). Similarly, *P. henkelii* may not be able to track suitable climatic conditions under climate due to its limited dispersal ability; however, *P. henkelii* seedlings will be able to tolerate elevated temperature and drought better than the widely distributed *A. falcatus* (Twala *et al.* 2022 [Chapter 3]). Therefore, populations will likely be maintained by drought tolerant adults (Brodribb and Hill 1998) that will persist in refugia until conditions improve (Adie *et al.* 2017). However, high genetic diversity within populations may facilitate adaptation to allow seedlings to occupy harsher environmental conditions than presently observed. The ability of *P. latifolius* to occupy and maintain a larger geographic extent and environmental niche in comparison to other podocarps (Chapter 2) is likely driven by its reproductive strategy and physiology. Podocarps produce recalcitrant seeds in the winter months when temperatures are cooler during the dry season. *Podocarpus henkelii* produces seeds with a waxy epimatium (coat) which limits water uptake acting as a germination barrier (Dodd *et al.* 1989a, 1989b), and reduces seed viability (Noel and Van Staden 1976). Reduced seed water content control and morpho-physiological dormancy in *P. henkelii* seeds limits regeneration in nature, thus ultimately limiting its distribution. Interestingly, *A. falcatus* and *P. latifolius* seeds are more drought tolerant than *P. henkelii* seeds (Geldenhuys 1993; Negash 2003). The ability of *A. falcatus* and *P. latifolius* to disperse over larger geographic distances before losing viability enables them to occupy larger geographic extents relative to *P. henkelii* seeds which lose viability quickly. *Podocarpus latifolius* is unable to disperse over long distances limiting gene flow between geographically distant populations (Chapter 4). Instead of adopting the masting strategy used by *A. falcatus* and *P. henkelii*, *P. latifolius* frequently produces seeds (Geldenhuys 1993; Wirminghaus *et al.* 2002; Hart *et al.* 2013), which increases its chances of colonising new areas. Once dispersed, the superior shade-tolerance of *P. latifolius* relative to *A. falcatus* allows it to regenerate and persist under the forest canopy and in the savanna-grassland matrix (Midgley *et al.* 1995; Adie and Lawes 2009a, b; 2011). The ability of podocarps to track favourable climates will be significantly influenced by their dispersal ability (Chapter 4), physiology (Chapter 3), recruitment, and establishment. In the savanna-grassland matrix podocarp recruitment is limited by fire (Adie *et al.* 2017). Seedlings and sapling mortality is high in the matrix if fires burn frequently due to seedlings being within the “fire trap” (Bond 2005). If conditions favour less frequent and less intense fires then podocarps will be able to establish in the savanna-grassland matrix (Bond *et al.* 2008; Adie and Lawes 2009b; Clark and Lawes 2013; Adies *et al.* 2017).

5.1.4. Environmental niche

Species with smaller geographic distributions are more vulnerable to climate change due to their narrow environmental niche (Malcolm *et al.* 2006; Bai *et al.* 2018). Both *P. elongatus* and *P. henkelii* were predicted to have the smallest geographic distributions of the four podocarps, interestingly *P. elongatus* was predicted to occupy the broadest environmental niche and *P. henkelii* the narrowest (Twala *et al.* 2023 [Chapter 2]). The mismatch between the geographic distribution and environmental niche of *P. elongatus* and *P. henkelii* may be an indication that the species are poor dispersing species preventing them from dispersing to suitable habitats and because podocarps are high persistence species, they may have the ability to persist in sites where conditions are currently unsuitable. Poor dispersing and persistent species are bad model species for correlative models making the model predictions less accurate, therefore species distribution modelling for poor dispersing and persistent species require mechanistic models that include other biophysical characteristics and demographic estimates.

Numerous studies have shown that other biophysical variables besides climate play an important role in predicting species distribution (Gardner *et al.* 2019; Silva *et al.* 2019; Zangiabadi *et al.* 2021), therefore making it difficult to predict species range shifts under climate change. Considering that our models were generated using climatic variables alone there is uncertainty in the accuracy of these predictions because of exclusion of variables like geology, topography, edaphic conditions and land use change which is uncertain in the future. Furthermore, there are disadvantages of predicting climate change outcomes which include the inability to include key variables that influence species distributions due to data only being available for well-studied species and the lack of projected datasets as well as the difficulty in data selection.

5.2. Conclusion and recommendations for future research

In summarising podocarp distribution (biogeography) and physiology (persistence) and dispersal ability (range shift) it is apparent that podocarps are not as sensitive to climate change as previously predicted. Podocarps are distributed in Afromontane forests in Limpopo, Mpumalanga, KwaZulu-Natal, Eastern Cape and Western Cape provinces, where two species (*A. falcatus* and *P. latifolius*) have a wide distribution and two species (*P. elongatus* and *P. henkelii*) have a constrained distribution and are endemic to South Africa. The distributions of *A. falcatus* and *P. latifolius* are not restricted to South Africa but extend to east and central Africa with *P. latifolius* also occurring in West Africa. Analyses were not conducted for data outside of South Africa owing to this study being South African based to get a fine scale perspective of podocarp distribution, physiology and dispersal. Podocarps were predicted to expand their distribution under climate change except for *P. henkelii* under RCP4.5 and *P. latifolius* under RCP8.5 where the distributions were predicted to contract under climate change. The ability of podocarps to track favourable climate will be determined by distance between patches because podocarp dispersal deteriorates with distance (Chapter 4). The genetics data suggests that podocarp populations are connected, but there may be dispersal barriers between some populations which could be due to land use or geographic barriers. However, these data should be interpreted with caution for several reasons: (1) leaf material from *A. falcatus, P. latifolius* and *P. henkelii* were not collected across their full extent therefore we might have missed some ghost populations that could suggest that podocarps are able to disperse over long distances or may suggest that there are geographic barriers which we could not decipher from this data set, (2) owing to the poor amplification of the microsatellites only a few microsatellites could be used which may have limited our ability to robustly detect genetic diversity and population structure and (3) the *P. henkelii* results were not robust because the microsatellites used to determine *P. henkelii* population diversity and structure were developed for *P. latifolius* which were already limited. This however does not invalidate these results as they give us a glimpse into podocarp population diversity and structure.

Ecophysiological experiments could only be conducted for *A. falcatus* and *P. henkelii* as only those species' seedlings were available (Twala *et al.* 2022 [Chapter 3]). Drought and elevated temperatures reduced *A. falcatus* and *P. henkelii* seedling physiological performances but did not have a significant effect on the plant structures due to the seedlings being slow growing, therefore 28 days was not sufficient to observe structural growth. Podocarp seedlings dealt with drought and heat stress by increasing their WUE. Interestingly, *P. henkelii* was observed to be more heat and drought tolerant. These findings suggest that *P. henkelii* distribution is not confined by its physiological thresholds but rather by seedling recruitment, while the larger distribution and environmental niche occupied by *A. falcatus* and higher geneflow suggests that the ability of *A. falcatus* is not solely determined by its drought and heat tolerance, which allows it to compete with angiosperms in the forest canopy, but also by its ability colonise new habitats. *Podocarpus elongatus* occupied the largest environmental niche but was predicted to have a restricted distribution. A mismatch between the environmental niche and geographic distribution suggests that *P. elongatus* occupies a wide range of habits within its distribution but its range is limited either by a dispersal barrier or competition as it cannot expand its geographic range. Ultimately, the life history traits and robustness of podocarps enables them to persist and compete with angiosperms waiting for conditions to improve. Although many species are at risk of extinction under climate change podocarps have proven to be hardy survivors that can persist in refugia when conditions are not favourable or dominate when conditions are.

Together, these data lay the groundwork for a better understanding of podocarp distributions, the factors that determine their distribution and the mechanisms that drive these patterns, particularly under climate change. The data collected in this study and the results obtained can be used to inform conservation projects where the podocarp species distribution model rasters can be used to inform models for other species distributions which can be used for conservation management. For example, the podocarp species distribution output layers from this thesis are being used to inform Cape Parrot distribution models to help with conservation efforts by identifying areas with podocarps which can be used as food and nesting sources. Even so, there is so much that remains to be understood particularly with regards to:

1. Interspecific differences in the physiological and morphological responses of *A. falcatus* and *P. henkelii* highlights the need for future studies to examine the impacts of elevated temperature and drought stress events on the growth, survival and physiology of podocarp in different age classes. This will provide a better understanding of podocarp physiological thresholds at different age classes and how these may influence podocarp recruitment and persistence, and ultimately distribution.
2. Bond (1989) stated that the slow growth rates of gymnosperms puts them at a competitive disadvantage against angiosperms. It will be interesting to examine through ecophysiological experiments on the two phyla if temperature and drought stress maintains this competitive dynamic between gymnosperms and angiosperms or if one will dominate.
3. Leaf material was collected for *A. falcatus, P. henkelii* and *P. latifolius* across South Africa, however, due to Covid restrictions sampling was limited. It is recommended that future studies collect leaf material across the entire distribution of all South African podocarps to provide insight into podocarp dispersal patterns. Furthermore, demographic data (age and

"gender") may be useful to tease out past and present dispersal patterns and aid in predicting future range shifts. Additional leaf material should be collected as follows:

   a. Collecting more leaf material for *A. falcatus, P. latifolius* and *P. henkelii* may be useful as a geographically widespread dataset can help explain some of the dispersal patterns we observed in this thesis. For example, it would be interesting to definitively explain why *P. latifolius* KrK and Umg populations occur in the same genetic cluster as the Western Cape populations and not with the other KwaZulu-Natal populations without depending on *prima facie* evidence. This requires future studies to collect leaf material from ghost populations.
   b. Collecting *P. elongatus* leaf material across its distribution will inform us about the genetic structure and diversity of the species. This could help us understand whether the populations are connected or whether the populations are geographically isolated and possibly contribute towards our understanding of why it is endemic to the Western Cape province. Understanding the connectivity of this species will give us insight into dispersal barriers that maybe limiting its distribution and inform us about its ability to persist under climate change.

4. The recent hyperactivity in the divergence of the African and Somali plates highlights the importance of commencing long-term monitoring studies which can be used to document podocarp demographics, physiology, and population genetics prior to the formation of a geographic barrier.

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