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The journal aims to disseminate, to a wide audience, knowledge, information and innovative approaches that promote and enhance the wise use and management of biodiversity in order to sustain the systems and species that support and benefit the people of Africa.

The journal publishes original research findings, as well as reviews, commentaries or perspectives, strategies and short communications. Special focus issues emanating from symposia or conferences that fall within the scope of the journal may also be published.

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2. Assessment of biodiversity, including the status of populations, species and ecosystems, the impacts of threats, harvesting and disturbance or of interventions on populations, species and ecosystems, and the value of the goods and services provided by biodiversity.
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The relationship between mammalian burrow abundance and bankrupt bush (*Seriphium plumosum*) encroachment

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Background: Much of the Grassland Biome in South Africa is prone to shrub encroachment, leading to loss of ecosystem services, habitat heterogeneity and species diversity. Burrowing mammals are an important component of grasslands as these animals create microhabitats for other taxa, including smaller mammal species, birds, reptiles and invertebrates. However, our understanding of how shrub encroachment affects burrowing mammals is poor.

Objectives: Here we assessed the relationship between burrow abundance and bankrupt bush, *Seriphium plumosum*, encroachment as well as burrowing mammal diversity in bankrupt bush encroached and non-encroached grasslands.

Method: Shrub density, medium and large mammal burrow abundance and density were measured in 24 encroached and 24 non-encroached areas randomly selected in the Telperion Nature Reserve, Mpumalanga, South Africa. In addition, burrowing mammal diversity was assessed using camera traps in a subset of six encroached and six non-encroached areas.

Results: Our results show that the abundance and density of medium and large burrows were significantly lower in encroached areas than in non-encroached areas ($p = 0.011$ and $p < 0.001$, respectively). The relationship between burrow abundance and bankrupt bush encroachment was negative (*rho* = -0.456, $p = 0.001$). However, burrowing mammal diversity had no significant difference between encroached and non-encroached areas.

Conclusion: Our data, therefore, suggest that with increasing bankrupt bush encroachment and a decreased abundance in burrowing mammal ecosystem services, a negative effect will occur on burrowing mammal communities, leading to the reduction in species-specific habitat heterogeneity and possibly animal biodiversity.

Keywords: burrowing mammals, burrow density, *Seriphium plumosum*, shrub density, Telperion Nature Reserve, Grassland Biome.

Introduction

The Grassland Biome is the second largest biome in South Africa, encompassing 28% of the land area, and supports a number of ecosystems inhabited by diverse vertebrate and invertebrate communities (Mucina & Rutherford 2006; Carbutt et al. 2011). For example, the small, medium and large burrowing mammals that live in grasslands are ecosystem engineers that play a vital role in sustaining the open habitats characterising grassland areas (Davidson et al. 2012; Jayadevan et al. 2018). Grasslands are of agricultural, ecological and conservation management importance; however, due to an increase in CO_2, inadequate fire regimes and poor management, grasslands are threatened

by an increase in shrub or bush encroachment (Ward 2005; Buitenwerf et al. 2011; Carbutt et al. 2011; Soto-Shoender et al. 2018; Graham et al. 2020). Shrub or bush encroachment is a phenomenon observed through an increase in woody biomass and cover, which in turn leads to a detriment in herbaceous layers (O'Connor et al. 2014). Shrub encroachment can thus lead to changes in ecological succession and ultimately influence biodiversity (O'Connor et al. 2014). Bankrupt bush, *Seriphium plumosum*, is a native, encroaching woody plant originating from the fynbos region in the Western Cape of South Africa and belonging to the Asteraceae family (Jordaan 2009; Snyman 2012; Van Zyl & Avenant 2018; Graham et al. 2020). The ability of *S. plumosum,* to encroach grasslands has interested researchers since the 1930s, as it reduces grazing potential of rangelands (Roux 1969; Avenant 2015). Nevertheless, little work has been done to examine the effect of shrub encroachment generally, or *S. plumosum* encroachment specifically, on mammals and mammalian ecosystem engineers (Avenant 2015; Rodgers et al. 2017; Jayadevan et al. 2018). This is despite *S. plumosum* being regarded as an aggressive encroacher of the Grassland Biome of South Africa (Mucina & Rutherford 2006). The encroaching aetiology of *S. plumosum* is well documented (Jordaan 2009; Buitenwerf et al. 2011; Snyman 2012; Van Zyl & Avenant 2018), with previous research indicating that *S. plumosum* follows a pioneer plant growth strategy by mainly encroaching grasslands after soil disturbance through overgrazing and trampling (Roux 1969; Jordaan 2009; Snyman 2012; Avenant 2015). With a root system ensconcing 1 m^2 of the soil surrounding the bush, and reaching depths of 1.8 m (Jordaan 2009; Snyman 2012; Van Zyl & Avenant 2018), this shrub allows for tight soil binding, which may limit the burrowing services of ecosystem engineers in densely encroached areas (Vahrmeijer 2017; Uys 2018).

Ecosystem services provided directly or indirectly by ecosystem engineers include soil development, increased soil fertility, reduction in soil erosion, an increase in nutrient cycling and even food provision (Laundré & Reynolds 1993; Gabet et al. 2003; James & Eldridge 2007; Martin 2017; Rodgers et al. 2017). Landscape development by bioturbation (the movement or reworking of soil by burrowing organisms) creates and transforms habitats by physically altering the species and community specific distribution of resources within ecosystems (Gabet et al. 2003; Martin 2017). However, soil characteristics and the availability of suitable food sources influence the abundance, ecology and behaviour of ecosystem engineers such as semi-fossorial mammals. Medium (15–30 cm) and large (34–100 cm) sized burrows created by, for example, yellow mongoose, *Cynictis penicillata*, suricates, *Suricata suricatta*, aardvark, *Orycteropus afer*, and Cape porcupine, *Hystrix africaeaustralis,* create microhabitats within their burrows (Ewacha et al. 2016; Rodgers et al. 2017) providing refuge to many different species such as invertebrates, rodents, birds and reptiles (Davidson et al. 2012; Rodgers et al. 2017). However, it is speculated that the abundance of burrows and associated microhabitats decrease as shrub growth intensifies and soils become less productive in open grassland habitats (James & Eldridge 2007; Rodgers et al. 2017). With a decrease in burrow abundance, it is expected that burrowing mammal diversity would decrease as shrub encroachment leads to a reduction in available resources such as productive soils and food sources (Iribarren & Kotler 2012; Kgosikoma et al. 2012; Jayadevan et al. 2018).

The thickening of *S. plumosum* in grassland habitat and its relationship with elusive burrowing mammals are of interest to us. Here we investigate if there is a correlation between burrow abundance and *S. plumosum* density and if there is a difference between burrowing mammal diversity in encroached and non-encroached areas. We predict that a negative correlation will exist between burrow abundance and *S. plumosum* density and that encroached areas will have lower burrowing mammal diversity than non-encroached areas.

Study area

The Telperion Nature Reserve (25°38'S, 29°01'E), measuring some 7 350 ha, is located northeast of the town Bronkhorstspruit on the border of the Gauteng and Mpumalanga provinces of South Africa (Figure 1). The vegetation on the Telperion Nature Reserve is described as Mesic Highveld Grassland, comprising grass plains, wooded areas and vegetated mountainous or rocky areas (Figure 1; Mucina & Rutherford 2006). The area is characterised by having a mean annual precipitation of 726 mm and a mean annual temperature of 14.7°C (Mucina & Rutherford 2006). Telperion Nature Reserve contains a variety of large mammal species, including giraffe, *Giraffa camelopardalis*, eland, *Tragelaphus oryx*, plains zebra, *Equus quagga*, waterbuck, *Kobus ellipsiprymnus* and blue wildebeest, *Connochaetes taurinus*, and carnivores such as leopard, *Panthera pardus,* and brown hyaena, *Parahyaena brunnea*. Smaller mammal species on the reserve include Cape porcupine, yellow mongoose, South African springhare, *Pedetes capensis*, and black-backed jackal, *Canis mesomelas* (Fagir et al. 2015).

Methods

Shrub density and burrow abundance measurements

Sampling took place from April 2018 to July 2018. We measured *S. plumosum* abundance in the northern and

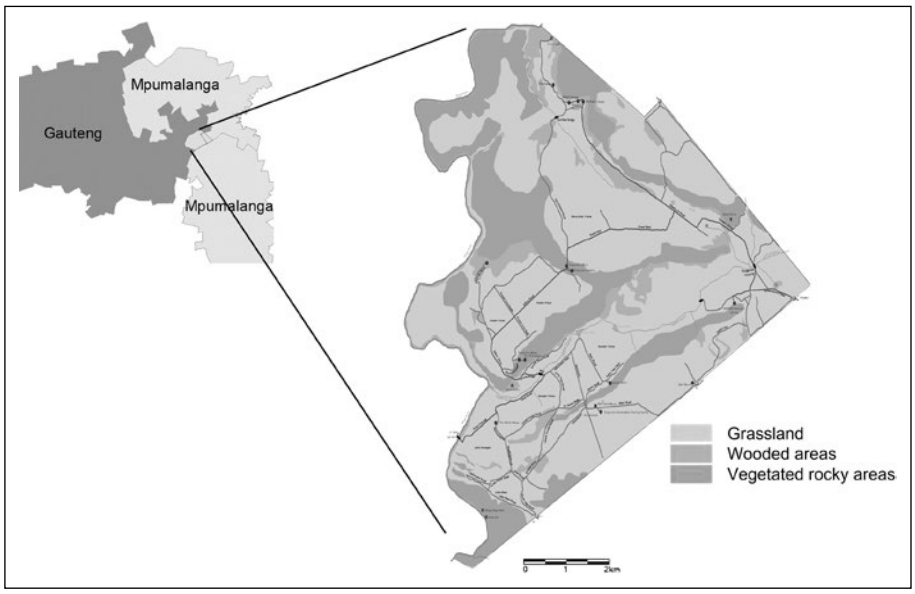

Figure 1. The Telperion Nature Reserve is situated on the border of the Gauteng and Mpumalanga provinces of South Africa. The enlarged area depicts the extent of the Telperion Nature Reserve, including the distribution of the vegetation types defined by Mucina and Rutherford (2006).

southern grasslands of the Telperion Nature Reserve. Here we classified the two grassland areas into two categories based on the estimated cover of *S. plumosum*. In doing so, we used an adapted Domin-Krajina (DK) cover abundance scale to select ground cover classes of the area occupied by *S. plumosum* in each area (Mueller-Dombois & Ellenberg 1974; Herrick et al. 2005). We demarcated 24 sampling plots, 12 encroached and 12 non-encroached plots, respectively, each measuring 25 × 25 m (625 m^2). All *S. plumosum* individuals in the plot were visualised into the centre of the plot and compared to the cover class (DK Class) (Figure 2). If the ground cover of *S. plumosum* was 10% or less, the area had little to no encroachment. The area was classified as densely encroached when the ground cover was estimated to exceed 10% (Westfall & Panagos 1984; Herrick 2005). We randomly allocated the centre position of each sampling plot within the encroached and non-encroached areas. All sampling plots were placed at least 50 m from the nearest road. To estimate the abundance of *S. plumosum,* we divided each quadrat into 25 blocks, 5 × 5 m in size, and counted the total number of adult shrubs in each sample plot. These data were expressed as the *S. plumosum* density (shrubs/m^2) per sampling plot. We did not count any of the shrubs with less than 50% of their base within the quadrat (Elzinga et al. 1998; Van Zyl & Avenant 2018). Following the work by Avenant (2015), we considered adult shrubs to have a stem height taller than 45 cm.

We counted the medium (15–30 cm) and large (34–100 cm) mammalian burrows in each sampling plot and calculated burrow abundance and density. We measured the width and length of each burrow entrance with a tape measure (model number 30-657, Stanley Black and Decker, USA) to identify medium and large mammalian burrows (Rodgers et al. 2017).

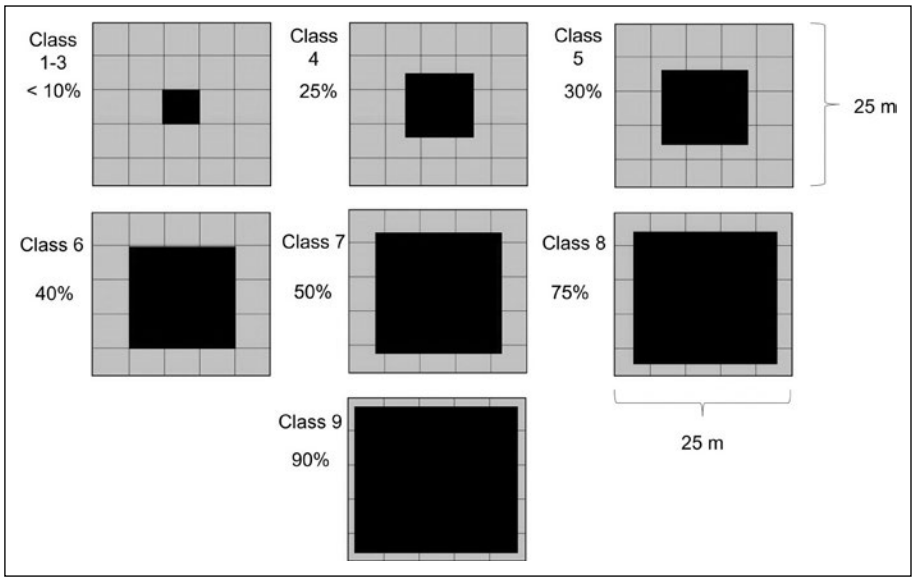

Figure 2. Adapted Domin-Krajina cover class (DK class) method indicating the major cover percentage for a 25 × 25 m plot. No encroachment is indicated by less than 10% cover (cover class of 1 to 3). Class 1 indicates a 0% cover; Class 2 indicates a cover of more than 0% but less than 10%; Class 3 indicates a cover of 10%. Encroached plots are represented by classes 4 to 9, respectively, according to the percentage cover in the 25 × 25 m plot (Mueller-Dombois & Ellenberg 1974; Herrick et al. 2005).

Mammalian diversity

We placed one camera trap (Browning Strike Force HD PRO, model BTC – 5HDP, Browning trail cameras, Alabama, USA) at the edge of each of the six plots in the encroached and non-encroached areas. Cameras were deployed from April 2018 to July 2018. Each camera trap was fixed onto a metal fence pole 50 cm above the surface of the ground and left to capture animal activity within the selected plot. Cameras were set to burst mode, with three pictures taken over 10 seconds when triggered. Pictures within 30 minutes of the same animals captured were not used in data analyses. The locations of all camera traps were recorded with a handheld GPS (Garmin eTrex® 10, Garmin Consumer Electronics, USA). We classified burrowing mammals into two categories based on their length and mass. We considered mammals with a reported total length ≤ 90 cm and mass ≤ 14 kg as medium sized, while those with a total length ≥ 100 cm and mass ≥ 15 kg were considered large burrowing mammals. We used Skinner and Chimimba (2005) as our reference work on burrowing mammal size.

Statistical analyses

Data were statistically analysed in R (version 3.4.0) and RStudio (version 1.0.143, RStudio: Integrated development environment for R, Boston, USA). We used paired t-tests to determine if the mean shrub density and burrow abundance differed between the encroached and non-encroached areas. We used Spearman's rank correlation to investigate the relationship between burrow abundance and *S. plumosum* encroachment, and the Shannon Wiener Index (H where $H=\sum[(pi)\times\ln(pi)]$) using the VEGAN package (version 2.4-3, RStudio (version 1.0.143) (Oksanen et al. 2013) to assess species diversity in the two areas. We also conducted an Analysis of Similarity (ANOSIM) (Jaccard) of the two areas using the VEGAN package (version 2.4-3, RStudio (version 1.0.143) (Oksanen et al. 2013).

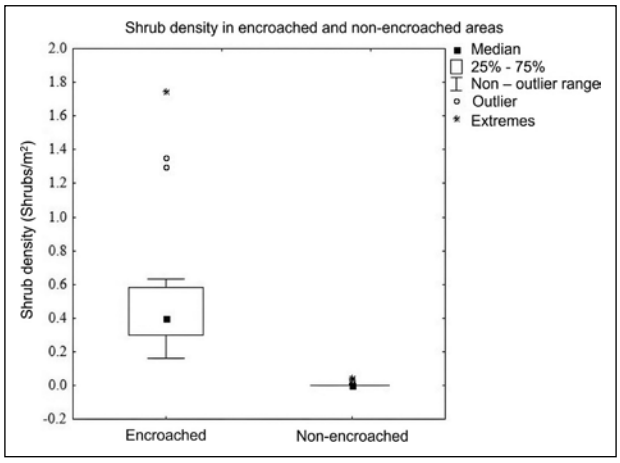

Figure 3. The observed *S. plumosum* density (shrub/m²) recorded in the encroached and non-encroached areas of the Telperion Nature Reserve.

Results

Shrub and burrow density

In the encroached and non-encroached areas, the *S. plumosum* shrub density ranged from 0.16 to 1.74 shrubs/m² and 0–0.04 shrubs/m², respectively (Figure 3). The recorded densities are equivalent to up to 17 400 *S. plumosum* shrubs in the encroached areas, while the non-encroached areas had up to 400 *S. plumosum* shrubs. The mean shrub density in the encroached areas (0.5 ± 0.4 shrubs/m²) was significantly higher than in the non-encroached areas (0.004 ± 0.01 shrubs/m²; $t = 6.59$, $p < 0.001$).

We found three burrows (two medium and one large-sized) spread across two (8.3%) of the 24 encroached sampling sites. We found 57 burrows distributed across 15 (62.5%) of the non-encroached sampling areas. Of these, 22 burrows were medium-sized and 35 were large-sized. Overall, the mean burrow density in the encroached areas (0.0002 ± 0.0007/m²) was significantly lower than in the non-encroached areas (0.004 ± 0.005/m²; $t = -3.48$, $p = 0.002$).

The mean abundance of medium sized burrows was significantly lower in the encroached areas (0.083 ± 0.408 per 625 m²) than in non-encroached areas (0.92 ± 2.02 per 625 m²; $t = -2.03$, $p = 0.05$). Similarly, the mean abundance of large burrows was lower in the encroached (0.041 ± 0.204 per 625 m²) than in the non-encroached areas (1.46 ± 1.95 per 625 m²; $t = -3.47$, $p = 0.002$). There was a significant moderate negative correlation between total burrow abundance and shrub density (Figure 4) (Spearman rank correlation test: $rho = -0.456$, $p = 0.001$).

Diversity of burrowing mammals

The total number of camera trap days equalled 64 in the encroached and 72 in the non-encroached areas. We obtained images of burrowing mammals from seven of 12 camera traps, i.e., from three of six cameras deployed in the encroached areas and from four of six cameras deployed in the non-encroached areas. The camera traps captured seven burrowing species, including three species of medium sized burrowing mammal and four species of large burrowing mammals (Table 1). The common warthog, *Phacochoerus africanus*, was the most sighted and the only burrowing species recorded in both the encroached and non-encroached areas. Burrowing mammal species richness was even for both areas, as four species were captured

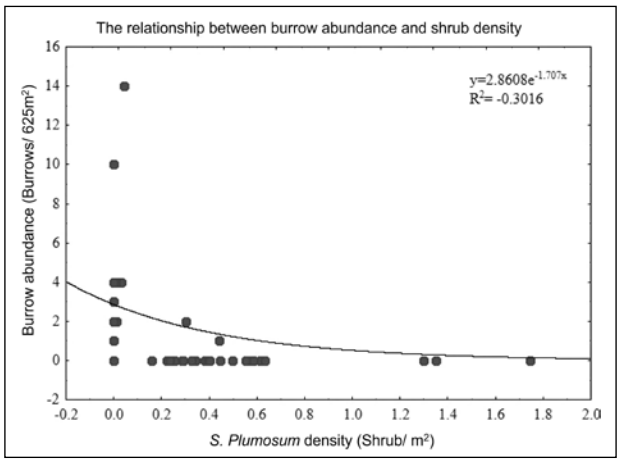

Figure 4. The negative relationship between burrow abundance (number of burrows per 625 m^2 sampling plot; grey dots) and *S. plumosum* density (shrubs/m^2) depicted for all sampling plots in both encroached and non-encroached areas in the Telperion Nature Reserve.

in non-encroached areas and four in encroached areas. The mean Shannon Wiener diversity index (H) for encroached and non-encroached areas was 0.45 ± 0.3 and 0.12 ± 0.22, respectively. Our ANOSIM indicated that the diversity of burrowing mammals had no significant difference (R = -0.231, p = 0.933) between the encroached and the non-encroached areas.

Discussion

Burrow and shrub relationship

Although absent in some of our sample plots, we found a mean *S. plumosum* density ranging from 40 plants per hectare in the non-encroached areas to 5 000 plants per hectare in the encroached areas. Our results on *S. plumosum* density align with those of Graham et al. (2020), who reported densities of up to 9 500 *S. plumosum* individuals per hectare on the Telperion Nature Reserve. Both medium and large mammalian burrows occurred at lower densities in the encroached than non-encroached areas, and we found a negative relationship between burrow abundance and shrub density. Despite our limited sampling across the Telperion Nature Reserve, these results support our first prediction. Moreover, our results correspond with earlier studies that reported a higher prevalence of medium and large sized burrows in open pastures and grasslands compared to woody, bushy areas (Butynski & Mattingly 1979; Melton & Daniels 1986; Augustine et al. 1995; Whittington-Jones 2006; Whittington-Jones et al. 2011; Rodgers et al. 2017). Many burrowing mammals prefer open grasslands to burrow in rather than dense shrublands, presumably because open grasslands increase predator detection and tend to have higher food and spatial availability (Melton & Daniels 1986; Davidson et al. 2012; Jayadevan et al. 2018).

Our camera trapping results revealed no significant similarity in burrowing mammal diversity between the encroached and non-encroached areas. Therefore, we cannot accept our second prediction that an increase in *S. plumosum* density would result in decreased burrowing mammal diversity. Nevertheless, Rodgers et al. (2017) recently reported higher burrowing mammal diversity on a non-encroached Namibian game reserve compared to an encroached livestock farm. While shrub and bush encroachment does not necessarily lead to the loss of habitat heterogeneity, and the encroachment effects are likely species, scale and environment specific (Eldridge & Soliveres 2014), some burrowing species have been affected negatively by bush encroachment. For example, shrub thickening in semi-arid parts of

Table 1. Burrowing species captured on the camera traps, reflecting where these species were sighted, the number of sightings for each species, the relative survey effort, and the time of the burrowing animal's activity

Species per sample area	Size*	Number of sightings	Relative survey effort	Time of activity
Encroached areas			64 days	
Orycteropus afer (aardvark)	Large	2		Night
Proteles cristata (aardwolf)	Large	1		Night
Mellivora capensis (honey badger)	Large	1		Night
Phacochoerus africanus (warthog)	Large	29		Day/Night
Non-encroached areas			72 days	
Hystrix africaeaustralis (Cape porcupine)	Medium	5		Night
Suricata suricatta (meerkat)	Medium	10		Day
Cynictis penicillata (yellow mongoose)	Medium	4		Day
Phacochoerus africanus (warthog)	Large	37		Day/Night

*Based on Skinner and Chimimba (2005)

North America has contributed to population declines in prairie dogs *Cynomys* spp. (Weltzin et al. 1997); shrub thickening in semi-arid regions of Australia has had similar effects on burrowing bettongs, *Bettongia lesueur* (Noble et al. 2007). In our study, predators and burrowing mammals were only captured together on one camera in the non-encroached area. In the encroached areas, no camera trap yielded a picture of a predator and a burrowing mammal in the same plot. Even though we did not calculate for predators in the vicinity of the burrowing mammal diversity, it was noted that black-backed jackals were sighted more in the non-encroached areas than in the encroached areas. Predators play an important role in the distribution of prey species such as ecosystem engineers, and we must investigate how predators can influence the distribution of ecosystem engineers in the Telperion Nature Reserve (Melton & Daniels 1986; Davidson et al. 2012; Jayadevan et al. 2018).

On the Telperion Nature Reserve, where *S. plumosum* is spread across an estimated 30% of the reserve (Brown, unpublished data), it was interesting to note that the less common burrowing species, such as aardvark and aardwolf (Table 1), which prefer feeding in open areas with termite mounds (Melton & Daniels 1986; Williams & Richardson 1997; Whittington-Jones 2006; Stuart 2015; Rodgers et al. 2017), were only recorded in our encroached sampling areas during the current study. Although we have not consistently quantified termite mound availability as part of this study, termite mounds were prevalent in the encroached areas that we sampled as part of this study. The presence of aardvark and aardwolf in only the encroached areas may, therefore, be an artefact of our sample site selection. Moreover, the extent to which the *S. plumosum* encroachment, which has not been quantified across the Telperion Nature Reserve, has affected these less-common species is not well-understood at present. However, elsewhere in South Africa, simulations have predicted likely negative effects of continued encroachment on local mammal diversity and abundance (Soto-Shoender et al. 2018). These predicted effects are in line with earlier work reporting that shrub thickening negatively influenced the abundance of medium sized burrowing carnivores (Blaum et al. 2007). It is not inconceivable, therefore, that increasing shrub cover and a decreased abundance in burrowing mammal populations could lead to a decrease in the ecosystem services that burrowing mammals provide (Carbutt et al. 2011; Davidson et al. 2012).

These services include changes in soil structure and nutrients through the creation of burrow mounds, burrow networks, and the presence of latrine sites close to burrow entrances (Melton & Daniels 1986; Davidson et al. 2012; Martin 2017; Rodgers et al. 2017), which could allow for improved water infiltration, seed germination, increased nutrients and landscape heterogeneity to occur (James 2009; Whittington-Jones et al. 2011; Davidson et al. 2012; Haussmann 2017; Louw et al. 2017). Burrowing mammals are vital in creating microhabitats (Blanco-Perez et al. in prep.) and thermal refuges for a range of other vertebrates (Weyer et al. 2020). Therefore, the disappearance of keystone species, including the burrowing ecosystem engineers, may result in the structural change of grassland habitats and can lead to cascading effects on burrowing mammal ecosystem services, other taxa and possibly biodiversity (Davidson & Lightfoot 2008; Martinez-Estevez et al. 2013; Meysman et al. 2006). In grassland areas it may, therefore, be beneficial to monitor burrowing mammal density and burrow occupancy to highlight potential changes in the ecosystem services provided. Furthermore, this will help elucidate how grassland structural changes occur and may facilitate the development of new management approaches to reduce the risk of grassland habitat change (Davidson & Lightfoot 2008; Martinez-Estevez et al. 2013; Meysman et al. 2006). Furthermore, quantifying the encroachment of *S. plumosum*, as well as the effects thereof on the burrowing mammal community, is therefore essential in the effective management of *S. plumosum* in the Telperion Nature Reserve.

A limiting factor of our study is that we did not consider burrowing networks, which may have resulted in us overestimating the number of burrows. Nevertheless, we have shown that *S. plumosum* encroachment, if not controlled in grassland areas, can likely lead to a loss in ecosystem services associated with burrowing mammals, and reduce the state of the landscape in encroached areas. However, further investigation regarding burrowing mammal populations, occupancy and behaviour on the Telperion Nature Reserve must be done to determine the influence of woody encroachment on ecosystem engineers.

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Competing interest

We have no competing interests concerning the writing of this article.

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Restoration of diversity and regeneration of woody species through area exclosure: the case of Maun International Airport in northern Botswana

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Background and objectives: Deforested and degraded areas can be cheaply and conveniently restored through establishment of exclosures. An area exclosure excludes animals and humans from accessing an area to promote natural regeneration of plants and rehabilitate ecological condition of the area. The study was aimed at: (1) determining the diversity (species richness, diversity and evenness); (2) assessing the stand structure (densities); and (3) assessing regeneration status of woody species inside and outside exclosed Maun International Airport, northern Botswana.

Methods: Vegetation sampling was conducted from April to May 2018. A total of 48 and 37 quadrats of 20 × 20 m were laid down at 50 m intervals along transect lines inside and outside Maun International Airport, respectively. Identity, number of all live individuals and height of all woody species were recorded in all the quadrats. The diversity of all woody species was analysed by using the Shannon Diversity Index (H') and regeneration status of each woody species was assessed using frequency distribution of height class.

Results: The diversity, evenness and species richness were significantly higher inside than outside Maun International Airport. *Colophospermum mopane* was the most abundant species both inside (75% of all woody species) and outside (96% of all woody species) Maun International Airport. More species showed more regeneration inside than outside Maun International Airport. The inside of Maun International Airport recorded more alien invasive woody species compared with the outside, owing to its original use as a residential area. The local communities might have introduced these species as ornamental trees.

Conclusion: This study, while limited in scale, contributes to understanding of the role of exclosures in enhancing woody species richness, diversity and evenness as well as facilitating regeneration of woody species. Degraded woodlands and other similar ecosystems could be cheaply and conveniently restored through establishment of exclosures, but more research and monitoring are required to fully understand the processes and impacts.

Keywords: density, evenness, population structure, regeneration.

Introduction

Tropical dry forests and woodlands, considered as savanna in Botswana, account for about 42% of all tropical and sub-tropical forest area (Hasnat & Hossain 2020). Forests and woodlands provide a suite of valuable ecosystem services that are important livelihood activities for most rural communities (Shackleton & Shackleton 2004), particularly the poor and vulnerable communities in sub-Saharan Africa (SSA) who strongly depend on forest and non-timber

forest products (NTFPs) for sustenance (Kabubo-Mariara 2013; Van Passel et al. 2020). The ecosystem services provided include provisioning services (e.g., fuelwood, timber, food) (Boy & Witt 2013), regulating services (e.g., carbon sequestration, erosion control and reduction of air pollution) (Morgenroth et al. 2016) and cultural services (spiritual, religious, cognitive effects and tree monuments) (Dallimer et al. 2012).

However, forests are being destroyed at an alarming rate worldwide (Elliot et al. 2013), with an estimated loss of about 1–4% of their current area per annum (Naidu & Kumar 2016). The destruction is attributable to increasing anthropogenic activities, deforestation and natural factors (Chow et al. 2013; Siyum 2020). The destruction of forests is largely driven by human and livestock populations, which result in land-use changes from forestry to agriculture and human settlement (Neelo et al. 2013), owing to their favourable climatic conditions (Ewel 1999). Moreover, climate change and its associated impacts on temperature and rainfall patterns are expected to affect dry woodlands and forests (IPCC 2014), and forests are overexploited for fuelwood, construction material and timber.

In Botswana unsustainable use and the ineffective management of mopane woodlands, as well as their conversion to other land-use types, are depriving the local communities of the full benefits that can be derived from the mopane woodlands (Makhado et al. 2014; Teketay et al. 2018). The reduction and degradation of forests calls for strategies to conserve and maintain the remaining forests and simultaneously restore deforested and degraded areas (Teketay et al. 2018). One such strategy that has been used recently to reverse deforestation and degradation is the establishment of area exclosures. An area exclosure is used as a fast, cheap and convenient approach to restoring degraded forest and woodland areas. The area exclosure is closed from animals and human access to promote natural regeneration of plants and to rehabilitate the ecological condition of the area (Teketay et al. 2018; Atsbha et al. 2019). Exclosures have been used in many countries across the world, e.g., China (Park et al. 2013; Liu et al. 2019), central America (Griscom & Ashton 2011), Australia (Bastin et al. 2003; Silcock & Fensham 2013), Scotland (Shaw et al. 2010), Iran (Ebrahimi et al. 2016), Ethiopia (Gebregerges et al. 2018; Ubuy et al. 2018; Atsbha et al. 2019; Asmare & Gure 2019), Kenya (Wairore et al. 2016) and South Africa (Mbatha & Ward 2010).

Various studies on the area exclosure have been conducted in Botswana, including in Mokolodi Nature Reserve (MNR) in southern Botswana (Flyman 1999; Käller 2003; Bengtsson-Sjörs 2006; Teketay et al. 2016) and also in northern (Neelo et al. 2015; Teketay et al. 2018) parts of the country. In the case of the studies in MNR, Flyman (1999) excluded herbivores to determine the fate of seedlings of woody species, Käller (2003) investigated growth pattern and reproduction of woody vegetation and Bengtsson-Sjörs (2006) studied establishment and survival of woody seedlings. Recently, Teketay et al. (2016) found that most woody species in MNR exhibited unstable population structure and hampered natural regeneration following exposure to overgrazing and other heavy anthropogenic impacts. In northern Botswana, studies on exclosure were conducted in sites close to the current study area by Neelo et al. (2015) and Teketay et al. (2018). Neelo et al. (2015) discovered that exclosure had similar diversity and density values compared with open areas and attributed such observations to heavy over-grazing and cutting of trees before establishment of the exclosure, as well as to seasonal flooding of a large portion of the exclosed area owing to its proximity to Thamalakane River. In the study reported by Teketay et al. (2018), mean density, population structure and regeneration status of woody species inside the exclosure was better than outside. All these studies on area exclosures in Botswana were conducted on formerly degraded grazing lands. Studies on the impact of exclosure on areas formerly used as residential areas or human settlement are limited in Botswana.

Therefore, this study aimed at conducting a comparative study on woody species diversity, stand structure of woodlands and regeneration status of the woody species in a ten-year area exclosure (inside Maun International Airport) and open area adjacent to Maun International Airport, northern Botswana. The specific objectives of the study were to: (1) determine the diversity (species richness, diversity and evenness); (2) assess the stand structure (densities and frequencies); and (3) assess regeneration status of woody species inside and outside the area exclosure.

Materials and methods

Study area

The study was conducted in Maun Village, Ngamiland District, northern Botswana (Figure 1). The village is located within the Okavango Delta, which is the distal part of the Okavango River Basin. The Delta originates in the Angolan highlands where the Cuito and Cubango river catchments receive 876 and 983 mm of rain per annum, respectively (Wolski & Murray-Hudson 2008). The Okavango River then discharges 10 km^3 into the alluvial fan of about 12 000 km^2 (McCarthy 2006). The flood wave peak discharge at the Panhandle is between April and May, and then meanders across 250 km of seasonal floodplains to arrive at Thamalakane River in Maun between July and August (McCarthy et al. 2000; Mazvimavi & Mmopelwa 2006).

The Okavango Delta is a globally renowned Wetland of International Importance and Ramsar site and was

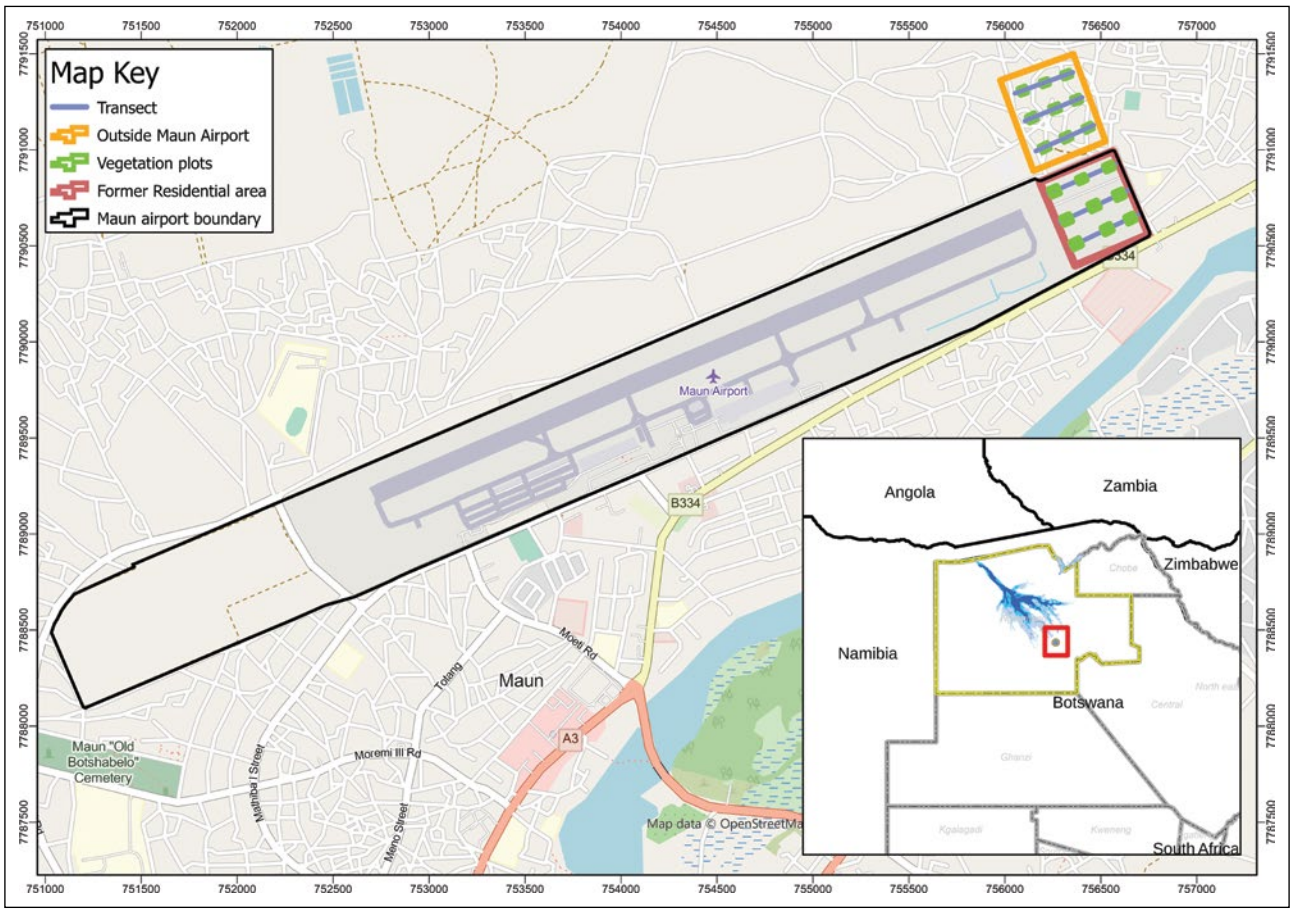

Figure 1. Map showing location of the study area.

inscribed as UNESCO's 1000th World Heritage Site in 2014. The stunning scenery of the Delta, characterised by an array of plant and animal life, swamps and islands, attract thousands of tourists who visit every year (Mbaiwa 2018). The tourism sector is only surpassed by mining and contributes about 4.5% to the country's Gross Domestic Product (Mbaiwa & Hambira 2020). The riparian communities depend on the Delta's ecosystem resources for their livelihoods. Common livelihood activities include dry and flood recession farming, fishing, collection of veld products, harvesting of thatching grass and reeds, basket making and tourism (Blackie & Casadevall 2019).

Study site

The first study site was Maun International Airport (MIA) (Figure 1), located on Kalahari sandveld with > 90% sand (Veenendaal et al. 2008). Annual rainfall is quite variable, averaging between 450 and 500 mm, falling in one distinct season between November and April (Moore & Attwell 1999). Maun is the fifth largest village in Botswana with a population of 60 263 (Statistics Botswana 2014). MIA is the second busiest airport to Sir Seretse Khama International Airport (SSKIA) in terms of the number of passengers (CAAB 2019). It caters for both domestic and international flights. Owing to its proximity to the Okavango Delta, it serves as the gateway for tourists visiting the delta. MIA traffic is dominated by small single- and twin-engine aircrafts that fly daily to and from airfields in the Delta. In 2014, there were 24 864 landings, 24 870 departures and 234 896 passengers (Mmolai 2015). In response to an increase in air traffic within MIA, the Government of Botswana relocated 1 595 families within the vicinity of the airport for expansion and upgrading of the airport in 2006. The area has been a village settlement since 1985 (21 years) when it was allocated for residential use. The area was fenced in 2010, enclosing the formerly residential areas (or human settlement) to be part of MIA. The study site was therefore exclosed by a fence for ten years at the time of the current study. The inside and outside sites of MIA were adjacent and only separated by a fence. The outside of MIA was a communal area that was used for grazing and harvesting of fuelwood. At the time of the study, the exclosure (inside MIA) represents a site with low anthropogenic disturbances and outside the exclosure (outside MIA) represents the site with high anthropogenic disturbances due to open access to grazing animals and harvesting of woody species.

Data collection

The species, genera, family, diversity (richness and evenness) and regeneration status of the woody species was

Table 1. Comparison of diversity indices and total density of woody species inside and outside of Maun International Airport

Site	Density (individuals ha^{-1})	H′	E	Species richness
Inside	2 642	1.06	0.43	26
Outside	6 435	0.21	0.07	12

determined in March 2020 by laying six and five parallel line transects, 50 m apart inside and outside MIA, respectively. The number of transect lines was informed by the size of the area and spatial heterogeneity of the vegetation. On the transect lines, quadrats measuring 20 × 20 m (400 m^2) were laid down at 50 m intervals, leading to a total of 48 quadrats inside and 37 quadrats outside MIA. The first quadrat was placed 20 m away from the first transect line to minimise the border effect. Following the procedure adopted by Neelo et al. (2013, 2015) and Teketay et al. (2016, 2018), the following parameters were recorded in each of the quadrats: identity of all woody species, number of all live individuals of each woody species and height of all woody species. A graduated 20 mm polyvinyl chloride (PVC) conduit was used to measure plant height.

The woody species were identified directly at the sites using books published on the flora of Botswana (Heath & Heath 2009; Setshogo 2002, 2005; Setshogo & Venter 2003) and with assistance from the forest officers and local communities familiar with the flora. Where species could not be identified, herbarium specimens were collected, and photographs were taken for later identification at the Peter Smith University of Botswana Herbarium (PSUB). In this article, woody species nomenclature follows that of Setshogo and Venter (2003) and Setshogo (2005).

Data analysis

The diversity of woody species was analysed using the Shannon Diversity Index (*H′*). Evenness (*E*), or equitability, measures similarity of the abundance of the different woody species in the different habitats and was analysed by using Shannon's Evenness Index. Its value ranges from 0 to 1, with 1 being complete evenness.

Regeneration status of each woody species in the two sites was assessed using frequency distribution of diameter classes. Histograms were constructed by using the density of individuals of each species categorised into five height classes i.e., 0.0–0.5 m; 0.5–1.0 m; 1.0–2.0 m; 2.0–4.0 m and > 4.0 m. The woody species were then grouped according to the pattern of the histograms.

Results

Species diversity and density

Species accumulation curves show that all species were likely to have been sampled in both areas (Figure 2). The comparison of diversity (*H′*), evenness (*E*), species richness and density are shown in Table 1.

The four most common woody species inside MIA were *Colophospermum mopane, Leucaena leucocephala, Vachellia erioloba* and *Dodonaea angustifolia*. These four species dominated outside MIA (Table 2), and *C. mopane* was found to be the most abundant species both inside (75% of all woody species) and outside (96% of all woody species) MIA (Figure 3). Five invasive alien plant species were recorded inside MIA.

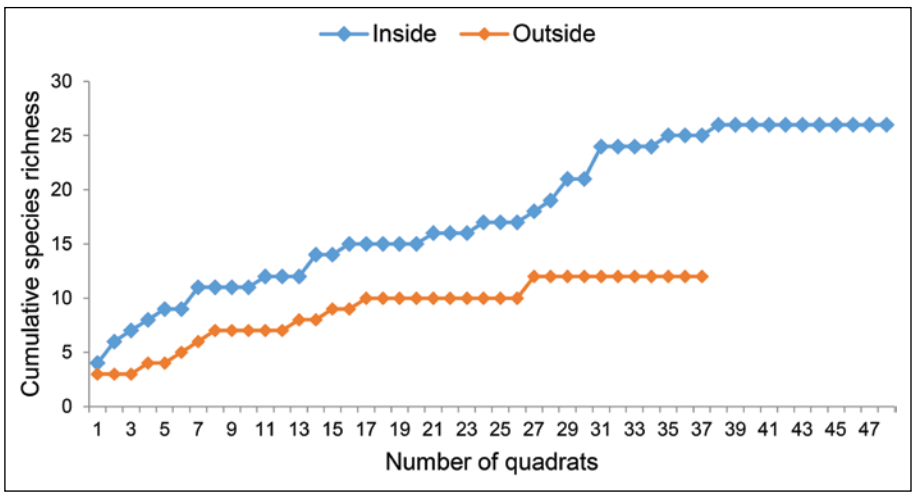

Figure 2. Species accumulation curves for woody species inside and outside MIA.

Table 2. Mean density per hectare, life form and family names of woody species recorded inside and outside MIA

Species	Life form	Family	Density (individuals ha^{-1}) ± SEM	
			Inside	Outside
Colophospermum mopane	Tree	Fabaceae	1971 ± 398	6201 ± 872
*Leucaena leucocephala**	Shrub/small tree	Fabaceae	266 ± 64	0
Vachellia erioloba	Tree	Fabaceae	150 ± 73	130 ± 41
*Dodonaea angustifolia***	Shrub	Sapindaceae	77 ± 20	5 ± 4
Sclerocarya birrea	Tree	Anacardiaceae	56 ± 31	0
Vachellia tortilis	Tree	Fabaceae	25 ± 18	44 ± 32
Berchemia discolor	Tree	Rhamnaceae	15 ± 9	2 ± 2
Philenoptera nelsii	Tree	Fabaceae	14 ± 11	1 ± 1
Grewia bicolor	Shrub	Malvaceae	8 ± 6	0
Phyllanthus reticulatus	Shrub	Phyllanthaceae	7 ± 4	0
*Ailanthus altissima**	Tree	Simaroubaceae	7 ± 7	0
Combretum mossambicense	Tree	Combretaceae	6 ± 3	0
Combretum imberbe	Tree	Combretaceae	5 ± 4	0
*Ricinus communis**	Shrub/small tree	Euphorbiaceae	5 ± 5	0
Senegalia mellifera	Tree	Fabaceae	5 ± 5	2 ± 2
Boscia albitrunca	Tree	Capparaceae	5 ± 4	19 ± 13
*Jatropha curcas**	Shrub/small tree	Euphorbiaceae	4 ± 3	0
Ziziphus mucronata	Shrub/small tree	Rhamnaceae	4 ± 3	18 ± 12
Dichrostachys cinerea	Tree	Fabaceae	3 ± 3	6 ± 6
Philenoptera violacea	Tree	Fabaceae	2 ± 2	1 ± 1
Hyphaene petersiana	Tree	Arecaceae	2 ± 2	0
Combretum collinum	Tree	Combretaceae	1 ± 1	0
Croton megalobotrys	Tree	Euphorbiaceae	1 ± 1	0
Terminalia prunioides	Small tree/shrub	Combretaceae	1 ± 1	6 ± 5
Terminalia sericea	Tree	Combretaceae	1 ± 1	0
*Jacaranda mimosifolia**	Tree	Bignoniaceae	1 ± 1	0
Total			2 642 ± 281	6 435 ± 688

* = Invasive alien species; ** = Exotic species

Interestingly, the invasive woody species, *L. leucocephala* was the second most abundant species inside and was absent outside MIA. Similarly, other invasive woody species (*Ricinus communis, Jatropha curcas, Ailanthus altissima* and *Jacaranda mimosifolia*) were encountered inside, but not found outside MIA. Some indigenous fruit-bearing woody species, such as *Berchemia discolor, Boscia albitrunca* and *Ziziphus mucronata* were found both inside and outside MIA. Other indigenous fruit-bearing species (*Sclerocarya birrea, Grewia bicolor* and *Hyphaene petersiana*) were only present inside MIA.

Regeneration status

Assessment of the regeneration structure of woody species inside MIA produced four regeneration patterns (Figure 4). The first pattern showed a high number of individuals in the shorter height classes and a gradual decline towards the tallest classes (Figure 4A, B, C and D). Such a 'reverse J-shaped' pattern was evident for *C. mopane, V. erioloba, Grewia bicolor, Phyllantus reticulatus, D. angustifolia* and *V. tortilis*. The species in the second pattern showed a lack of individuals in the shortest height class and no individuals in the tallest height classes (Figure 4E,

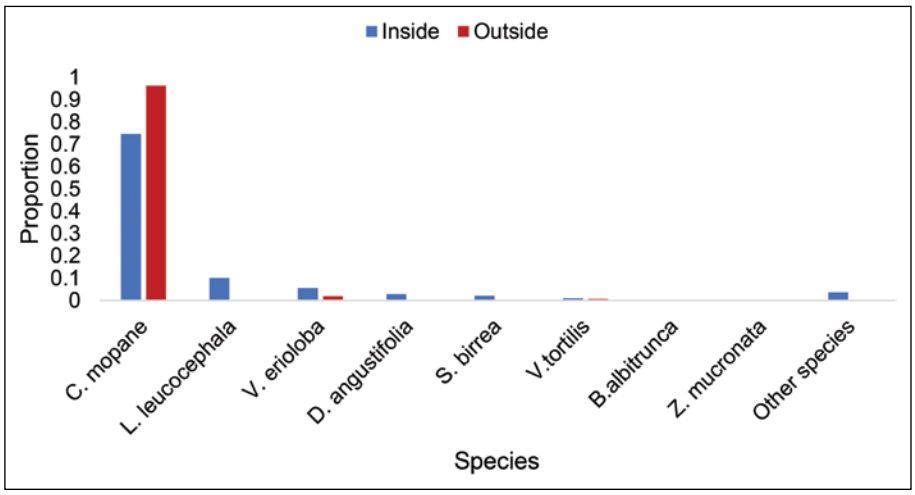

Figure 3. Proportion of individual woody species recorded in total across all quadrats.

and F). This pattern was illustrated by *B. albitrunca, Combretum mossambicense, J. curcas, R. communis, Ziziphus mucronata* and *Berchemia discolor.* The third pattern was composed of species that showed a high number of individuals in the short height class and a low number of individuals in the tall height class, with no individuals in the middle height class (Figure 4G and H). Species showing this pattern were *L. leucocephala* and *Sclerocarya birrea*. The fourth pattern showed hampered seedling recruitment with no individuals in the taller height classes (Figure 4I and J). This pattern was exemplified by *Philenoptera nelsii* and *Senegalia mellifera*. The fifth pattern was found in rare species, and it showed species with one middle or higher height class (Figure 4K and L). This pattern was represented by *A. altissima* and *D. cinerea*.

The distribution of the woody species outside MIA was categorised into four regeneration patterns. The first group showed high numbers of individuals in the shortest height classes and a progressive decline towards the middle and upper height classes (Figure 5A, B, C and D). This group was represented by *D. cinerea, Z. mucronata, V. erioloba* and *V. tortilis*. The second group showed interrupted 'reverse J-shaped' pattern, i.e., high numbers of individuals in the shortest height class and few or no individuals in the middle and upper height classes (Figure 5E). This pattern was only exhibited by *C. mopane*. The third group showed no individuals in the shortest height classes (i.e., seedlings) and in the taller height classes (Figure 5F, G and H). To this group belong *D. angustifolia*, *T. prunioides* and *B. albitrunca*. The species in the fourth group showed dominance of individuals in a single height class (Figure 5I, J, K and L). This group included *B. discolor, C. hereroense, P. nelsii, S. mellifera, H. petersiana* and *P. violacea*.

Discussion

The study revealed a substantial difference between the inside and outside of MIA in terms of species diversity, evenness and regeneration status of the woody species. The Shannon Diversity Index (*H'*) of the woody species inside was five times greater than outside MIA. The difference in anthropogenic disturbances in the two areas resulted in the differences in species richness and evenness. The presence of five alien invasive plant species also partly contributed to higher richness inside MIA. There were more woody species and more even distribution of individuals of different species inside compared with outside MIA. Continuous harvesting of woody species for fuelwood and construction as well as annual fires may account for lower species richness and diversity outside MIA. The site outside the exclosure is part of communal rangelands, and therefore subjected to heavy browsing, mainly by domestic animals, but also by wild animals. Layers of grass were absent at both areas and are not likely to have influenced recruitment or regeneration. Wildfires are common in Botswana (Maabong & Mphale 2021), and therefore fire might have influenced species diversity and richness at either site.

The diversity and evenness observed inside and outside MIA are lower than those recorded from open areas in Botswana, e.g., in Shorobe ($H' = 2.18$ and $E = 0.6$) and Xobe ($H' = 1.5$ and $E = 0.5$) (Neelo et al. 2013) and also from an exclosed woodland at Island Safari Lodge ($H' = 2.16$ and $E = 0.6$) and Okavango Research Institute compound ($H' = 2.42$ and $E = 0.75$) (Teketay et al. 2018). The lower diversity recorded for MIA could be explained by its historical use for human settlement or residential purposes. It can be argued that during its time as a residential area, most woody species inside MIA were frequently harvested for fuelwood and construction. Regeneration of woody species subjected to such disturbances is influenced by several factors (Teketay et al. 2018). When a heavily harvested site is excluded from anthropogenic disturbances and herbivory impacts, as was the case with MIA, vegetation regenerates quickly through seedling recruitment from the soil seed bank and coppicing from stumps (Teketay 2005). The soil seed bank is recognised as the main pathway of regeneration of most woody species (Whitmore 1996).

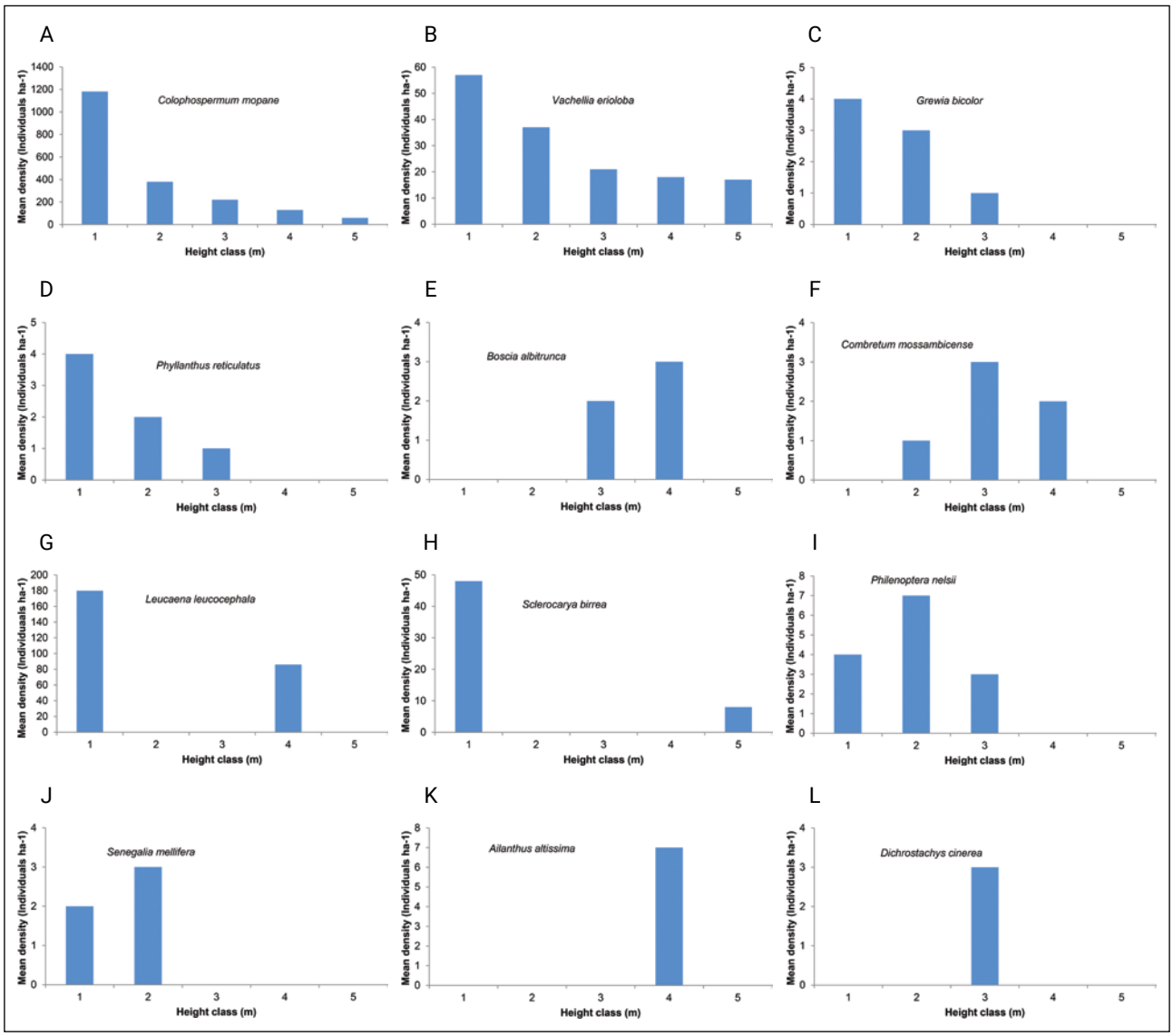

Figure 4. Population structure of woody species inside Maun International Airport. Height classes: 1 = 0–0.5; 2 = 0.5–1; 3 = 1–2; 4 = 2–4; and 5 = > 4 m.

In the current study, large numbers of seedlings were recorded for the dominant species (*C. mopane*) inside and outside MIA, suggesting that seed rain is the major regeneration strategy.

Woody species examined revealed substantial variations in their height class distributions, which indicates different adaptation capacity of the species to the prevailing environmental conditions and disturbances. Six and four species inside and outside MIA, respectively, exhibited a 'reverse J-shape' curve with continuous height class distributions, which implies healthy regeneration (Inoussa et al. 2017; Teketay et al. 2018; Asmare & Gure 2019). It also confirms the role of the exclosure in conservation of natural resources. However, in a mopane woodland area, exclosure may facilitate regeneration of *C. mopane*, resulting in thickets of mopane with low herbaceous productivity and diversity. This implies that the use of exclosures in rehabilitation of degraded woodlands should be used with caution.

The dominant species *C. mopane* displayed healthy regeneration inside and hampered regeneration outside MIA, even though there were very high densities of seedlings outside the exclosure area, suggesting that human impacts, such as cutting and logging, are disrupting regeneration of this species. It is commonly used as firewood because it burns slowly and produces good charcoal (Tietema et al. 1991), as well as for construction and fencing due to its resistance to rotting, termite and powderpost beetle ((https://www.wood-database.com/mopane/ accessed on 02-07-2020).

Dichrostachys cinerea and two species of *Vachellia*, namely *V. erioloba* and *V. tortilis*, exhibited healthy regeneration outside MIA despite pressure from anthropogenic activities and herbivory impacts. This may indicate bush encroachment due to overgrazing (Neelo et al. 2015; Teketay et al. 2016). The leaves of *V. tortilis* and *V. erioloba* are nutritious (Tolsma et al. 1987; Moleele 1998), but the presence of thorns limit browsing by herbivores

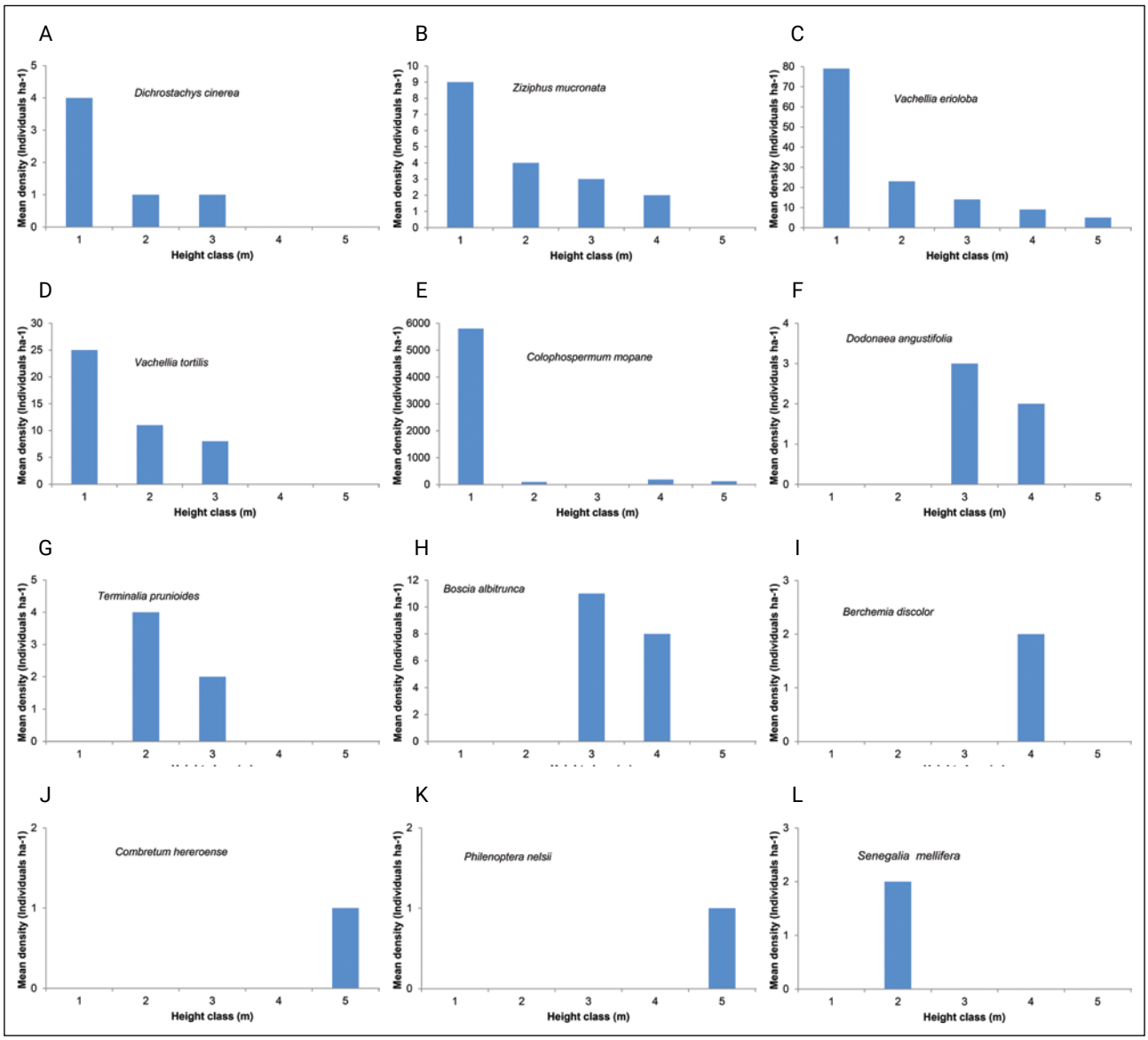

Figure 5. Population structure of woody species outside Maun International Airport. Height classes: 1 = 0–0.5; 2 = 0.5–1; 3 = 1–2; 4 = 2–4; and 5 = > 4 m

and, as a result, the species proliferate into trees and shrubs (Moleele & Perkins 1998). *Dichrostachys cinerea* is an aggressive invader particularly in overgrazed areas (Teketay et al. 2016). It is reported to be, most likely, stimulated by disturbances, such as fire and browsing (Wakeling & Bond 2007; Wigley et al. 2014).

Invasive woody species were only present inside MIA and showed either hampered regeneration (*L. leucocephala*) or hampered seedling recruitment (*J. curcas*, *R. communis* and *A. altissima*). The presence of invasive species is not surprising as the site was previously a residential area and local communities might have introduced them as ornamental trees, whereas the area outside the MIA was a communal area that was used for animal grazing and harvesting of fuelwood. *Ailanthus altissima* (also known as prison tree and tree-of-heaven) is planted in many countries as an ornamental tree (Iverson et al. 2019). It is an aggressive invader that spreads from root sprouts and grows rapidly to produce large quantities of seeds (Call & Nilsen 2005) that are wind dispersed (Bory & Clair-Maczulajtys 1980). *Ricinus communis* (also known as castor bean) is a fast-growing small tree that produces large quantities of toxic seeds (Kuete 2014) and has been rejected for use as biofuel crop due to its high invasive potential (Gordon et al. 2011). *Leucaena leucocephala* is a nitrogen-fixing tree-legume (Bageel et al. 2020) that grows vigorously to colonise disturbed vegetation. It has spread aggressively around the world (De Sousa Machado et al. 2020), and it is declared a Category 2 weed in South Africa (Henderson 2001). *Jatropha curcas* has spread rapidly in Asia and Africa where it is promoted as an ornamental and hedge plant (www.cabi.org/isc/data sheet). It is classified as a high-risk plant (Gordon et al. 2011; Negussie et al., 2013). Its cultivation is prohibited in Australia (PIER 2008), South Africa (GISP 2008) and Hawaii (USDA-NRCS 2008).

Edible fruit-bearing woody species displayed healthy regeneration (*G. bicolor*), hampered seedling recruitment (*B. albitrunca* and *B. discolor*) and regeneration (*S. birrea*) in the exclosure. Lack of seedlings in *B. albitrunca* and *B. discolor* may imply that fruits of these species are consumed by birds and rodents. Fruits of *B. albitrunca* and *B. discolor* are widely eaten by mammals and birds as well as by humans (Heath & Heath 2009). The fruits of *S. birrea* and *B. discolor* are rich in vitamin C, which is higher than that of exotic species (Leakey, 1999; Chivandi et al. 2012), signifying their importance as a source of food. *Sclerocarya birrea* fruits have been traditionally used to make a beer (Shackleton 2002) as well as other products (Wynberg et al. 2002).

Conclusion

While the present study is limited in scale and requires replication, preferably where the historical use of the land inside and outside the exclosure is the same, it has demonstrated that exclosure may play a role in enhancing woody species richness, diversity and evenness as well as facilitating regeneration of woody species for this particular area. The current study may indicate that degraded woodlands and other similar ecosystems can be cheaply and conveniently restored through establishment of exclosures. However, there are many factors that could influence the results, and a larger number of similar studies are recommended to verify the findings. Based on our findings, the following recommendations are provided for sustainable management of woodlands inside MIA:

1. Develop and implement a plan for eradication of invasive woody species.
2. Study the reproductive ecology of individual trees (seed production, dispersal and germination).
3. Conduct research on herbaceous species richness, diversity, evenness and density for long-term monitoring.
4. Initiate a programme for management of bush encroachment by *D. cinerea*, *V. tortilis* and *V. erioloba*.

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Competing interests

All the authors declare that they have no competing interests

Authors' contributions

KK planned, designed and conducted field work. He also analysed the data and developed the first draft of the manuscript. DT revised the MS with significant contributions to all sections of the manuscript. MM and MKG were actively involved in data collection and entry. All the authors have approved the final manuscript.

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Impact of poaching on the population structure and insect associates of the Endangered *Encephalartos eugene-maraisii* from South Africa

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Background: South Africa is an important centre of cycad diversity in Africa, however, the country's cycads face extinction. Among the primary causes is the poaching of plants from the wild, even within protected areas.

Objectives: This study examined poaching patterns in a local population of the Endangered *Encephalartos eugene-maraisii* I.Verd. and how it might affect the population structure, sex ratios, as well as interactions with associated insects.

Methods: The population was surveyed in 2008 and 40% of this population was resurveyed between 2021 and 2022. We mapped missing cycads and generated heatmaps. Lastly, we investigated whether the proportion of stems from different size classes, sex ratios and abundance of insect associates varied between areas with a high and low poaching incidence.

Results: Poaching, defined as the illegal removal of individuals from the wild, occurred 1.5 times more frequently along the border fence line than areas further away. Medium-sized stems (21–80 cm) are primarily targeted (likely as they can be carried more easily) and low proportions of these stems remain in areas with a high poaching incidence. While *E. eugene-maraisii* exhibits some resilience against poaching through basal suckering, it takes several decades for suckers to mature and replace harvested stems. No effect on sex ratios were recorded in areas with a high poaching incidence, suggesting poachers have not deliberately selected female or male cycads at this site. No pollinating insects were detected on *E. eugene-maraisii*, and no seedlings were observed.

Conclusion: Cone production may be too rare in diminished populations to support pollinators that utilise cones as brood sites. The presence of insects that use other plant parts, including leaves, dried leaf stalks and cycad trunks, in the larger population suggests that they are more resilient to diminishing host populations. However, these insects were absent in smaller populations, and their abundances were lower in low-density sites and smaller clump sizes of their host in the larger population. This suggests these insects may be vulnerable to the decline of their host populations due to poaching.

Keywords: cycads, conservation areas, herbivore-plant interactions, population decline, impact.

Introduction

South Africa is a major centre of cycad diversity in Africa, with the monotypic *Stangeria eriopus* (Kunze) Baill. and 37 species of *Encephalartos* Lehm., of which 29 species are endemic to South Africa (Calonje et al. 2023). However, South Africa's cycads face extinction. Many species have limited distributions and small populations, and their numbers are continually declining (Table 1). South African species include four that are already Extinct in the Wild, 11 that

Table 1. Status and threats of South African *Encephalartos* species according to the IUCN Red List of Threatened species (IUCN 2023)

Species	Status	Individuals (N)	Populations (N)	Threats
E. brevifoliolatus Vorster	EW	0	0	1
E. heenanii R.A.Dyer	EW	0	0	1, 2, 5
E. nubimontanus P.J.H.Hurter	EW	0	0	1
E. woodii Sander	EW	0	0	7
E. inopinus R.A.Dyer	CR (?EW)	1	1	1, 4
E. dolomiticus Lavranos & D.L.Goode	CR (?EW)	0–4	1	1, 4
E. cerinus Lavranos & D.L.Goode	CR (?EW)	0–5	1	1, 4
E. hirsutus P.J.H.Hurter	CR (?EW)	0–10	1	1, 4
E. laevifolius Stapf & Burtt Davy	CR	6–11	3	1, 4
E. cupidus R.A.Dyer	CR	50	1	1, 4
E. latifrons Lehm.	CR	70	4	1, 4
E. msinganus Vorster	CR	100–200	1	1, 2, 4
E. middelburgensis Vorster, Robbertse & S.van der Westh.	CR	184–200	4	1, 2, 4, 5
E. dyerianus Lavranos & D.L.Goode	CR	350–400	1	1, 4
E. aemulans Vorster	CR	600	1	1
E. eugene-maraisii I.Verd.	EN	400–620	3	1, 4
E. arenarius R.A.Dyer	EN	850–1500	5	1, 2
E. horridus (Jacq.) Lehm.	EN	Unknown	Unknown	1
E. lebomboensis I.Verd.	EN	5 000	5	1, 3, 6
E. ngoyanus I.Verd.	VU	2 500–5 000	10	1, 2, 5
E. paucidentatus Stapf & Burtt Davy	VU	3 000–5 000	6	1, 2
E. princeps R.A.Dyer	VU	3 500–5 000	6	1, 2, 6
E. altensteinii Lehm.	VU	4 000–10 000	10–15	1, 2, 3
E. ghellinckii Lem.	VU	8 000–10 000	Unknown	1, 5
E. senticosus Vorster	VU	5 000–10 000	12	1, 2
E. humilis I.Verd.	VU	4 500–9 500	10–12	1, 5
E. lanatus Stapf & Burtt Davy	VU	70 000–80 000	8	1
E. lehmannii Lehm.	VU	Unknown	12	1, 2, 6
E. natalensis R.A.Dyer & I.Verd.	VU	Unknown	Unknown	1, 3
E. trispinosus (Hook.) R.A.Dyer	VU	Unknown	8	1, 2
E. friderici-guilielmi Lehm.	NT	5 000–10 000	Unknown	1, 3
E. ferox G.Bertol.	NT	>10 000	Unknown	1, 2, 5
E. caffer (Thunb.) Lehm.	NT	10 000–30 000	12–20	1, 2
E. transvenosus Stapf & Burtt Davy	NT	20 000–50 000	Unknown	1, 2, 3
E. longifolius (Jacq.) Lehm.	NT	Unknown	11–20	1
E. cycadifolius (Jacq.) Lehm.	LC	Stable	Unknown	5
E. villosus Lem.	LC	Stable	Unknown	1, 2

EW – Extinct in the Wild; CR – Critically Endangered; EN – Endangered; VU – Vulnerable; NT– Near Threatened; LC – Least Concern; 1 – Over-collecting/poaching; 2 – habitat destruction for agriculture, livestock impact, etc.; 3 – Harvesting for traditional medicine; 4 – Reduced recruitment; 5 – Natural system modification (e.g., altered fire regimes); 6 – Invasive plant species; 7 – Naturally rare? The last remaining stems were all deliberately removed from the wild.

are Critically Endangered, four that are Endangered, 11 that are Vulnerable, and five that are Near Threatened (IUCN 2023). Relatively more cycads in South Africa are Extinct in the Wild or Critically Endangered than in other centres of cycad diversity (Donaldson 2008).

In most regions of the world, the primary cause of the decline in cycad numbers is habitat loss, but in South Africa the poaching of wild plants has played an even greater role, affecting nearly all species (Okubamichael et al. 2016; Table 1). Established cycads from the wild are targeted because cycads are notoriously slow growing and can take decades to reach desirable sizes (Donaldson 2003). Consequently, many plants have been collected for botanical gardens and private collections (Osborne 1995). Those that become rare increase in value, making them even more desirable to collectors and increasing the pressure on species in the wild (Courchamp et al. 2006; Okubamichael et al. 2016). Some species have suffered dramatic declines; for example, in Kaapsehoop, 1 700 *Encephalartos laevifolius* Stapf & Burtt Davy plants were present in the 1970s, but there are now fewer than five remaining (Government Gazette 2017). Despite various conservation measures, restrictive legislation, and the use of novel technologies (such as microchips and microdots), poaching continues relentlessly because large, rare specimens are in high demand (Donaldson 2003).

South African cycads are also harvested for traditional medicine (Ravele & Makhado 2010; Cousins et al. 2011, 2012, 2013; Williamson et al. 2016; Ndou et al. 2021). Traditional medicine has experienced significant commercialisation in recent years and there has been an increase in the sale of stem sections and bark strips of *Encephalartos* species at traditional markets, which puts more pressure on wild *Encephalartos* populations (Cousins et al. 2011). Intensive harvesting of bark strips and stem sections can be destructive and often result in the death of plants (Donaldson 2003; Bamigboye & Tshisikhawe 2020).

Other threats include the destruction of habitats and invasive plant species. Historically, habitat destruction has contributed to a decline in South African cycad populations. For example, the clearing of dune thicket for agriculture directly reduced *E. arenarius* R.A.Dyer populations (Donaldson 2003). Alien plants such as *Lantana camara* L. have invaded the habitat of cycads such as *E. princeps* R.A.Dyer and *E. lebomboensis* I.Verd. and can potentially affect recruitment by smothering young plants (Donaldson 2003; Government Gazette 2017).

Those involved in illicit trade with cycads often claim that their goal is conservation, even though the illicit collection is the main threat (Torgersen 2017). It is important to conserve cycads not only as part of South Africa's natural heritage but also as a component of ecosystem function. They provide food and shelter for birds and animals (Donaldson 2008), host complex mutualistic relationships with insects (Toon et al. 2020), and host arbuscular mycorrhizae that shape biogeochemical processes in their microhabitats (Marler & Calonje 2020).

Cycads recover slowly from poaching due to their slow growth (Raimondo & Donaldson 2003). Poaching can affect the size of the cycad population, age structure and sex ratio. For example, *Cycas circinalis* L. populations subjected to pith harvesting completely lacked individuals greater than 50 cm tall (Krishnamurthy et al. 2013). The expected sex ratio for a healthy cycad population is 1:1 but in small populations, male-biased sex ratios are often observed, and it has been speculated that this results from selectively harvesting female plants since they produce seeds (Donaldson 2008). The rarest species are now often represented only by small populations, making them vulnerable to stochastic events (e.g., drought, fire), inbreeding depression and reduced natural recruitment (Donaldson 2003). Cycads and their pollinators exhibit brood-site mutualism, making them vulnerable to coextinction (Toon et al. 2020). There may be too few cones produced by diminished cycad populations to support insect pollinators (Oberprieler 1995). South African species of *Encephalartos* also have a high diversity of other specialised insects, for example, female cone specialists and leaf consumers, which are also threatened by declining host populations (Oberprieler 1995).

Encephalartos eugene-maraisii I.Verd. is listed as Endangered under Red List criteria A2ad + 4ad; B1ab(v) (IUCN 2023). This species has a limited distribution in the Waterberg range and lacks natural recruitment, making it extremely vulnerable to poaching (Bezuidenhout et al. 2020). The impact of poaching on this cycad has not been studied before. A lack of scientific information constrains decision support systems and the development of management decisions that can effectively ensure the survival of *E. eugene-maraisii* in the wild (Bezuidenhout et al. 2020). This study aimed to 1) identify poaching patterns of *E. eugene-maraisii* in one of its last remaining populations, 2) assess its impact on the size class structure and sex ratio of the population, and 3) how this might impact insects closely linked with *E. eugene-maraisii*.

Materials and methods

Study site

Encephalartos eugene-maraisii is endemic to the Waterberg range in Limpopo, South Africa (Bezuidenhout et al. 2020). The majority of individuals remain in two main conservation areas, located at either end of its

geographical range. Marakele National Park (Marakele) is located at the southwestern extreme of the Waterberg cycad distribution. The Entabeni Safari Conservancy (Entabeni) is at the northeastern edge of its range, where the majority of *E. eugene-maraisii* plants still exist. There have been no reports of cycad poaching in Marakele since its proclamation in 1994 (Bezuidenhout et al. 2020). However, at that time, very few plants (< 50) remained in Marakele and they are extremely difficult to reach (Bezuidenhout et al. 2017). We have also failed to record the presence of any cycad-associated insects in Marakele. Therefore, sampling was confined to Entabeni where the majority of plants remain.

Most plants grow on the rocky mountain plateaus and scarps in the Waterberg-Magaliesberg Summit Sourveld (Gm 29) at high altitudes (1500–1750 m.a.s.l.) (Mucina et al. 2006). The vegetation is characterised by patches of open woodland of *Protea caffra* Meisn. and open shrubland of *Englerophytum magalismontanum* (Sond.) T.D.Penn. and *Ancylobotrys capensis* (Oliv.) Pichon (Steyn & Bezuidenhout 2020). The climate is warm in summer and cold and prone to frost in winter. Historically, fires were frequent in the study areas due to the very high frequency of lightning strikes, and fire scars were visible on the cycads in both populations.

Study species

Encephalartos eugene-maraisii has aerial stems (up to 4 m long) that become procumbent as they age. Individual plants are multi-stemmed through the production of basal suckers (Figure 1). Individual plants can persist over long periods of time due to vegetative production of suckers and stem longevity. Like all cycads, *E. eugene-maraisii* is dioecious although cones are produced infrequently.

Patterns of poaching

Poaching was assessed in Entabeni, where the majority of plants remain. The main driver behind the poaching in Entabeni is the horticultural trade, which requires that whole stems are removed. We did not find evidence that plant parts are being harvested for the traditional medicinal trade. This is also supported by previous authors (Bezuidenhout et al. 2020). *Encephalartos eugene-maraisii* has also not been recorded in traditional medicine markets (Cousins et al. 2011, 2012, 2013).

In 2008, Entabeni conducted a cycad census on its property to determine the population size and distribution

Figure 1. Typical architecture of an *Encephalartos eugene-maraisii* plant. Photographer: P.D. Janse van Rensburg.

within the reserve (De Klerk, 2008). GPS coordinates were provided to facilitate the retracing of individual plants. Given the scattered distribution of plants, it was unlikely to mistake them for those in similar locations. The original census took months to complete and many of the plants are in areas difficult to reach. Given time limitations, we only re-surveyed the most densely populated area between 2021 and 2022. Approximately 40% of the plants identified in the original census (De Klerk 2008) were revisited. The studied plants occurred in a small area (~8 km^2), which accounted for approximately 30% of the total area. We recorded plants as present, dead or missing. Remains of dead cycads are visible for a very long period. It was rarely possible to determine the cause of mortality, but common causes include stems falling over, baboon damage and poachers damaging and excavating large stems to get to smaller stems that they could carry. If no remains were found they were classified as missing. Missing cycads were mapped and heatmaps produced. We recorded the number of stems for the plants present, and for each individual stem, we measured its height. The survey areas were also searched for seedlings to confirm the presence or absence of natural recruitment.

Impact of poaching

The plants grew in areas along the fence line and areas further from the fence. The fence stretches over a distance of approximately 3 km over rocky terrain that is difficult to patrol. Other areas are more easily visible and accessible from roads within the reserve. It appears that plants have been poached from across the entire population. However, poaching has historically been more intense along the fence line (Entabeni reserve manager, pers. comm.). Therefore, analyses were conducted by categorising areas along the fence line as 'high poaching incidence' and areas further from the fence line as 'low poaching incidence'. A ridgeline divides the two areas. The high poaching incidence area consists of plants along the fence line and the western slope of the ridgeline, which faces the fence. The low poaching incidence area consists of plants on the eastern slope of the ridgeline and further away.

All analyses were done using SPSS version 28 (IBM Corp 2021). To assess the impact of poaching on the size structure of the population we compared the distribution of stem height of individual stems in areas with high and low poaching incidence using the Kolmogorov-Smirnov test for goodness of fit (e.g., Botha et al. 2004a, 2004b). The studied plants occurred in a small area and experienced similar climatic conditions and fire regimes. Additionally, cycad stem growth is positively correlated with stem height, therefore shrinkage of stems is ruled out (Griffiths et al. 2005; Marler et al. 2020).

Medium-sized stems seemed to be primarily targeted because large stems may be too heavy to carry over the large distances that poachers need to cover over neighbouring properties (Entabeni reserve manager, pers. com.). To test this, we investigated whether the proportion of stems from different size classes varied between areas with high poaching incidence and low poaching incidence. We classified all stems into five size classes based on their length: suckers (no visible stem); visible stems (> 0 cm); small stems (1–20 cm); medium-sized stems (21–80 cm) and large stems (> 80 cm). The proportion of each size class in the areas of high and low poaching incidence was compared using Chi-square analyses (χ^2). Finally, to show how the clump size of individual plants might be affected by poaching we tested for significant differences with a Kruskal-Wallis test, between the mean number of stems per plant for each category in areas with high vs low poaching incidence.

During the 2008 census, a small proportion of plants were sexed. To gather more data, we examined cycad plants for cone material to determine the sex of the plants. A binomial test was conducted to see if the proportion of male and female plants are different from the expected 1:1 sex ratio in cycads. To test whether poaching affects the sex ratio through selective harvesting of female plants, we compared the proportions of male and female plants between areas with a high poaching incidence and low poaching incidence using Chi-square analysis. We also tested for significant differences in the mean number of stems between male and female plants using a Kruskal-Wallis test.

Insect abundance

We recorded three insect species associated with *E. eugene-maraisii* in Entabeni (Figure 2), but, as per previous extensive surveys, none of these are pollinators. Reference collections (accession numbers: *PDJVR Morpho 6* and *7*) are stored at the Biosystematics Division, South African National Collection of Insects (SANC), Agricultural Research Council, Pretoria, South Africa.

Amorphocerus cf. *setosus* Boheman, 1838 (Coleoptera: Curculionidae) bore into the trunk of *E. eugene-maraisii*. The trunks exhibit characteristic emergence holes made by beetles. All stems except those out of reach or pinned between rocks were assessed for beetle emergence holes. This was done by placing a 10 cm wide piece of clear plastic, from top to bottom on each stem and counting the number of exit holes made by *A.* cf. *setosus* adults. The number of holes per square centimetre was calculated by dividing the number of holes by the area recorded (the length of the stem × 10 cm).

Apinotropis verdoornae Jordan, 1945 (Coleoptera: Anthribidae) breed in dead leaf stalks of *E. eugene-maraisii*. It has overlapping life history stages and so it is

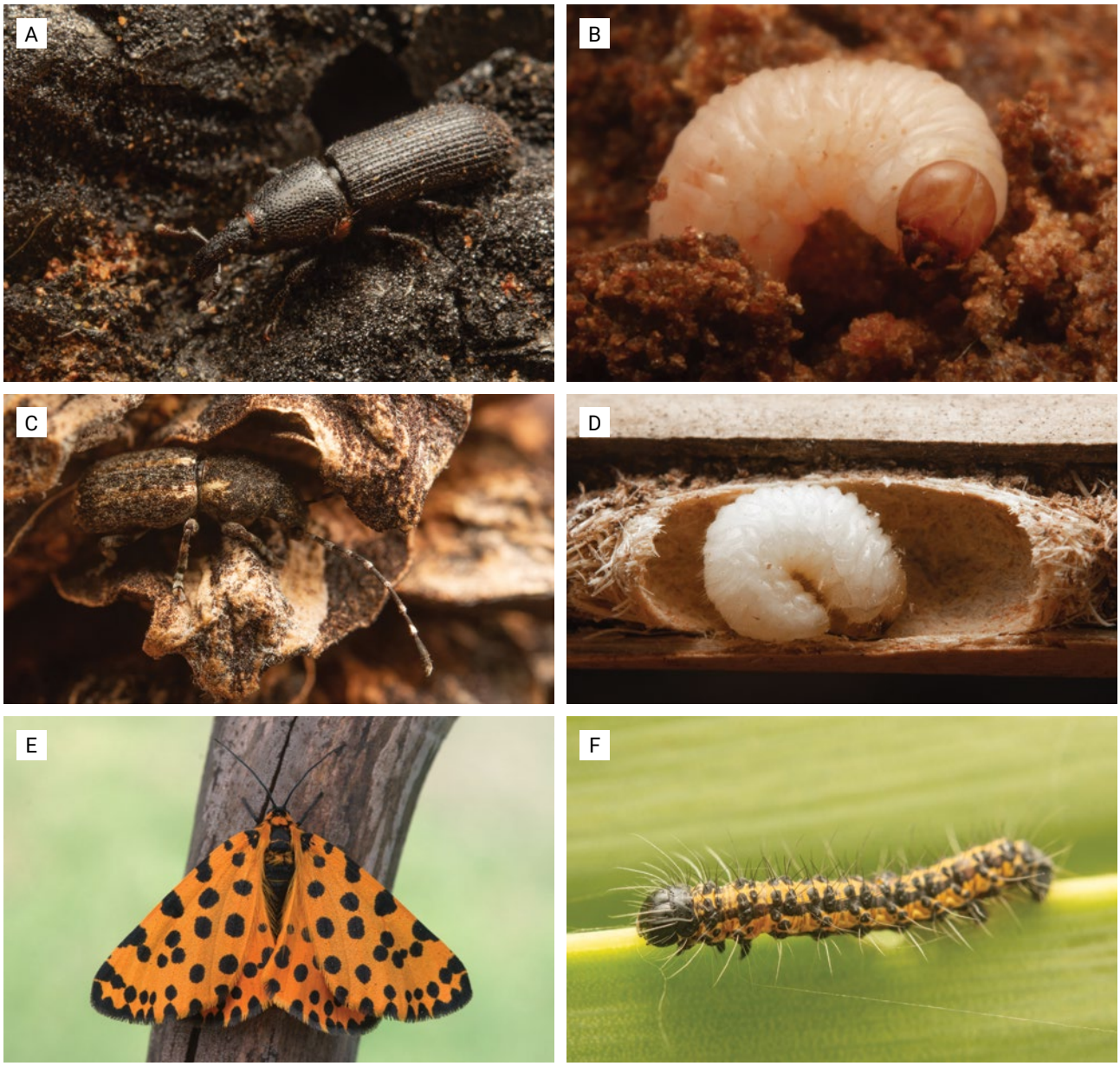

Figure 2. Insects associated with *Encephalartos eugene-maraisii* in Entabeni: A, B, *Amorphocerus* cf. *setosus*; C, D, *Apinotropis verdoornae*; E, F, *Zerenopsis lepida*. Photographs: P.D. Janse van Rensburg.

usually possible to find adults and larvae within the same leaf stalk at any time of the year (personal observations). If dead leaf stalks were present, five dead leaves were randomly selected from each stem, cut off and dissected after which the numbers of larvae and adults were determined.

Larvae of *Zerenopsis lepida* (Walker, 1854) (Lepidoptera: Geometridae) consume new leaf flushes (Janse van Rensburg et al. 2023). Herbivory damage was used as an estimate of the abundance of *Z. lepida*. To determine the level of damage, the percentage of leaf area removed for each leaf was visually estimated using different damage classes: 0%, 1–25%, 26–50%, 51–75%, and > 75%. We calculated the percentage of leaf area consumed by larvae by multiplying the number of leaves from each damage class with the midpoint of each damage class category, e.g., 13% for the 1–25% class. The values of all classes were then summed and divided by the total number of leaves per stem. Only new leaf flushes are damaged and only a small portion of plants flush leaves each season. For a more complete sample of the entire population, we combined leaf damage estimates from consecutive years, 2021 and 2022.

Kruskal-Wallis tests were used to determine whether there were significant differences in mean insect abundances between plant sex, altitude and aspect. Additionally, we recorded whether the stems had fire scars and tested for significant differences between the abundance of insects on the burned and unburned stems. To assess the potential impacts of poaching we compared insect abundance between high poaching

incidence and low poaching incidence areas. Also, because poaching can lead to lower plant densities and smaller clump sizes due to removed stems, we compared insect abundance between different densities of *E. eugene-maraisii* and analysed correlations between the abundance of insects and the clump size (number of visible stems) of *E. eugene-maraisii* using Spearman rank correlation analysis. Using heat maps of existing plants, we rated areas with dense plant density (dark spots on the heatmap), sparse plant density (light spots on the heatmap), and intermediate plant density (areas between dense and sparse areas).

Ethical considerations

Ethics approval (no.: NWU-01552-20-A9) for this study was granted by the North-West University, Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC). A permit (no.: ZA/LP/111179) to do research on plants in the Limpopo province of South Africa was granted by the Limpopo Department of Economic Development, Environment and Tourism (LEDET).

Results

Patterns of poaching

We were unable to find any seedlings in the survey areas. A total of 297 plants recorded in 2008 were revisited. Out of those, 246 (83%) plants were still present, eight (~3%) plants were dead and 43 (~14%) plants could not be relocated. This represents a reduction of ~17% (51 plants) in 14 years, equivelant to an annual intrinsic population growth rate of -0.013. The estimation is based only on completely missing plant individuals and does not include missing stems from plant individuals that were still present. Most of the missing plants were those that occurred adjacent to the border fence of the conservation area and are assumed to have been removed from the wild by poachers (Figure 3).

Impact of poaching

The Kolmogorov-Smirnov test for goodness of fit only indicated a marginally significantly different stem size distribution between areas with high poaching incidence (n = 70) and low poaching incidence (n = 176) (K-S = 1.443, p = 0.031). Chi-square analysis indicated that there was a higher proportion of stemless suckers in areas with high poaching incidence (35.8%) compared to areas with low poaching incidence (24.3%) (χ^2 = 10.457, df = 1, p = 0.001) (Table 2). There was a higher proportion of visible stems (> 0 cm) in the areas with low poaching incidence (75.7%) than areas with a high poaching incidence (64.2%) (χ^2 = 10.805, df = 1, p = 0.001). Of the visible stems, the frequency of medium-sized stems was lower in the areas with a high poaching incidence (18.7%) compared to areas with a low poaching incidence (28.5%) (χ^2 = 5.596, df = 1, p = 0.018). The proportions of small stems (χ^2 = 0.454, df = 1, p = 0.500) and large stems (χ^2 = 0.006, df = 1, p = 0.937) did not differ significantly between areas.

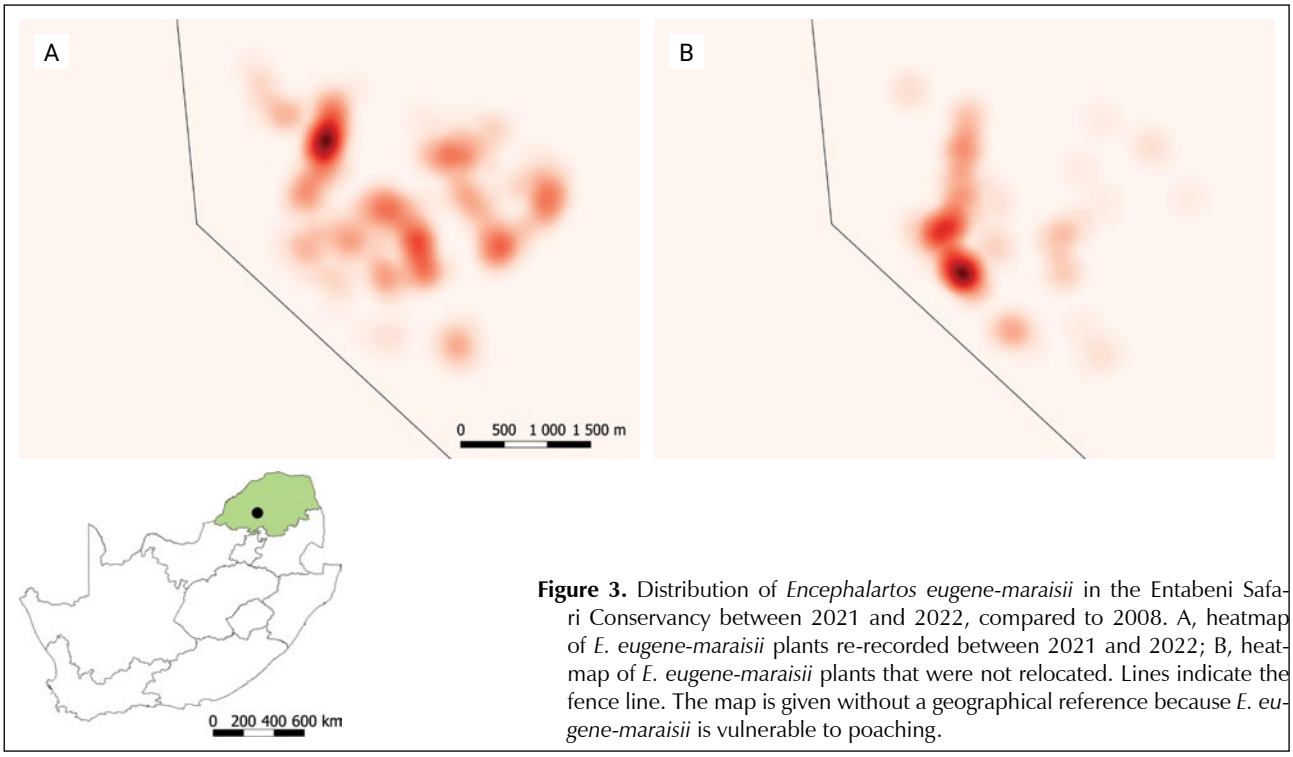

Figure 3. Distribution of *Encephalartos eugene-maraisii* in the Entabeni Safari Conservancy between 2021 and 2022, compared to 2008. A, heatmap of *E. eugene-maraisii* plants re-recorded between 2021 and 2022; B, heatmap of *E. eugene-maraisii* plants that were not relocated. Lines indicate the fence line. The map is given without a geographical reference because *E. eugene-maraisii* is vulnerable to poaching.

Table 2. The relative frequency of stems from different height classes of *Encephalartos eugene-maraisii*, and the mean number of stems per plant for each height class, in areas with high poaching incidence and low poaching incidence

Stem length (cm)	Relative frequencies (%) of height classes			Mean (range) number of stems of height class per plant		
	High poaching incidence	Low poaching incidence	Chi Squared	High poaching incidence	Low poaching incidence	Kruskal-Wallis
Stemless suckers (0)	35.83	24.27	0.001	0.96 (3)	0.76 (4)	0.042
Visible stems (> 0)	64.17	75.73	0.001	1.73 (6)	2.38 (7)	0.003
Small stems (1–20)	15.51	17.70	0.500	0.41 (2)	0.55 (4)	0.287
Medium-sized stems (21–80)	18.72	28.47	0.018	0.50 (3)	0.89 (3)	< 0.001
Large stems (> 80)	29.95	29.56	0.937	0.80 (3)	0.92 (4)	0.433
All	100	100	-	2.61 (6)	3.09 (7)	0.080

The range indicates the difference between the lowest and highest values.

This was further reflected by a significantly higher mean number of medium-sized stems (21–80 cm) and lower stemless suckers (0 cm) per plant in areas with a low poaching incidence (Table 2).

Sex ratios

We were able to determine the sex of 90 (39.3%) of the remaining plants, of which 52 were male and 38 were female. A two-tailed binomial test indicated that the observed proportion of female plants did not differ significantly from the expected value 0.50 ($p = 0.142$). There were no differences in the frequency of male and female plants between areas ($\chi^2 = 0.552$, $p = 0.457$). We found no difference between the mean number of visible stems and suckers between female (2.83) and male plants (2.85) (H = 0.029, $p = 0.865$) and the mean numbers of medium-sized stems (primarily targeted for harvesting) between female (0.33) and male plants (0.69) (H = 1.216, $p = 0.270$).

Insect abundance

There was no significant association between the abundance of insects and aspect, altitude and the sex of plants (Tables S1, S2; Supplementary material). A total of 78% of stems had visible fire scars. This did not significantly affect the abundance of *A. verdoornae* or herbivory by *Z. lepida*. However, the mean number of *A.* cf. *setosus* exit holes was significantly higher on burned stems (0.018 holes/cm^2) than on unburned stems (0.005 holes/cm^2) ($p < 0.001$).

The mean number of *A. verdoornae* individuals were higher in areas with a low poaching incidence, but not significantly so (Table 3). The abundance of *A. verdoornae* was significantly lower in areas with low plant density, and plants with smaller clump sizes also had a lower abundance of *A. verdoornae* (Table 3). The level of herbivory from *Z. lepida* was significantly higher in areas with a low poaching incidence and decreased significantly with decreasing plant density and decreasing

Table 3. The relationship between density and clump size of *Encephalartos eugene-maraisii* and the abundance of *Amorphocerus* cf. *setosus* (measured by the number of exit holes on the cycad trunk), *Apinotropis verdoornae* (measured by the number of individuals in dead leaf stalks) and *Zerenopsis lepida* (measured by the level of herbivory)

Insects	Mean (range) abundance between area			Mean (range) abundance between plant density				Correlation with clump size	
	High poaching incidence	Low poaching incidence	*p*	Sparse	Intermediate	Dense	*p*	r_s	*p*
Amorphocerus cf. *setosus*	0.016 (0.05)	0.017 (0.06)	0.399	0.017 (0.05)	0.015 (0.06)	0.019 (0.06)	0.137	0.067	0.305
Apinotropis verdoornae	2.72 (13)	3.49 (22)	0.138	2.46 (11)[a]	2.81 (13)[a]	4.49 (22)[b]	0.010	0.158	0.018
Zerenopsis lepida	6.12 (50.29)	12.12 (88)	< 0.001	5.57 (29.96)[a]	10.50 (88)[ab]	13.20 (88)[b]	0.024	0.406	< 0.001

The range indicates the difference between the lowest and highest values.

clump sizes of *E. eugene-maraisii* (Table 3). We did not record any significant difference in the number of exit holes of *A.* cf. *setosus* between the areas nor any correlation with the density or clump sizes of *E. eugene-maraisii* (Table 3).

Discussion

This is the first investigation into poaching patterns in a local population of the Endangered *E. eugene-maraisii* and how it might affect its population structure and interactions with associated insects. Supporting the observations made by the reserve manager, a slightly higher intensity of poaching was observed along the border fence line of the conservation area. Areas along the border fence line had a low proportion of medium-sized stems (21–80 cm), which seems to be the primary target for poachers. We found no difference in the sex ratios between areas with high poaching incidence and low poaching incidence and found no evidence for selective harvesting of female plants. The abundance of *A. verdoornae* and herbivory by *Z. lepida* decreased in lower densities and clump sizes of *E. eugene-maraisii*, indicating they may be sensitive to the decline of their host plant.

Poaching of *E. eugene-maraisii* has been a major problem for the past 30 years, with estimates suggesting a 50% reduction in the population (Government Gazette 2017). As a result, the distribution has shrunk, and the remaining subpopulations can be found mostly in Entabeni and Marakele (formally protected area managed by SANParks) (Bezuidenhout et al. 2020). There have been no reports of cycad poaching in Marakele since its proclamation in 1994 (Bezuidenhout et al. 2020). There were, however, very few plants left by then. Entabeni's plants were some of the most difficult to reach. The eradication of cycads in most areas in the Waterberg, caused by poaching, has led to an increase in poaching incidents in Entabeni.

Although sampling only 40% of the population was a limitation, we concentrated on the locations with the highest density of plants that were most often targeted by poachers. Other plants on the reserve are more randomly distributed and harder to reach. In this study, a slightly higher poaching rate (1.5 times) was observed along the border fence line. Besides being the first cycads encountered, it also falls in a difficult-to-patrol area with many places for poachers to hide. The majority of medium-sized stems (21–80 cm) had been intensively harvested. Cycads are generally removed indiscriminately of size (Okubamichael et al. 2016). While larger stems fetch higher prices, smaller stems are also targeted because they are easier to transport (Okubamichael et al. 2016). The reason for targeting medium-size stems in Entabeni may be case specific. To reach cycads, poachers must walk long distances (up to 12 km) through neighbouring properties, and larger stem sizes are often too heavy to carry. However, large stems are sometimes damaged or excavated to reach the smaller stems that can be carried with relative ease (Figure 4).

The selective harvesting of medium-sized stems, in this case, has led to significantly different size class distribution of stems between areas with a high poaching incidence and low poaching incidence. Poachers often only target a subset of individuals in a population, for example, individuals with the largest tusks in the case of elephants (Chiyo et al. 2015) or selective harvesting of certain tree species in higher size classes (e.g., Botha et al. 2004a, 2004b). Selective harvesting may result in changes to the population structure, possibly causing declines. Poaching is the main threat to cycads in South Africa (Table 1); however, it is also very difficult to detect in populations that are not closely monitored. The stem size structure may potentially be used as a flexible and cost-effective indicator to track changes in wild populations of cycads and provide insight into the status of poaching. For example, *C. circinalis* populations subjected to pith harvesting have resulted in a complete absence of plant individuals taller than 50 cm (Krishnamurthy et al. 2013).

As evidenced by the large number of stemless suckers produced by plants in the areas targeted by poachers, *E. eugene-maraisii* has been able to survive despite severe poaching pressures and the absence of natural recruitment because it reproduces vegetatively. Cycads grow extremely slowly, with reports generally indicating around 1–3 cm per year (Vovides 1990; Cabrera-Toledo et al. 2019; Marler et al. 2020). Consequently, cycads are not particularly resistant to poaching because suckers will take a long time to reach the size of their poached counterparts. Modelling different poaching scenarios for two South African species with contrasting life histories revealed that poaching even small numbers of adult plants can cause rapid population declines (Raimondo & Donaldson 2003).

Moreover, *E. eugene-maraisii* lacks natural recruitment and has a complete lack of subadults, which suggests that the absence of natural recruitment has been a long-standing problem. Even in the presence of natural recruitment, species such as *E. cycadifolius* (Jacq.) Lehm. that have highly persistent adult plants and infrequent recruitment events are unable to recover within a reasonable time frame (< 100 years) even from small losses of adult plants (Raimondo & Donaldson 2003). Reinforcement (adding individuals to an existing population) efforts have been made in a proactive attempt to limit the decline of *E. eugene-maraisii* (Bezuidenhout 2019; Bezuidenhout et al. 2020). However, rare opportunities to hand-pollinate female cones and the slow growth of reintroduced seedlings

Figure 4. Large *Encephalartos eugene-maraisii* stem that was pushed over by poachers. Photographer: P.D. Janse van Rensburg.

have not been able to stem the tide of poaching (Bezuidenhout et al. 2020).

Limited conclusions can be drawn on the sex ratios because only 39% of the plants could be sexed. The sex ratio was slightly male-biased; however, female plants are less likely to be identified because they cone less frequently. If a larger proportion of the population was sexed the sex ratio may have been closer to 1:1. We found no evidence that female plants are selectively harvested. Due to the slow coning rate of *E. eugene-maraisii,* there is little material (live cones, dry cone material and seeds) poachers can use to sex plants. The destruction of cones, especially female cones, by baboons also removes evidence of coning. Moreover, individual stems are usually harvested rather than whole multi-stemmed plants, which will maintain the sex ratio.

No pollinators have been recorded on *E. eugene-maraisii* despite extensive surveys. Cycad pollinators are dependent on cones for reproduction and the time between coning events can become too long for pollinators to be sustained in diminished host populations (Oberprieler 1995). The presence of other insects on *E. eugene-maraisii* may indicate higher resilience to decreasing host populations because they depend on more abundant plant parts (Figure 2). However, herbivory by *Z. lepida* and abundance of *A. verdoornae* decreased at lower plant densities and smaller clump sizes of *E. eugene-maraisii*, indicating that they are still sensitive to the decline of their host. South Africa hosts a rich diversity of insects associated with cycads (Oberprieler 1995). However, several cycad species are now only represented by single populations containing few individuals (Table 1). Several cycad species also exhibit reduced reproductive success, which might indicate rarity or absence of their pollinators (Table 1). Specialist herbivores are often absent in small host populations due to the higher probability of herbivore extinction (Kéry et al. 2001; Colling & Matthies 2004). Chance events (droughts, floods, fires, etc.) puts small insect populations at greater risk of extinction (Thomas & Jones 1993). Smaller ranges have fewer patches (refuges) that escape disturbance from where insect populations can recolonise cycad host plants.

Apinotropis verdoornae is of particular interest. It is a detritivore that feeds on dead leaf stalks and dried cone material. It is the only genus of Anthribidae exclusively associated with cycads (Oberprieler 1999). Entabeni is the only known locality where *A. verdoornae* occurs, as we have not recorded it from other *E. eugene-maraisii* population or closely related species such as *E. middelburgensis* Vorster, Robbertse & S.van der Westh. and *E. dyerianus* Lavranos & D.L.Goode. To conserve *E. eugene-maraisii*

and its associated insects, it is crucial to maintain as many refuges of suitable habitat as possible.

Conclusion

This study examined poaching patterns in one of the last remaining populations of *E. eugene-maraisii* and its impact on population structure and insect interactions. Higher poaching incidence was observed along the border fence line, primarily targeting medium-sized stems. These findings can inform decision making processes, helping determine areas that require increased patrolling and prioritise stem sizes for interventions like micro-dotting. Lower insect abundance in areas with lower host densities and smaller clump sizes of their host highlights the potential impact of poaching, emphasising the need for protection against poaching. Conserving *E. eugene-maraisii* in the wild will require several actions that may include increased protection from poaching, species recovery techniques (reinforcement and reintroductions), identifying other threats (e.g., climate change) and further research on the disappearance and reintroduction of pollinators.

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Author contributions

All authors contributed to the study's conception and design. Data collection and analysis were performed by PDJvR with assistance from JvDB and HB. The first draft of the manuscript was written by PDJvR and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Disclaimer

The views expressed in the submitted article are our own and not an official position of the institution or funder.

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Supplementary material

Table S1. Kruskal-Wallis results indicating the impact of aspect, altitude and plant sex on the abundance of *Amorphocerus* cf. *setosus* and *Apinotropis verdoornae,* and level of herbivory by *Zerenopsis lepida*

Insect species	Aspect		Altitude		Plant sex	
	H	P	H	P	H	P
Amorphocerus cf. *setosus*	0.304	0.983	4.108	0.128	0.391	0.532
Apinotropis verdoornae	2.777	0.596	4.222	0.121	0.317	0.573
Zerenopsis lepida	8.237	0.083	0.113	0.945	0.116	0.734

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
1	1	148	1	2	F	0.007432	3	0
	2	0						
	3	0						
	4	0						
2	5	76	1	2		0.003947	2	0
	6	69						
3	7	145	1	2	F	0	0	46.4375
	8	6						
4	9	112	1	2	M	0.003571	5	27.27073
	10	102						
	11	0						
	12	0						
5	13	98	1	2		0.030612	3	0
	14	0						
	15	0						
6	16	146	1	2		0.024051	2.5	3.150327
	17	156						
	18	150						
	19	14						
	20	6						
	21	39						
7	22	187	1	2	M	0.017792	1	2.830688
	23	60						
	24	32						
8	25	107	1	2		0	4.5	25.42857

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
	26	6						
	27	0						
9	28	197	1	2		0	4	0
10	29	78	1	2	M	0.003968	2.5	24.47869
	30	242						
11	31	200	1	2	F	0	3	50.28571
	32	0						
12	33	84	1	2		0.020238	0	0
	34	0						
13	35	57	1	2		0.026316	0	0
	36	0						
14	37	340	1	2	M	0	1	3.171171
	38	170						
	39	45						
15	40	220	1	2	M	0.011818	6	0
	41	15						
16	42	260	1	2		0.000513	1	0
	43	14						
	44	18						
17	45	180	1	2	M	0.012222	1	0
18	46	236	1	2	M	0.029429	2	15.13499
	47	62						
	48	0						
	49	0						
	50	61						
19	51	62	1	2	F	0.022177	1.5	0
	52	60						
20	53	230	1	2		0.021014	3.5	22.37235
	54	138						
	55	0						
21	56	16	1	1		0	0	0
	57	0						
22	58	227	1	1		0	0	10.65688
	59	0						
	60	0						
	61	0						

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
23	62	89	1	1		0.008989	4	12.95172
	63	0						
24	64	6	1	1			10.5	11.60455
	65	0						
	66	3						
	67	0						
	68	0						
25	69	14	1	1		0.011688		15.1
	70	0						
	71	0						
	72	11						
	73	0						
26	74	61	1	2		0.011475	0	17.95249
	75	0						
	76	0						
27	77	26	1	1		0		8.6875
	78	0						
	79	0						
	80	0						
28	81	31	1	1		0.041935		0
	82	0						
29	83	192	1	1	F	0.015104	0	0
30	84	114	1	1	M	0.05209	3.5	7.916667
	85	17						
	86	0						
31	87	150	1	1	M	0	2	7.111111
	88	11						
32	89	98	1	1		0.011224	0	0
	90	0						
33	91	249	1	1		0.035352	1.5	0
	92	57						
	93	0						
34	94	75	1	1		0	1	0
	95	10						
35	96	135	1	1		0	4	0
36	97	0	1	1				0

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
	98	0						
	99	0						
37	100	12	1	1		0	0	0
	101	0						
38	102	99	1	1		0.037374	2	0
	103	0						
39	104	73	1	1		0.024658	3	0
	105	0						
40	106	30	1	1		0.013333	2	0
	107	18						
41	108	105	1	1		0.022024	6	0
	109	16						
	110	8						
	111	0						
42	112	132	1	1	M	0.023485	0	0
	113	0						
43	114	242	1	1		0	7	0
44	115	0	1	1				0
45	116	0	1	1				0
46	117	158	1	1	M	0.026461	3	1.857143
	118	117						
	119	0						
47	120	0	1	1				0
48	121	155	1	1	F	0.026452	6	0
	122	0						
49	123	171	1	1	F	0		10.82937
	124	17						
	125	0						
	126	0						
50	127	300	1	1	F	0.016667	1	3.320755
	128	107						
	129	6						
	130	6						
51	131	278	1	1	F	0.017648	4.5	0
	132	97						
	133	20						

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
	134	0						
	135	0						
52	136	160	1	1	F	0.043072	3	6.45614
	137	40						
	138	39						
	139	0						
53	140	180	1	1		0.0175	5	0
	141	60						
	142	0						
	143	8						
	144	2						
54	145	19	1	1		0.036842		29.96591
	146	0						
	147	0						
	148	0						
55	149	202	1	1	F	0.011139	3	4.195652
	150	6						
56	151	181	1	1		0.024309	0	0
	152	0						
	153	0						
57	154	181	1	1	M	0.011602	2	9.087719
	155	0						
	156	0						
58	157	32	1	2		0.028125	6	0
59	158	39	1	2		0.020513	0	14.5294
60	159	53	1	2	M	0.032415		1.537879
	160	66						
	161	0						
	162	18						
61	163	55	1	2		0.02	3	0
	164	0						
62	165	109	1	2		0.010092	1	0
	166	0						
63	167	30	1	2		0.050417	13	2.043478
	168	40						
	169	0						

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
64	170	149	1	2		0.020418	3	0
	171	0						
	172	52						
65	173	199	1	2		0.012141	9	19.75987
	174	28						
	175	0						
66	176	160	1	2		0.02211	1.5	12.2107
	177	190						
	178	46						
	179	0						
67	180	250	1	2		0.015733	3	0
	181	70						
	182	30						
68	183	108	1	2		0.00463	0	0
69	184	127	1	2	F	0	0	0
	185	0						
70	186	107	1	2		0.007477		0
71	187	100	2	2	F	0	0	0
72	188	0	2	2				0
	189	0						
	190	6						
73	191	160	2	2	M	0.010545	0	4.454586
	192	26						
	193	17						
	194	35						
	195	48						
	196	310						
74	197	112	2	2		0.017857	4	0
75	198	170	2	2	F	0.023855		5.423377
	199	32						
	200	51						
	201	70						
	202	2						
76	203	210	2	2	M	0	3.5	7.9375
	204	67						
77	205	62	2	3	M	0.035484	6	0

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
78	206	285	2	3	F	0.02162		8.911974
	207	112						
	208	41						
	209	6						
	210	40						
	211	170						
	212	114						
	213	20						
	214	0						
79	215	160	2	3		0.020069	0	4.153846
	216	15						
	217	12						
80	218	98	2	3	M	0.010703		8.573775
	219	87						
	220	86						
	221	17						
	222	50						
	223	0						
81	224	74	2	3		0.010811	21	0
82	225	71	2	3		0.010664	7	67.99947
	226	42						
83	227	330	2	3		0.017652	1	4.605652
	228	86						
	229	78						
	230	18						
	231	9						
84	232	40	2	3		0.0075	10	0
	233	0						
85	234	144	2	3		0.020985		19.14874
	235	104						
	236	60						
	237	85						
	238	98						
86	239	147	2	3		0.002268	2	30.36134
	240	6						
	241	3						

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
	242	17						
87	243	210	2	3		0.019538	12	11.36932
	244	41						
	245	34						
	246	46						
88	247	235	2	3	F	0.011874	2	0.597701
	248	52						
	249	45						
89	250	150	2	3	M	0.002972	0	1.588889
	251	128						
	252	6						
90	253	214	2	3	F	0.006128	3	4.656917
	254	91						
	255	68						
91	256	287	2	3	M	0.025784	0	0
	257	112						
	258	17						
92	259	146	2	2		0.015068	2	0
	260	0						
	261	0						
	262	0						
	263	0						
93	264	145	2	2		0.02069	4	27.5
	265	0						
94	266	148	2	2	M	0	2	13.14583
	267	10						
	268	0						
95	269	88	2	2		0	0	8.03268
	270	0						
	271	0						
96	272	30	2	2		0.013333	0	6.888889
	273	0						
	274	0						
97	275	50	2	2		0.046346	0	21.375
	276	52						
98	277	3	2	2		0.006522	4	0

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
	278	0						
	279	184						
99	280	160	2	2		0	5	0
	281	0						
100	282	127	2	2	M	0.028346	0	3.203125
	283	0						
	284	0						
	285	0						
101	286	170	2	2		0.01794	12	11.45195
	287	145						
	288	13						
	289	0						
	290	11						
102	291	213	2	2		0.001408	0	3.478261
	292	6						
	293	0						
103	294	56	2	2		0.025794	4	0
	295	6						
	296	3						
104	297	112	2	2		0.002679	4	4.846154
	298	0						
	299	25						
105	300	90	2	3	F	0.022222		8.84783
106	301	37	2	3		0.035135	5	0
107	302	51	2	3		0.038133	5	4.041667
	303	77						
	304	0						
108	305	190	2	3		0.028889	2	10.40345
	306	25						
	307	6						
109	308	0	2	3		0.013656	0	0
	309	0						
	310	0						
	311	0						
	312	227						
110	313	130	2	3		0.006923		0

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
111	314	124	2	3		0.022581		16.83333
	315	0						
	316	0						
112	317	211	2	3		0	0	0
113	318	50	2	3		0.020254	1.5	10.86275
	319	21						
	320	15						
114	321	219	2	2	M	0.017294	3.5	1.105263
	322	59						
	323	12						
115	324	76	2	2	F	0.019737	1	4.53333
116	325	24	2	2		0		16.00706
	326	12						
	327	6						
	328	203						
	329	59						
117	330	183	2	2	F	0.062061	7	0.727273
	331	7						
	332	0						
	333	0						
	334	0						
118	335	57	2	2		0.02807	1	0
	336	0						
119	337	57	2	2	F	0.007212	5	88
	338	131						
	339	0						
120	340	233	2	2	F	0.023646	2	3.984615
	341	34						
	342	34						
	343	3						
	344	79						
121	345	130	2	1	M	0.026355	4	20.38333
	346	63						
122	347	310	2	2	F	0	4	23.3383
	348	110						
	349	15						

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
	350	8						
	351	6						
123	352	65	2	1		0.010769	6	0
124	353	46	2	1		0.027464	1.5	0
	354	45						
	355	0						
	356	0						
125	357	118	2	1	M	0.017797	0	4.573333
	358	0						
	359	0						
126	360	77	2	1		0	5	4.511111
127	361	80	2	3	M	0.013276	0	1.039414
	362	191						
	363	0						
	364	0						
128	365	175	2	3		0.003354	9	34.39175
	366	180						
	367	18						
129	368	92	2	3	M	0.034783	2.5	0
	369	0						
130	370	137	2	3		0.023122	5	27.26236
	371	17						
131	372	165	2	3		0.024848	5	9.803571
	373	0						
132	374	170	2	3		0	6	88
133	375	61	2	3		0.034426		0
134	376	40	2	3	F	0.01	7	0
135	377	80	2	3		0.01625	0	0
136	378	118	2	3		0.018968	8	8.668023
	379	12						
	380	17						
	381	0						
	382	0						
137	383	197	2	3		0.03212	2.5	4.883721
	384	74						
138	385	150	2	3	M	0.005561	5	10.93401

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
	386	127						
	387	42						
	388	6						
	389	27						
	390	0						
139	391	91	2	3		0.021978	4	29.1894
140	392	110	2	3		0.011096	16	0
	393	17						
141	394	127	2	3		0.013386	6	12.35021
	395	12						
	396	6						
	397	17						
142	398	68	2	3		0.019678	14	5.792683
	399	193						
143	400	30	2	3		0.026667	0	0
144	401	80	2	3		0.037222	8	6.428111
	402	80						
	403	30						
145	404	43	2	3		0.009432	3	9.3375
	405	6						
	406	12						
	407	0						
146	408	181	2	3		0	0	0
	409	61						
	410	0						
147	411	21	2	3		0.063571	1.5	43.25833
	412	30						
148	413	140	2	3	M	0.047527	8	1.894258
	414	42						
	415	30						
	416	26						
149	417	236	2	2	M	0.00374	6	9.736488
	418	112						
	419	99						
	420	0						
	421	2						

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
150	422	107	2	2		0.043925	4	16.5385
151	423	120	2	2		0.002222	2	41.10632
	424	17						
	425	0						
	426	40						
152	427	115	2	2	M	0.023921	1	12.35606
	428	56						
	429	0						
	430	0						
	431	0						
	432	0						
153	433	278	2	2		0.033351	2	16.08912
	434	31						
	435	15						
	436	17						
154	437	33	2	2		0.004545	0	12.66667
	438	6						
155	439	136	2	2		0.009559	0	0
156	440	87	2	2	M	0.006897	4	5.263889
	441	0						
	442	0						
	443	0						
157	444	25	2	2		0.019783	5	0
	445	46						
158	446	135	2	2	M	0.00963		0
159	447	58	2	2		0.011207	4	0
	448	0						
	449	6						
	450	12						
160	451	97	2	2	F	0.018557	3	0
161	452	114	2	2		0	2	0
	453	17						
162	454	91	2	2	M	0.022955	3.66	6.595238
	455	78						
	456	294						
163	457	95	2	2		0.023158	2	0

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
164	458	77	2	2		0.038961	4	11.17328
	459	0						
	460	0						
165	461	49	2	2		0.024567	0	21.17544
	462	22						
	463	8						
	464	0						
	465	12						
	466	0						
166	467	182	2	2		0.030769	9	0
167	468	55	2	2		0.016292	0	20.98148
	469	0						
	470	5						
	471	55						
	472	95						
	473	28						
168	474	119	2	2		0.012131	2	7.115217
	475	65						
	476	12						
169	477	40	2	3		0.045357	4	0
	478	42						
	479	0						
	480	0						
170	481	180	2	3		0.013653	4	6.728889
	482	58						
	483	30						
	484	60						
	485	95						
	486	0						
171	487	180	2	3	F	0.030556	1	0
	488	0						
172	489	170	2	3		0.000606	6	4.571429
	490	0						
	491	0						
	492	0						
	493	0						

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
	494	30						
	495	220						
173	496	71	2	2		0.013651	0	23.15476
	497	250						
174	498	35	2	2	M	0.025714	0	0
175	499	180	2	2	M	0.02132	2	10.37517
	500	44						
	501	0						
	502	0						
	503	0						
	504	0						
	505	12						
	506	65						
176	507	118	2	2		0.011299	4	0
	508	12						
177	509	102	2	2		0.044608	0	25.97549
	510	12						
	511	0						
	512	21						
178	513	80	2	2		0.00875	0	0
179	514	370	2	2	M	0.01629		18.30393
	515	135						
	516	160						
	517	24						
	518	34						
	519	0						
180	520	148	2	2	F	0.018946	4	30.28832
	521	100						
	522	0						
181	523	91	2	2		0.005249	3	6.419815
	524	82						
	525	0						
	526	2						
	527	0						
182	528	253	2	2		0.020059	8	14.19576
	529	46						

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
183	530	93	2	2	M	0.009005	3	0
	531	12						
184	532	185	2	2		0.011755	1	37.96697
	533	57						
	534	17						
	535	15						
	536	8						
185	537	68	2	2		0	6	0
186	538	141	2	2	F	0.030083	9	11.22222
	539	12						
	540	6						
187	541	125	2	2		0.0128	0	10.79279
	542	0						
	543	0						
188	544	49	2	3		0.013025	0	8.277778
	545	17						
189	546	251	2	3		0.01903	1.5	36.10498
	547	40						
	548	54						
190	549	170	2	3	F	0.028157	2	40.2472
	550	75						
	551	0						
191	552	25	2	3		0.056		0
192	553	115	2	3		0.011833	5	51.14618
	554	34						
	555	0						
	556	0						
	557	6						
193	558	282	2	3	F	0.024069	3	17.97643
	559	145						
	560	48						
	561	48						
194	562	242	2	3		0.05286	4	23.25926
	563	21						
	564	18						
195	565	78	2	3		0.014505	9	19.2381

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
	566	14						
	567	10						
196	568	259	2	3		0.01945	10	75.43377
	569	0						
	570	20						
197	571	92	2	3		0.028125	6	2.5375
	572	48						
	573	0						
	574	0						
	575	0						
198	576	160	2	3		0.015	6	8.96774
199	577	127	2	3	M	0.006299	0	0
200	578	79	2	3		0.011392	2	0
	579	0						
	580	0						
	581	0						
201	582	226	2	3	F	0.027655	1.5	4.063889
	583	22						
	584	16						
202	585	245	2	3		0.004592	4	13.6667
	586	105						
	587	12						
	588	98						
	589	0						
	590	0						
203	591	81	2	3		0.024027	22	60.72222
	592	26						
	593	0						
204	594	67	2	1	M	0.01194	1	18.25
	595	0						
	596	0						
205	597	130	2	1	M	0	0	13.95833
	598	0						
206	599	125	2	2	M	0.0092	1.5	5.654828
	600	125						
	601	6						

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
	602	0						
	603	6						
207	604	124	2	2		0.007279	2	22.96032
	605	13						
	606	6						
208	607	108	2	2	M	0.028704	1	0
	608	6						
209	609	148	2	2	F	0.017657	5	14.5976
	610	88						
	611	50						
	612	5						
	613	24						
	614	17						
	615	6						
	616	7						
210	617	54	2	2		0.009259	3	7.200311
	618	6						
	619	0						
	620	0						
211	621	293	2	2	M	0.016864	3	9.049829
	622	149						
	623	25						
	624	60						
	625	12						
212	626	143	2	2		0	2	17.29032
	627	16						
	628	0						
213	629	285	2	2		0.006316	6	6.97619
	630	40						
214	631	48	2	2		0.03125	5	0
	632	0						
215	633	69	2	2	F	0.002899	0	5.886364
	634	12						
216	635	125	2	2	M	0.011767	2	51.53628
	636	86						
	637	70						

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
	638	0						
	639	0						
217	640	183	2	2	M	0	4.5	18.5625
	641	30						
	642	0						
218	643	73	2	2		0.014578	9	51.86147
	644	54						
	645	54						
	646	0						
	647	0						
219	648	170	2	2		0.007647	2	16.73529
	649	0						
220	650	230	2	1	F	0.011812	0	29.25556
	651	42						
	652	0						
221	653	78	2	2		0.020513	3	0
222	654	122	2	2	F	0.031987	3.33	23.38477
	655	17						
	656	10						
	657	10						
	658	7						
	659	27						
223	660	79	2	2	M	0.010127	4	3.960317
	661	0						
	662	0						
224	663	160	2	2	F	0.018826	0	21.05729
	664	37						
	665	0						
	666	0						
225	667	77	2	2		0.028571	4	0
	668	0						
	669	0						
226	670	137	2	3		0.026141	3	27.86111
	671	21						
	672	12						
	673	0						

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
227	674	131	2	3	F	0.005344	1	28.6746
	675	14						
	676	0						
228	677	200	2	3	M	0.017158		2.970588
	678	295						
	679	47						
	680	22						
	681	20						
229	682	115	2	3		0.004348	2	6.41667
230	683	189	2	3	F	0	4	10.69231
	684	0						
231	685	250	2	3		0.0112	3	8.72
232	686	33	2	3		0.004545	7	0
	687	6						
233	688	113	2	3		0.002655	0	0
234	689	128	2	3	M	0.028013	1	26.08056
	690	64						
	691	0						
	692	0						
	693	21						
	694	0						
235	695	178	2	3		0.017301	2.5	7.275983
	696	6						
	697	37						
	698	78						
236	699	75	2	3	M	0.059333	3	31.4011
	700	4						
	701	0						
237	702	85	2	3		0.009412	4	0
238	703	59	2	2		0.011864	1	0
	704	0						
239	705	45	2	2	M	0	3	39.75
240	706	26	2	2		0.038462	0	0
	707	0						
241	708	128	2	2		0.001042	1.5	24.84286
	709	32						

Table S2: Recorded plant traits and insect abundance data of *Encephalartos eugene-maraisii* between 2021 and 2022. Areas are categorised as 1) areas with a high poaching incidence and 2) low poaching incidence. Plant densities are classified as 1) sparse, 2) intermediate and 3) dense (continued)

Plant number	Stem number	Stem height (cm)	Area	Plant density	Plant sex	Mean number of *Amorphocerus* cf. *setosus* exit holes/ cm^2 per plant	Mean *Apinotropis verdoornae* per plant	Mean *Zerenopsis lepida* leaf herbivory per plant
	710	0						
	711	0						
	712	26						
242	713	0	2	1		0.015908	0	2.515152
	714	54						
	715	56						
	716	0						
	717	0						
	718	60						
243	719	41	2	1		0.026829	2	0
	720	0						
	721	0						
244	722	25	2	1	M	0.02	0	28.8333
245	723	107	2	2	M	0.004862		2.918154
	724	25						
	725	223						
	726	108						
246	727	225	2	2		0.01064	2	13.24661
	728	82						
	729	112						
	730	160						
	731	4						
	732	30						
	733	0						

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The non-acarine Arachnida of the Amathole Mountains, South Africa

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Background: The Eastern Cape province of South Africa has a rich floral diversity, with seven of the country's eight floral biomes represented in the province. The non-acarine arachnid fauna of the province is largely understudied and considerable gaps exist in our knowledge of the distribution, diversity and levels of endemism of the arachnid fauna.

Objectives: To address this, non-acarine arachnids were sampled intensively in the Afromontane forests and surrounding biotopes in the Amathole Mountains over the course of a decade.

Methods: In the present contribution, comprehensive checklists of the non-acarine arachnids (specifically, the orders Amblypygi, Araneae, Opiliones, Pseudoscorpiones and Scorpiones) of the region are presented, based on a combination of field sampling, provenance data from museum specimen databases, and a review of the historical literature.

Results: In total, 398 species of non-acarine arachnids have been recorded from the Amathole Mountains, with spiders (Araneae; 324 species from 51 families) and harvestmen (Opiliones; 41 species from four families) the richest groups. The region is exceptionally rich in harvestmen and pseudoscorpions (Pseudoscorpiones; 24 species from 11 families), and might be considered a hotspot of biodiversity and endemism for these taxa.

Conclusion: As the sampling was concentrated around Hogsback, and most other areas remain undersampled, further efforts should be made to sample all representative biotopes more comprehensively in the mountain range. This will improve understanding of the distribution and endemism of the arachnid fauna and assess the conservation significance of the region from a national perspective.

Keywords: Amblypygi, Araneae, conservation, forests, hotspot, Opiliones, Pseudoscorpiones, SANSA, Scorpiones.

Introduction

South Africa contains a particularly rich arachnid fauna, which has been well studied compared to other parts of the continent (e.g., Staręga 1992; Jocqué et al. 2013). Since 1997, the South African National Survey of Arachnida (SANSA) has co-ordinated research on the non-acarine arachnids in the country and surveyed many undersampled areas. This greatly improved knowledge of the distribution of described species, provided material for taxonomists to describe new taxa, and offered insights into the community composition of different biotopes. The project resulted in the production of national checklists, catalogues or taxonomic treatments of the Pseudoscorpiones (Dippenaar-Schoeman & Harvey 2000), Scorpiones (Prendini 2005), Solifugae (Dippenaar-Schoeman et al. 2006; Dippenaar-Schoeman & González Reyes 2006), Opiliones (Lotz 2009), Amblypygi (Prendini et al. 2005) and Araneae (Dippenaar-Schoeman

et al. 2010). These works laid the foundation for understanding the biodiversity and biogeography of the non-acarine arachnid fauna.

The Eastern Cape is South Africa's second largest province by area and contains the greatest representation of the country's floral biomes. Seven of the eight biomes fall within its borders; only the Desert Biome is absent (Mucina & Rutherford 2006). Nevertheless, the Eastern Cape is among the most undersampled for non-acarine arachnids (Janion-Scheepers et al. 2016; Foord et al. 2020). In reviews of the savanna (Foord et al. 2011) and grassland (Haddad et al. 2013) spiders of the country, gap analyses revealed that the Eastern Cape was considerably undersampled. Although 13 protected areas in the Eastern Cape contain more than fifty specimen records, checklists have only been published for the Mountain Zebra National Park (Dippenaar-Schoeman 1988, 2006), Addo Elephant National Park (Dippenaar-Schoeman et al. 2020), Mkambati Nature Reserve (Dippenaar-Schoeman et al. 2011), Asante Sana Nature Reserve (Midgley 2012), Silaka Nature Reserve (Forbanka & Niba 2013), and Thyspunt (Dippenaar-Schoeman & Wiese 2021). There is considerable scope to conduct more intensive sampling in the province to determine the non-acarine arachnid diversity, particularly in highly threatened biotopes such as Afromontane Forest.

The Amathole Mistbelt Forest of the Amathole Mountains (at 642.2 km^2 remaining, the second largest forest type in South Africa according to Berliner (2009)), is regarded as an emblematic example of Afromontane Forest due to its relatively large extent and its unique fauna (Lawrence 1953). Unfortunately, most of the sampling in the Amathole Mountains has concentrated on the forests near Hogsback, whereas other areas are comparatively poorly sampled, particularly for spiders. Consequently, the arachnid fauna is suggested to consist of numerous endemic or near-endemic taxa, although these may indeed be more widespread in the nearby forests and adjacent biomes of the Eastern Cape. Further, the considerable undersampling of grassland, fynbos, thicket and savanna biomes in the region suggests that much of the regional species pool may not yet have been sampled. For example, no Solifugae have been sampled from the Amathole Mountains, despite 28 species being recorded from the Eastern Cape in more xeric biotopes to the south and west of this mountain range (Dippenaar-Schoeman et al. 2006).

The first known spiders described from the Amathole Mountains were *Stasimopus insculptus* Pocock, 1901, described from King William's Town (now Qonce) (Pocock 1901), *Spiroctenus flavopunctatus* (Purcell, 1903), originally placed in *Hermachastes* Pocock, 1900, and *Thomisus weberi* Lessert, 1923, later synonymised with *T. stenningi* Pocock, 1900 by Dippenaar-Schoeman (1983). Subsequently, ten harvestmen species were described from Hogsback by Lawrence (1931, 1934), including the genus *Amatola* Lawrence, 1931, with the type species *A. dentifrons* Lawrence, 1931. Species were occasionally described from the area in later papers (e.g. Lawrence 1940; Griswold 1985), but most new taxa from the area were described in the last two decades (Supplementary Table 1). Hogsback was also one of two South African sites included in the first studies of tree canopy arthropods in the country, as part of comparative studies with the U.K. fauna (Moran & Southwood 1982; Southwood et al. 1982).

In the present contribution, we provide a comprehensive overview of the records of non-acarine arachnids (specifically, the orders Amblypygi, Araneae, Opiliones, Pseudoscorpiones and Scorpiones) in the literature, include records from museum specimen databases, and incorporate all data on recently sampled arachnids from the Amathole Mountains to prepare a checklist of the fauna of the region. We further detail the biology of the common taxa of the region and their habits, supplemented by habitus photos of selected species. Lastly, we comment on the significance of the Amathole Mountains as a biodiversity hotspot for particular taxa within a national context.

Research method and design

Study area

The Amathole Mountains are located in the south-central part of the Eastern Cape and lie north of the provincial capital, Qonce (formerly King William's Town). The approximate limits of this mountain range are between the Kat and Esk rivers in the northwest and the Keiskamma and Thomas rivers in the east. The northern limits of the range fall south of the towns of Cathcart, Whittlesea and Tarkastad, and its southern limits to the north of the towns along the R63 road between Bedford and Qonce, covering an area of approximately 900 km^2 (Phillipson 1987).

The Afromontane Forests of the Amathole Mountains can be classified as Southern Mistbelt Forests (Mucina & Geldenhuys 2006), or Amathole Mistbelt Forests (Von Maltitz et al. 2003). These forests are regarded as being well conserved and classified as Least Threatened (Mucina & Geldenhuys 2006). The indigenous forests of South Africa have been widely exploited for fuel wood, timber, traditional medicine, clearing for agriculture and silviculture, and the florist industry (Mucina & Geldenhuys 2006), and the Amathole Mountains are no exception. Large tracts of land have been converted to pine and *Eucalyptus* plantations, bordering on indigenous forests in the area, as well as causing loss of grassland,

shrubland and fynbos vegetation. Most of the forests in the Amathole Mountains are state-owned and managed under a formal multiple-use system to ensure sustainable utilisation of resources (Von Maltitz et al. 2003). The grasslands surrounding forest patches are also under severe threat from silviculture and overgrazing by cattle (McMaster 2003). Although 1 215 plant species were recorded in the Amathole Mountains by Phillipson (1987), the majority of these are associated with grasslands and more open habitats, whereas Berliner (2009) indicated that only 161 species were found in the Amathole Mistbelt Forests, representing the lowest species richness among the six mistbelt forest types in the country.

Field sampling

Many of the arachnid specimens on which the present contribution is based, were collected during student field excursions to Hogsback between 2006 and 2013. Entomology third-year students from the University of the Free State were divided into groups of two or three students, with equal numbers of samples taken by sweep-netting, beating and leaf litter sifting in Afromontane forests, pine plantations, and the mixed forest (with exotic trees) at the Hogsback Arboretum (Figure 1). Students sorted all arthropods from the samples, with the first author sorting, identifying and tallying the

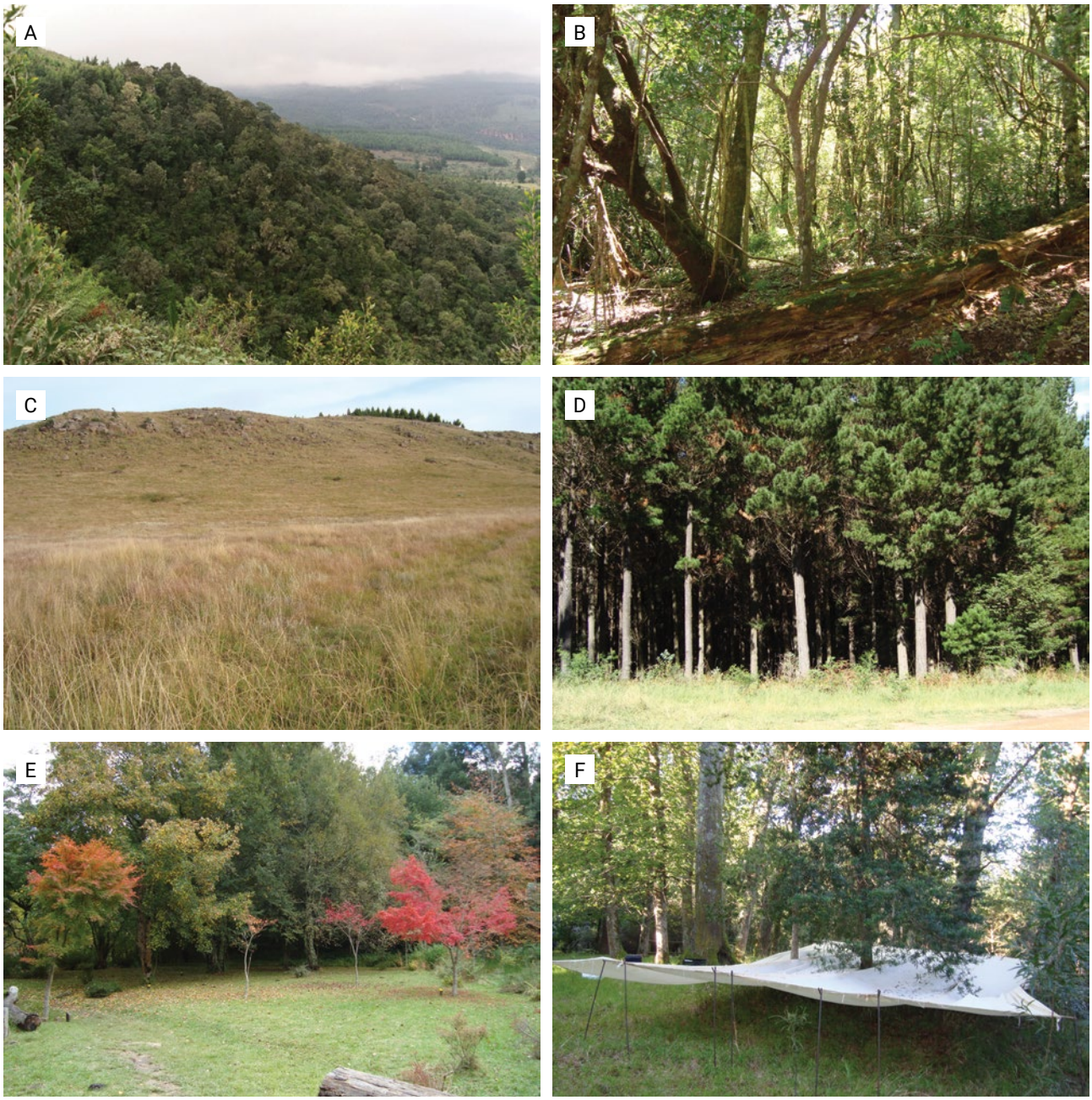

Figure 1. Biotopes sampled in the Hogsback area of the Amathole Mountains, Eastern Cape, South Africa; A, Part of Tyume Forest on a south-facing slope (Afromontane Forest); B, Understorey of Afromontane Forest; C, Open mesic grassland north of Hogsback; D, Pine plantation; E, Hogsback Arboretum, mixed indigenous and exotic trees; F, Canopy fogging a yellowwood sapling at Hogsback Arboretum. Photos: C. Haddad.

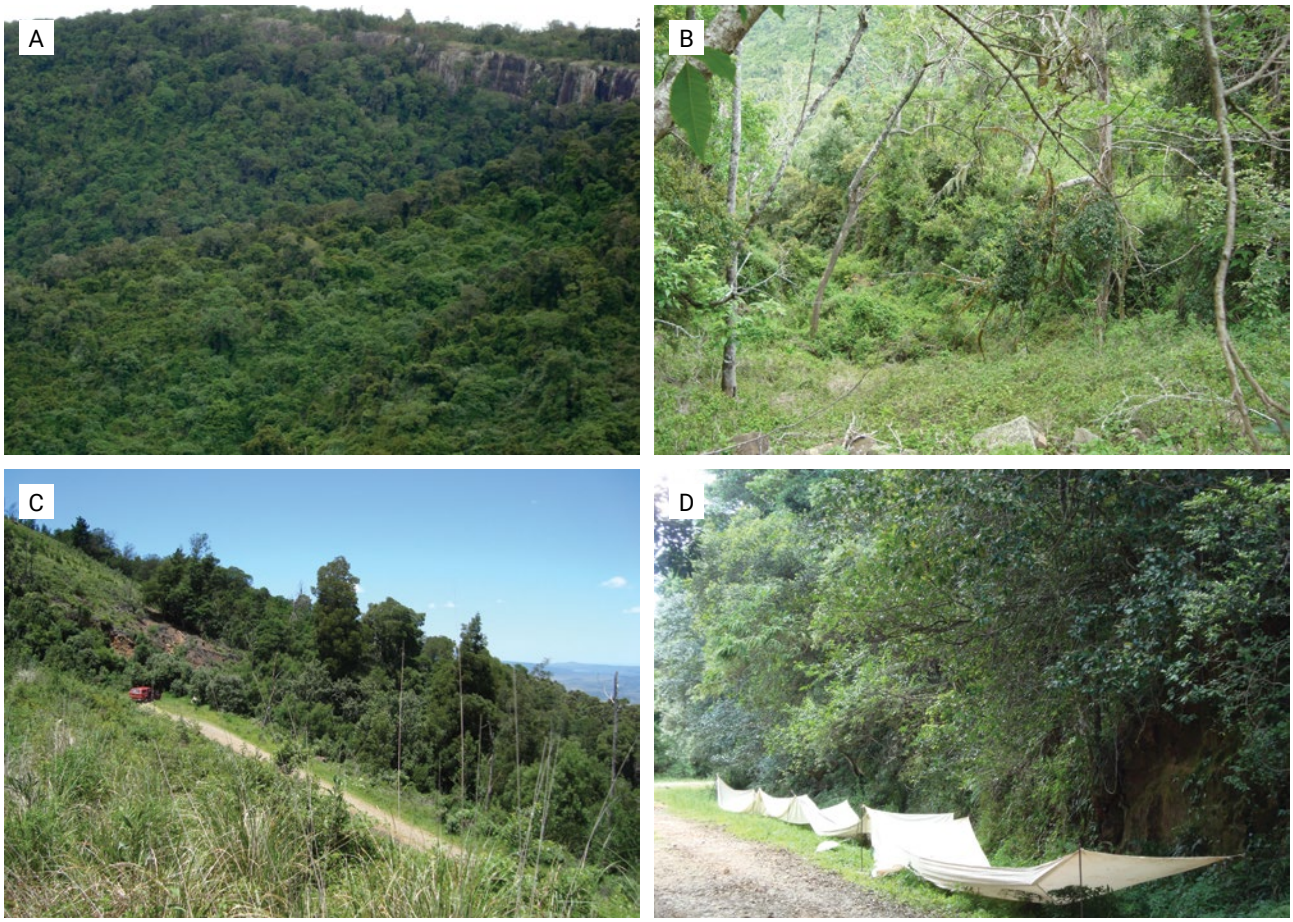

Figure 2. Afromontane Forest sampled at Mpofu Fort Fordyce Nature Reserve (A, B) and Katberg State Forest (C, D) in the Amathole Mountains, Eastern Cape, South Africa. Photos: C. Haddad.

non-acarine arachnids. Additional sampling was conducted by hand collecting, sweeping and beating in grassland and fynbos to the north and east of Hogsback, in gardens, and by tree canopy fogging (Figure 1F). Subsequently, the first and third authors also conducted sampling at Mpofu Fort Fordyce Nature Reserve and Katberg State Forest (Figure 2), including canopy fogging (Figure 2D). Additional scorpion material was collected by the second author over the course of several visits to the area during larger field excursions in the Eastern Cape.

Data mining

Additional records from the vicinity of the Amathole Mountains were obtained from the arachnid databases of the National Collection of Arachnida (Pretoria, NCA), KwaZulu-Natal Museum (Pietermaritzburg, NMSA), National Museum (Bloemfontein, NMBA), the Ditsong National Museum of Natural History (Pretoria, TMSA) and the Iziko South African Museum (Cape Town, SAMC). When this work was undertaken, the Albany Museum (Grahamstown) arachnid collection had not yet been digitised, and material could not be obtained from the California Academy of Sciences (San Francisco), preventing the inclusion of data from these collections.

A thorough survey of the primary taxonomic literature was also performed to source records for non-acarine arachnids. The *First Atlas of the Spiders of South Africa* (Dippenaar-Schoeman et al. 2010) served as the primary source for determining records of spiders, which were checked against the existing literature for all recorded species. Staręga (1992) and Lotz (2009) served as the preliminary sources for harvestmen, with additional records identified by Leon Lotz incorporated into the specimen database of the NMBA. Scorpions were identified and data sourced by the second author, and pseudoscorpions by the third author, with some identifications provided by Mark Harvey (Western Australian Museum, Perth, Australia) and Danilo Harms (Museum der Natur, Hamburg, Germany).

Results and discussion

To date, 398 species of arachnids have been recorded from the Amathole Mountains (Supplementary Table 1), with spiders (324 species from 51 families) and harvestmen (41 species from four families) the richest orders (Table 1). A brief overview of the arachnid fauna of the region, documenting some of the common species likely to be encountered, identifying taxa for which the

Table 1. Recorded species richness (# spp.) of arachnid orders (total number of species in parenthesis) and families in the Amathole Mountains, Eastern Cape, South Africa

AMBLYPYGI (1)	**# spp.**	**% of order**			
Phrynichidae	1	100.0			
ARANEAE (324)	**# spp.**	**% of order**		**# spp.**	**% of order**
Agelenidae	1	0.3	Oecobiidae	1	0.3
Amaurobiidae	5	1.6	Oonopidae	4	1.2
Anapidae	1	0.3	Orsolobidae	2	0.6
Araneidae	26	8.0	Oxyopidae	3	0.9
Bemmeridae	2	0.6	Palpimanidae	1	0.3
Cheiracanthiidae	5	1.6	Penestomidae	1	0.3
Clubionidae	10	3.1	Philodromidae	5	1.6
Corinnidae	5	1.6	Pholcidae	3	0.9
Ctenidae	2	0.6	Phyxelididae	3	0.9
Cyatholipidae	3	0.9	Pisauridae	4	1.6
Cyrtaucheniidae	2	0.8	Salticidae	49	15.1
Deinopidae	1	0.3	Scytodidae	12	3.7
Dictynidae	3	0.9	Segestriidae	2	0.6
Entypesidae	1	0.3	Selenopidae	5	1.6
Euagridae	1	0.3	Sparassidae	4	1.2
Gallieniellidae	2	0.6	Stasimopidae	2	0.6
Gnaphosidae	15	4.6	Tetragnathidae	9	3.6
Hahniidae	4	1.2	Theraphosidae	1	0.3
Hersiliidae	1	0.3	Theridiidae	28	8.6
Linyphiidae	12	3.7	Thomisidae	28	8.6
Lycosidae	16	4.9	Trachelidae	16	4.9
Microstigmatidae	1	0.3	Trochanteriidae	2	0.6
Migidae	1	0.3	Uloboridae	5	1.6
Mimetidae	4	1.2	Zodariidae	3	0.9
Miturgidae	2	0.6	Zoropsidae	3	0.9
Nesticidae	1	0.3			
OPILIONES (41)	**# spp.**	**% of order**		**# spp.**	**% of order**
Biantidae	5	12.2	Phalangiidae	8	19.5
Pettalidae	1	2.4	Triaenonychidae	27	65.9
SCORPIONES (8)	**# spp.**	**% of order**		**# spp.**	**% of order**
Buthidae	3	37.5	Scorpionidae	1	12.5
Hormuridae	4	50.0			
PSEUDOSCORPIONES (24)	**# spp.**	**% of order**		**# spp.**	**% of order**
Atemnidae	1	4.2	Gymnobisiidae	3	12.5
Cheliferidae	6	25.0	Olpiidae	1	4.2
Chthoniidae	2	8.3	Pseudochiridiidae	1	4.2
Feaellidae	1	4.2	Pseudotyrannochthoniidae	2	8.3
Garypinidae	1	4.2	Withiidae	3	12.5
Geogarypidae	3	12.5			

region represents a biodiversity hotspot, and providing information about their biology and endemism, is presented in Table 1 and Supplementary Table 1.

Amblypygi (whip spiders)

Only three whip spider species have been recorded from South Africa, all belonging to Phrynichidae. One of these, *Damon annulatipes* (Wood, 1869), has only been recorded in the Qonce area (Weygoldt 1999; Prendini et al. 2005), but probably occurs elsewhere in the Amathole Mountains. Whip spiders are secretive nocturnal arachnids that typically reside under logs or in rock crevices, so they may have evaded past collecting efforts.

Araneae (spiders)

South Africa has the richest known spider fauna on the continent, with 2 268 described species currently recorded and many more awaiting description (Foord et al. 2020). Much of the current knowledge benefits from a rich collecting history, a well-developed museum infrastructure, and active local and international taxonomists that made sizable contributions to describing the fauna (Dippenaar-Schoeman et al. 2015).

Overall, the levels of spider endemism in South African forests are surprisingly low in terms of number of species (< 10%), but when the area of each biome is considered, then forests have the highest level of endemism proportionally (Foord et al. 2020). The relatively low number of endemic species is unusual, considering the high levels of endemism reported for other invertebrates, such as snails (Perera et al. 2021), millipedes (Janion-Scheepers et al. 2016), harvestmen (e.g., De Bivort & Giribet 2010) and velvet worms (e.g., Daniels et al. 2009). This could possibly be attributed to: 1) the greater dispersal ability of spiders compared to the other invertebrate groups, and 2) undersampling of forests and other biomes in the Eastern Cape, which when improved, could provide more accurate data on spider biodiversity and levels of endemism. Despite its small area (< 0.3% of South Africa), forests still possess the fourth highest spider species richness (646 species) among the eight South African biomes (Dippenaar-Schoeman et al. 2015).

In total, 324 species of spiders were recorded from the Amathole Mountains, with Salticidae (49 spp.), Theridiidae and Thomisidae (28 spp. each) and Araneidae (26 spp.) the most species-rich families. In considering the various datasets used to assess the fauna of the Amathole Mountains, there is a clear sampling bias towards Hogsback (1 357 records), followed by Mpofu Fort Fordyce Nature Reserve (188 records), Qonce (formerly King William's Town) (105 records) and Katberg (63 records), with the remaining sites all represented by fewer than 30 records (Table 2). Hogsback has by far the highest recorded species richness (254 species), but even here the bulk of the records originate from forest habitats and plantations, and the grassland and fynbos biomes in the area remain comparatively poorly sampled. Increased sampling effort in these biomes, as well as the undersampled thicket and savanna biomes to the south, will likely result in the discovery of many new records for the area.

The Amathole Mountains fall within one of the areas with a moderate number of endemic South African spider species, none of which are considered rare or endangered (Foord et al. 2020). However, the apparent absence of certain 'typical' forest taxa from these mountains is perplexing. For example, all South African species of the family Archaeidae are endemic to the country, with three endemic to the Eastern Cape; most *Afrarchaea* Forster & Platnick, 1984 are forest-dwellers (Dippenaar-Schoeman et al. 2021). However, none have been sampled from Afromontane forests or grasslands in the interior of the Eastern Cape, despite several species occurring in these biomes in the KwaZulu-Natal Drakensberg and eastern Free State (Dippenaar-Schoeman et al. 2021). Similarly, the tiny

Table 2. Summary of collecting effort and species richness of spiders from localities in the Amathole Mountains, Eastern Cape, South Africa [FB = Fort Beaufort; HB = Hogsback; KB = Katberg; MF = Mpofu Fort Fordyce Nature Reserve; QO = Qonce (formerly King William's Town); ST = Stutterheim; OT = other Amathole localities]

Collection	FB	HB	KB	MF	QO	ST	OT	Sum
National Collection of Arachnida	29	1 249	61	188	102	10	11	1 649
National Museum, Bloemfontein	–	45	–	–	–	–	–	45
KwaZulu-Natal Museum	–	14	–	–	3	1	–	18
Ditsong National Museum of Natural History	–	49	2	–	–	5	19	75
Total records	29	1 357	63	188	105	16	30	1 788
Total species richness	21	254	49	95	82	12	10	324
Type locality	0	19	1	1	1	0	0	22

litter-dwelling corinnid genus *Hortipes* Bosselaers & Jocqué, 2000 is represented by 14 species in the country, most of which occur in forest and savanna habitats, some in the Eastern Cape, but none have been recorded to date from the Amathole Mountains (Bosselaers & Jocqué 2000). The web-building Eresidae are widespread throughout the country but have not yet been recorded from the region (Dippenaar-Schoeman et al. 2022).

Wandering spiders

More than two-thirds of the spiders from the Amathole Mountains (219 species in 35 families) are wandering species that actively search for prey or hunt from burrows. The ground-dwelling species most commonly collected in Afromontane forest litter include *Copa kei* Haddad, 2013 (Corinnidae; Figure 3A), *Drassodella amatola* Mbo & Haddad, 2019 (Gallieniellidae; Figure 3D),

Figure 3. Selected wandering spiders from the Amathole Mountains, Eastern Cape, South Africa: A, *Copa kei*, female (Corinnidae); B, *Lepthercus mandelai*, female (Entypesidae); C, Same, burrow opening among leaves; D, *Drassodella amatola*, female (Gallieniellidae); E, *Proevippa bruneipes*, female (Lycosidae); F, *Microstigmata amatola*, female (Microstigmatidae); G, *Asemonea amatola*, male (Salticidae); H, *Myrmarachne lesserti*, female (Salticidae); I, *Myrmarachne* sp., female (Salticidae); J, *Rumburak hilaris*, female (Salticidae); K, *Rumburak mirabilis*, male (Salticidae); L, *Thyenula alotama*, female (Salticidae). Photos: C. Haddad.

Microstigmata amatola Griswold, 1985 (Microstigmatidae; Figure 3F), tiny oonopid spiders of the genera *Australoonops* Hewitt, 1915 and *Opopaea* Simon, 1890, and the jumping spiders *Rumburak hilaris* Wesołowska et al., 2014 (Figure 3J), *Thyenula alotama* Wesołowska et al., 2014 (Figure 3L) and *Euophrys bifida* Wesołowska et al., 2014 (Salticidae). *Pachygnatha* Sundevall, 1823 (Figure 4E) is among the few genera of tetragnathids that do not build webs (Levi 1980), and is represented by a new species that is common in the litter of all forest types. Many of the aforementioned species are also frequently sampled in pine plantations and mixed forest.

In the grassland and fynbos biomes, a very different ground- and grass-dwelling fauna is encountered, which includes *Chumma foliata* Jocqué & Alderweireldt, 2018 (Amaurobiidae), various lycosids including *Proevippa bruneipes* (Purcell, 1903) (Figure 3E) and *Trabea* Simon, 1876 spp., several species of *Scytodes* Latreille, 1804 (Scytodidae), *Heliophanus* C.L.Koch, 1833 and *Thyenula* Simon, 1902 (Salticidae).

Some species are commonly associated with rocks and logs in forest habitats and plantations, including *Leptheracus mandelai* Ríos-Tamayo & Lyle, 2020 (Entypesidae; Figure 3B), which build a silk-lined burrow often covered with dead leaves (Figure 3C), the flat wall spiders *Anyphops amatolae* (Lawrence, 1940) (Selenopidae; Figure 4A) and *A. gilli* (Lawrence, 1940) (Figure 4B, C), and the scorpion spider *Platyoides walteri* (Karsch, 1886) (Trochanteriidae; Figure 4I).

A very rich fauna of arboreal spiders has been collected by beating vegetation and canopy fogging, including various jumping spiders such as *Asemonea amatola* Wesołowska & Haddad, 2013 (Figure 3G), *Dendryphantes purcelli* Peckham & Peckham, 1903 and *D. silvestris* Wesołowska & Haddad, 2013, species of *Myrmarachne* MacLeay, 1839 (Figure 3H, I), *Rumburak mirabilis* Wesołowska et al., 2014 (Figure 3K), and two species of *Wandawe* Azarkina & Haddad, 2020. Sac spiders of the families Clubionidae (10 spp.), Cheiracanthiidae (5 spp.) and Trachelidae (16 spp.) are especially species-rich compared to

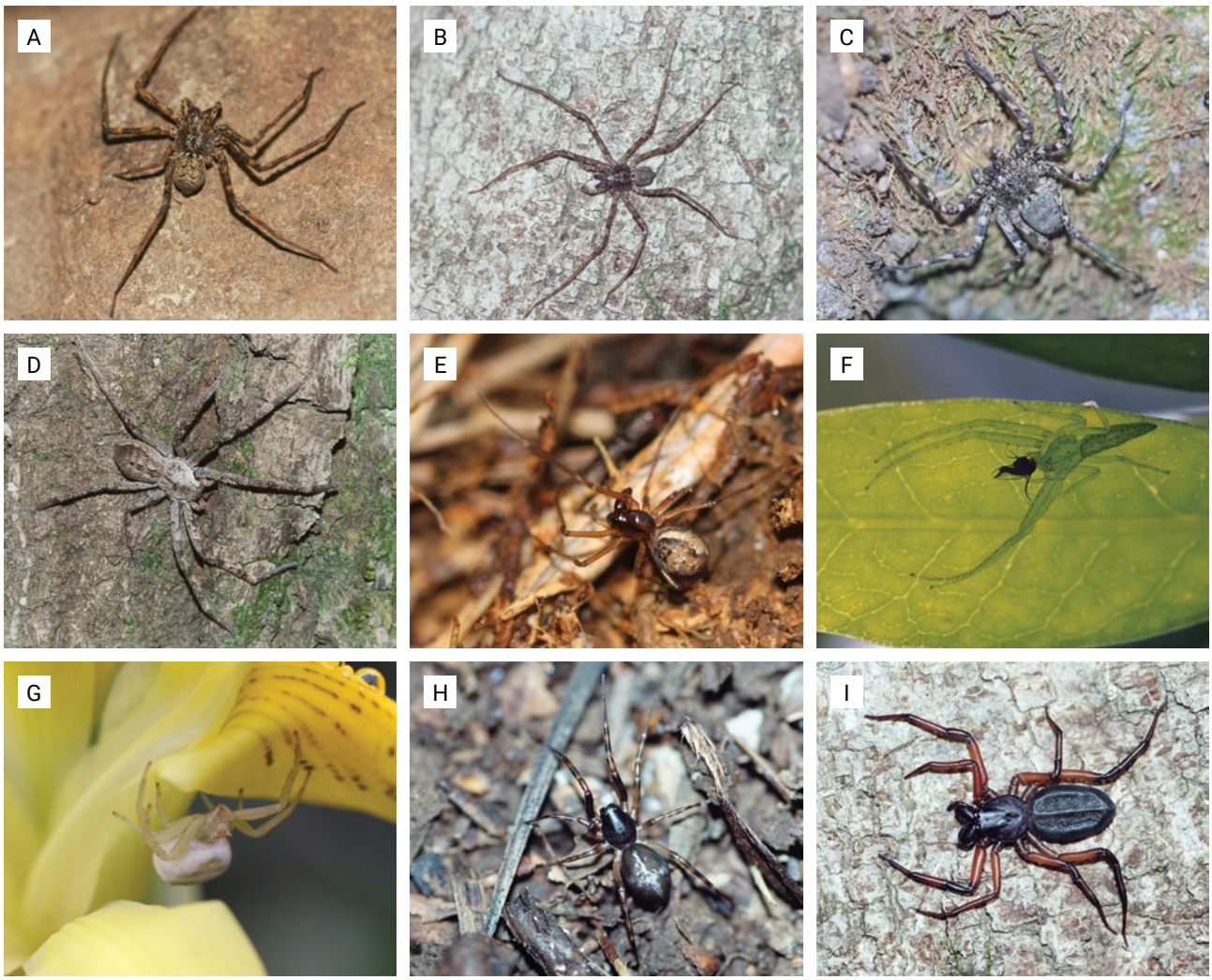

Figure 4. Selected wandering spiders from the Amathole Mountains, Eastern Cape, South Africa: A, *Anyphops amatolae*, female (Selenopidae); B, *Anyphops gilli*, male (Selenopidae); C, Same, female; D, *Palystes superciliosus*, female (Sparassidae); E, *Pachygnatha* sp., female (Tetragnathidae); F, *Oxytate ribes*, female (Thomisidae); G, *Thomisus australis*, female (Thomisidae); H, *Afroceto martini*, female (Trachelidae); I, *Platyoides walteri*, female (Trochanteriidae). Photos: C. Haddad.

other parts of South Africa. Species such as *Afroceto martini* (Simon, 1897) (Trachelidae; Figure 4H) are some of the most abundant wandering spiders on shrubs and in trees. Two species of rain spiders, *Palystes perornatus* Pocock, 1900, and *P. superciliosus* L. Koch, 1875 (Figure 4D), can be easily recognised by their large size and by their nests, comprising a ball of leaves, woven together with silk, to accommodate their egg sacs. Certain taxa prefer particular kinds of trees, such as *Oxytate ribes* (Jézéquel, 1964) (Thomisidae; Figure 4F), which was only collected from broad-leaved trees and shrubs, whereas others have very flexible habitat requirements, such as crab spiders of the genus *Thomisus* Walckenaer, 1805 (Thomisidae; Figure 4G), which occur in grasses, herbs, shrubs and trees.

Web-builders

Approximately one-third of the spiders (105 species in 17 families) are web-builders. Species of *Agelena* Walckenaer, 1805 (Agelenidae; Figure 5A) and *Hippasa* Simon, 1885 (Lycosidae) build funnel-webs close to

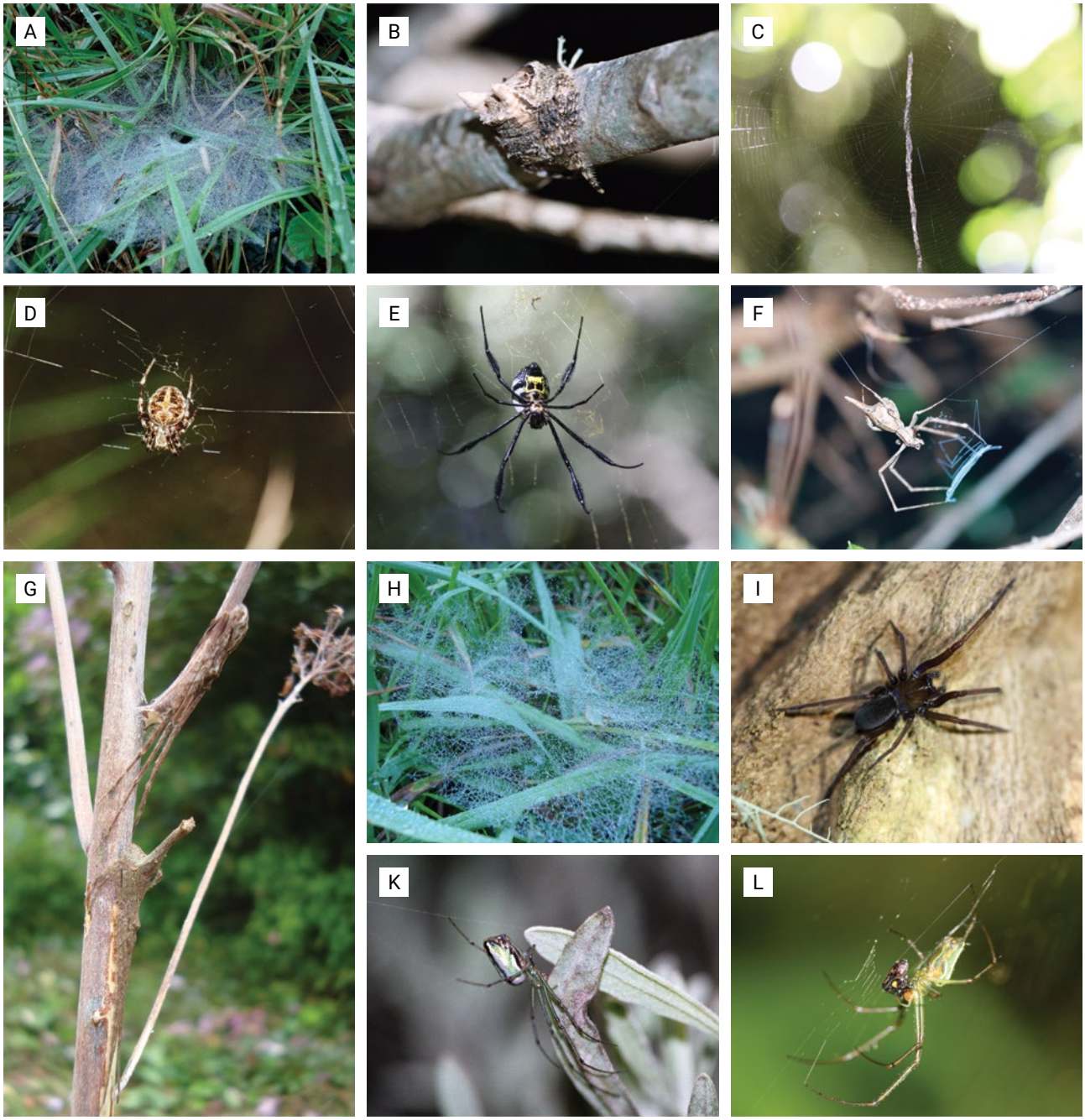

Figure 5. Selected web-building spiders from the Amathole Mountains, Eastern Cape, South Africa; A, Funnel-web of *Agelena* sp. (Agelenidae); B, *Caerostris sexcuspidata*, female (Araneidae); C, *Cyclosa insulana*, female in web (Araneidae); D, *Neoscona subfusca*, female (Araneidae); E, *Trichonephila fenestrata*, female (Araneidae); F, *Menneus camelus*, female, with net-casting web (Deinopidae); G, Same, male (above) and female (below), resting on a twig; H, *Microlinyphia sterilis*, female (Linyphiidae); I, *Themacrys* sp., female (Phyxelididae); J, *Leucauge decorata*, female (Tetragnathidae); K, *Leucauge festiva*, female (Tetragnathidae). Photos: C. Haddad.

the ground in open grassy areas that are obvious when covered by dew-drops in the mornings. Other species common in grassy areas include various hammock-web spiders (Linyphiidae; Figure 5H), *Neoscona subfusca* (C. L. Koch, 1837) (Araneidae; Figure 5D), and tetragnathid orb-weavers of the genera *Leucauge* White, 1841 (Figure 5K, L) and *Tetragnatha* Latreille, 1804.

Araneid orb-weavers are a diverse group (26 species), with several large and charismatic species that can be seen in the forests, constructing orb-webs often 50 cm or more in diameter. These include the bark spider *Caerostris sexcuspidata* Fabricius, 1793 (Figure 5B), species of *Neoscona* Simon, 1885 (Figure 5D), and the golden orb-web spider *Trichonephila fenestrata* (Thorell, 1859) (Figure 5E). Garbage-line spiders, *Cyclosa insulana* (Costa, 1834) (Araneidae; Figure 5C), build fine orb-webs with a vertical line of prey remains down the centre among which the spider rests. However, the most common orb-weavers seen in the forests are undoubtedly the species of *Leucauge* (Figure 5K, L), which construct obliquely orientated orb-webs in low foliage and the herbaceous layer, and are brightly coloured in shades of green, silver, orange and red.

Few web-building species are encountered on bark, predominantly including mesh web-building species of *Themacrys* Simon, 1906 (Phyxelididae; Figure 5I) and tiny Cyatholipidae, which construct small orb-webs in the buttresses and crevices of trees.

The assemblage of forest litter web-builders is dominated by Amaurobiidae, a group of small spiders < 4 mm in length that build mesh-webs between dead leaves. Several web-builders construct webs under rocks and logs, including *Vidole capensis* (Pocock, 1900) and species of *Xeviosa* Lehtinen, 1967 (Phyxelididae), *Steatoda* Sundevall, 1833, and *Theridion* Walckenaer, 1805 (Theridiidae).

Opiliones (harvestmen)

In total, 41 species representing 16 genera and four families of harvestmen have been collected in the Amathole Mountains (Table 1; Supplementary Table 1). Consistent with overall patterns in southern Africa (Lotz 2009), the most genus- and species-rich family is Triaenonychidae, followed by Phalangiidae. Most harvestmen sampled are associated with leaf litter, but species of *Rhampsinitus* Simon, 1879 (Figure 6A, B)

Figure 6. Selected harvestmen and scorpions from the Amathole Mountains, Eastern Cape, South Africa; A, *Rhampsinitus* sp., male (Phalangiidae); B, *Rhampsinitus* aff. *silvaticus*, female (Phalangiidae); C, possible *Adaeum* sp., female (Triaenonychidae); D, *Parabuthus planicauda*, female (Buthidae); E, *Uroplectes formosus*, female (Buthidae); F, *Uroplectes triangulifer*, male (Buthidae); G, *Hadogenes trichiurus*, juvenile (Hormuridae); H, *Opistacanthus validus*, male (Hormuridae); I, *Opistophthalmus latimanus*, female (Scorpionidae). Photos: A–C, G, H, C. Haddad; D, C. Hobson; E, I, S. Christie; F, G. Diedericks.

and Biantidae occasionally wander onto the foliage of grasses, herbs and shrubs, where they may be collected by sweeping or beating vegetation. Triaenonychids are mainly slow-moving cryptic species that blend in with the colour of the soil and litter (Figure 6C).

Surprisingly, harvestmen appear to be minimally impacted by silviculture (pines and *Eucalyptus*) near Hogsback and were more abundant there than in the indigenous forests. Preliminary indications suggest that harvestmen would be an ideal candidate taxon to include in studies evaluating the effects of silviculture on different animal groups, particularly given their high abundance in forest habitats, exceptionally high species richness in the Amathole Mountains (Supplementary Table 1), and the restricted ranges of many of the species (Lotz 2009).

Scorpiones (scorpions)

Eight species, representing six genera and three families of scorpions have been recorded in and around the Amathole Mountains (Table 1; Supplementary Table 1). These include three species of thick-tailed scorpions (genera *Parabuthus* Pocock, 1890 and *Uroplectes* Peters, 1861) in the family Buthidae, one species of flat rock scorpion (genus *Hadogenes* Kraepelin, 1894), three species of creeping scorpions (genera *Cheloctonus* Pocock, 1892 and *Opisthacanthus* Peters, 1861) in the family Hormuridae, and one species of burrowing scorpion (genus *Opistophthalmus* C. L. Koch, 1837) in the family Scorpionidae.

The scorpions of the Amathole Mountains may be classified into three ecomorphotypes (Table 3) based on their morphology and microhabitat requirements (Prendini 2001, 2005). The thick-tailed scorpions of the genus *Uroplectes* (Figure 6E, F) are lapidicolous, sheltering under stones, logs and other surface debris. These morphologically generalist scorpions are ecologically eurytopic. All other scorpion taxa occurring in and around the mountain range are morphologically specialist and ecologically stenotopic. The thick-tailed scorpion, *Parabuthus planicauda* (Pocock, 1889) (Figure 6D), is also fossorial and pelophilous, constructing scrapes or shallow burrows, usually under stones. The flat rock scorpion, *Hadogenes trichiurus* (Gervais, 1843) (Figure 6G), and one of the creeping scorpions, *Opisthacanthus validus* Thorell, 1876 (Figure 6H), are lithophilous, inhabiting the narrow cracks and crevices of rock outcrops. The burrowing scorpion, *Opistophthalmus latimanus* C. L. Koch, 1841 (Figure 6I), and the creeping scorpions of the genus *Cheloctonus* are fossorial and pelophilous, constructing burrows in hard, clayey soil, usually in open ground [*Cheloctonus crassimanus* (Pocock, 1896) and *O. latimanus*] or under stones (*C. glaber* Kraepelin, 1896). The burrows of *Opistophthalmus* are usually constructed at an angle to the ground surface, with a semi-circular entrance opening, whereas the burrows of *Cheloctonus* are usually vertical, with a more slit-like entrance opening.

The method of burrow construction differs among the four fossorial scorpion taxa. The scorpionid, *O. latimanus*, is a cheliceral burrower, which uses the chelicerae to loosen the soil, and the legs and, to a lesser extent, the metasoma, to scrape it away. The hormurids, *C. crassimanus* and *C. glaber*, are pedipalpal burrowers, which use the pedipalps to loosen and scrape the soil away. The buthid, *P. planicauda*, is a metasomal burrower, which uses the metasoma to loosen the soil and the legs and metasoma to scrape it away.

Different scorpion taxa inhabit distinct geographical areas in and around the Amathole Mountains. Three species with lower tolerance for aridity occupy mesic

Table 3. Habitat preferences of scorpions occurring in and around the Amathole Mountains, Eastern Cape, South Africa (Eco = ecomorphotype)

	Eco	Habitat	Elevation	Location
Buthidae				
Parabuthus planicauda (Pocock, 1889)	pelophilous	savanna	low	valleys, N/S slopes
Uroplectes formosus Pocock, 1890	lapidicolous	grassland, thicket	high	summit, N/S slopes
Uroplectes triangulifer (Thorell, 1876)	lapidicolous	savanna	low	valleys, N/S slopes
Hormuridae				
Cheloctonus crassimanus (Pocock, 1896)	pelophilous	savanna, thicket	low	valleys, S slope
Cheloctonus glaber Kraepelin, 1896	pelophilous	grassland	high	summit, N slope
Hadogenes trichiurus (Gervais, 1843)	lithophilous	savanna	low	valleys, N slope
Opisthacanthus validus Thorell, 1876	lithophilous	forest, thicket	high	summit, S slope
Scorpionidae				
Opistophthalmus latimanus C.L. Koch, 1841	pelophilous	savanna, thicket	low	valleys, S slope

habitats at higher elevations on the mountain range. *Opisthacanthus validus* inhabits forests and thicket on the summit and southern slopes whereas *C. glaber* inhabits grasslands on the summit and northern slopes. *Uroplectes formosus* Pocock, 1890 inhabits grasslands and thicket on the summit, northern and southern slopes. Five species with higher tolerance for aridity occupy xeric habitats, primarily savanna, thicket and, in places, Nama Karoo, in the warm, dry valleys intersecting the mountains. *Parabuthus planicauda* and *U. triangulifer* (Thorell, 1876) occur in valleys intersecting both the northern and southern slopes of the mountain range, whereas *C. crassimanus* and *O. latimanus* are restricted to valleys intersecting the southern slopes and *H. trichiurus* to valleys intersecting the northern slopes.

Pseudoscorpiones (false scorpions)

Pseudoscorpions are a morphologically homogenous group, with small differences in body shape, proportions and fine structures often determining their taxonomic placement (Figure 7). There are currently 165 species of pseudoscorpions described from South Africa (Dippenaar-Schoeman & Harvey 2000; Harvey et al. 2016; Neethling & Haddad 2016; Neethling & Neethling 2023), with the Amathole Mountain range containing 18 described species, representing 16 genera and nine families. An additional three species and two families are represented by possibly undescribed species (Table 4; Supplementary Table 1).

As elsewhere in South Africa, data on pseudoscorpion diversity are somewhat limited for the Amathole Mountains. The area is of particular historical significance with regard to South African pseudoscorpion taxonomy, however, as many of the earliest species descriptions came from material collected by Reverend Robert Godfrey, a missionary and naturalist stationed at the Pirie Mission near Qonce, then known as King William's Town (Ellingsen 1912). Indeed, eight of the area's described species have type localities at, or around, the Pirie Mission (Supplementary Table 1). For the Amathole region, historical records are concentrated around the Pirie Forest area, whereas modern sampling has only recently been conducted in the forests around Hogsback, Fort Fordyce, Katberg and Stutterheim. Barely any data are available on the presence of species outside these forests.

The indigenous forests around Hogsback are of particular interest. Not only do 11 described species occur there, but recent sampling has yielded as of yet unidentified species of *Ectactolpium* Beier, 1947 (Olpiidae), *Ectromachernes* Beier, 1944 (Withiidae), and *Parallowithius* Beier, 1955 (Withiidae). Another three new species of *Gymnobisium* Beier, 1931 (Gymnobisiidae) (Figure 7D, E) were recently described (Neethling & Neethling 2023).

Table 4. Habitat preferences (Hab) of pseudoscorpions occurring in and around the Amathole Mountains, Eastern Cape, South Africa (AR = arboreal; BE = associated with beehives; FA = facultative arboreal; LL = leaf litter)

Species	Hab
Atemnidae	
Cyclatemnus globosus Beier, 1947	LL
Cheliferidae	
Aperittochelifer minusculus (Ellingsen, 1912)	AR
Beierius walliskewi (Ellingsen, 1912)	LL
Ellingsenius sculpturatus (Lewis, 1903)	BE
Hansenius torulosus (Tullgren, 1907)	LL
Lophochernes mucronatus (Tullgren, 1907)	AR
Microchelifer minusculoides (Ellingsen, 1912)	AR
Chthoniidae	
Anaulacodithella mordax (Tullgren, 1907)	LL
Tyrannochthonius contractus (Tullgren, 1907)	LL
Feaellidae	
Feaella mucronata Tullgren, 1907	LL
Garypinidae	
Garypinidius capensis (Ellingsen, 1912)	AR
Geogarypidae	LL
Afrogarypus excelsus (Beier, 1964)	LL
Afrogarypus impressus (Tullgren, 1907)	LL
Afrogarypus triangularis (Ellingsen, 1912)	FA
Gymnobisiidae	
Gymnobisium cuneatum Neethling & Neethling, 2023	LL
Gymnobisium hogsbackense Neethling & Neethling, 2023	FA
Gymnobisium prionotogladiatum Neethling & Neethling, 2023	FA
Olpiidae	
Ectactolpium sp.	AR
Pseudochiridiidae	
Pseudochiridium lawrencei Beier, 1964	FA
Pseudotyrannochthoniidae	
Afrochthonius godfreyi (Ellingsen, 1912)	LL
Selachochthonius serratidentatus (Ellingsen, 1912)	LL
Withiidae	
Afrowithius paradoxus (Ellingsen, 1912)	FA
Ectromachernes sp.	LL
Parallowithius sp.	LL

As forest-dwellers, most pseudoscorpions occur in leaf litter, under dead logs or stones. Others are arboreal, hiding in holes or crevices in the trunks or under loose bark. Many of the species, in particular those of the families Chthoniidae, Feaellidae, Pseudotyrannochthoniidae and Tridenchthoniidae, are ecologically stenotopic, having adapted to the humid environment of the forest floor, whereas *Ellingsenius sculpturatus*

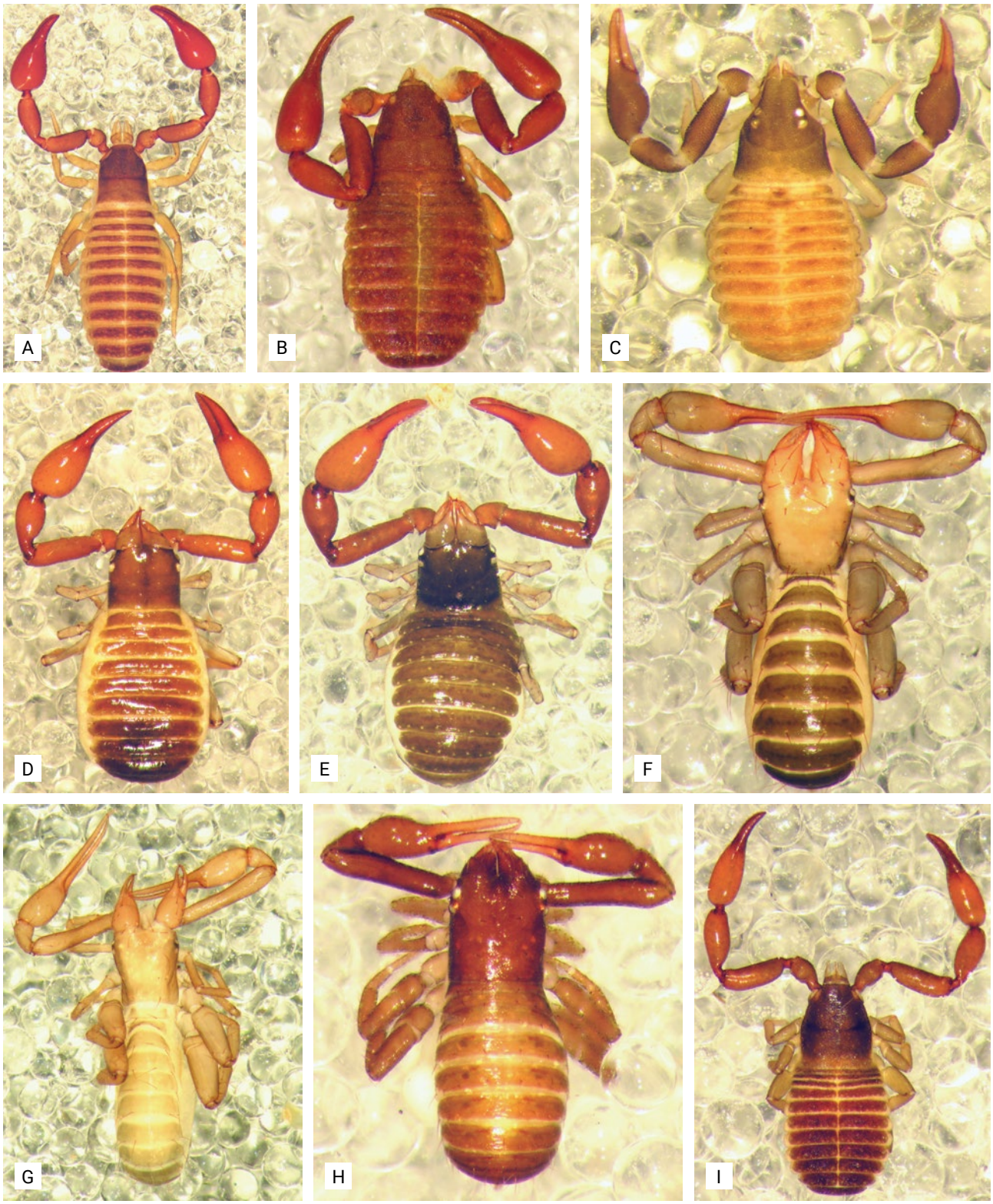

Figure 7. Selected Pseudoscorpiones from the Amathole Mountains, Eastern Cape, South Africa; A, *Catatemnus* sp., female (Atemidae); B, unidentified species of Cheliferidae, female; C, *Afrogarypus triangularis*, female (Geogarypidae); D, *Gymnobisium cuneatum*, female (Gymnobisiidae); E, *Gymnobisium hogsbackense*, male (Gymnobisiidae); F, *Afrochthonius godfreyi*, female (Pseudotyrannochthoniidae); G, *Afrochthonius* aff. *inequalis*, female (Pseudotyrannochthoniidae); H, *Anaulacodithella mordax*, female (Tridenchthoniidae); I, unidentified species of Withiidae, female. Photos: C. Haddad.

(Lewis, 1903) (Cheliferidae) are found exclusively on bees or in beehives (Hewitt & Godfrey 1929). Arboreal or semi-arboreal species, such as the families Atemnidae, Cheliferidae, Olpiidae, Pseudochiridiidae and Withiidae, are more widespread, as many have thicker cuticles and can tolerate a greater range of environmental conditions (Beier 1947). Some pseudoscorpions disperse via phoresis, enabling them to establish populations in a greater variety of habitats.

Amathole Mountains as a hotspot for Arachnida

The Amathole Mountains appear to be a hotspot for particular arachnid taxa, based on the data available, but the importance of conserving this mountain range will only be fully appreciated when many of the species in poorly studied taxa have been described and the fauna more comprehensively sampled. Although the current spider diversity for the area is 324 species (Table 1), many more species may occur there. For comparison, 276 species in 47 families were recorded from the Addo Elephant National Park (Dippenaar-Schoeman et al. 2020), the northern limit of which is about 80 km south of the western margin of the Amathole Mountains, and which has five biomes represented within its borders.

Among spiders, Hogsback is the type locality for 19 species, nine of which have not been recorded elsewhere: *Chumma foliata*, *Spiroctenus flavopunctatus* (Bemmeridae), *Lepthercus mandelai*, *Drassodella amatola* and *D. tolkieni* Mbo & Haddad, 2019, *Afraflacilla imitator* (Wesołowska & Haddad, 2013), *Asemonea amatola* and *Thyenula splendens* Wesołowska & Haddad, 2018 (Salticidae), and *Anyphops amatolae*. *Chumma subridens* Jocqué & Alderweireldt, 2018 has only been recorded from its type locality, Mpofu Fort Fordyce Nature Reserve, and *Stasimopus insculptus* Pocock, 1901 (Stasimopidae) is only known from Qonce. Sampling at various sites in the Amathole Mountains also provided considerable range extensions for many species (Dippenaar-Schoeman et al. 2010), particularly in the family Salticidae (Wesołowska & Haddad 2013, 2018).

Several groups are understudied taxonomically, and a large proportion of the Amathole species are new, e.g., both species of *Parapostenus* Lessert, 1923 (Miturgidae), 11 of 12 species of *Scytodes*, 10 of 16 species of Trachelidae, six of 10 species of *Clubiona* Latreille, 1804 (Clubionidae), and most Theridiidae. Only with additional taxonomic effort can these taxa be described and a more accurate representation of their distribution in South Africa be presented.

Despite the taxonomic shortfall for spiders, it is somewhat surprising that the Amathole Mountains were not identified as an area of endemism in Griswold's (1991) analysis of Afromontane biogeography. This could be explained by inclusion of only three currently recognised spider families, Microstigmatidae, Migidae and Phyxelididae, in his analysis. *Microstigmata amatolae* and two new species of Phyxelididae belonging to the genera *Themacrys* and *Xevioso* Lehtinen, 1967 may be endemic to the region, but only the former was known at the time of Griswold's (1985) study and neither of the latter two species was recorded in his revision of Phyxelididae (Griswold 1990). The Amathole Mountains may have emerged as an area of endemism if these or other spider taxa had been included in Griswold's (1990) analysis.

The Amathole Mountains have by far the richest Opiliones fauna of any part of South Africa, with more than 40 species already recorded, representing more than 20% of the species known from the country (Lotz 2009, 2010; De Bivort & Giribet 2010). A remarkable 27 species of Triaenonychidae and eight species of the phalangiid genus *Rhampsinitus* have been recorded, an exceptional diversity. Although most of the records (32 spp.) are concentrated around Hogsback, suggesting a very strong sampling bias, other sites, particularly forest biotopes, will probably have a similarly rich fauna if sampled thoroughly.

Hogsback is the only place in the Amathole Mountains that is a type locality for harvestmen; 11 species, of which one was subsequently synonymised (Staręga 1984), were described from material collected there (Lawrence 1931, 1934; De Bivort & Giribet 2010). Six of these have been recorded elsewhere in the Amathole Mountains and beyond (Lotz 2009), but four remain known only from Hogsback: *Parapurcellia amatola* De Bivort & Giribet, 2010 (Purcellidae), and *Adaeulum brevidentatum* Lawrence, 1934, *Larifuga mantonae* Lawrence, 1934 and *Roewerania lignicola* Lawrence, 1934 (Triaenonychidae).

Outside the Afromontane Forests, the pseudoscorpions of the Amathole Mountains are poorly studied. The region appears to possess a high degree of endemism, with some species, such as the Gymnobisiidae, occurring exclusively within the isolated forest patches, though, due to the lack of sampling in the region, the true extent of the distributions of many species is unknown.

The other two arachnid orders, Amblypygi and Scorpiones, are represented by species more widespread in the Eastern Cape or South Africa. None of these species is endemic to the Amathole Mountains, although the hormurid scorpion, *Cheloctonus glaber* may be considered near-endemic, with a distribution restricted to the Amathole and ranges to the north.

Conclusions

The Amathole Mountains contain an impressive arachnid biodiversity and are a hotspot for several taxa,

particularly harvestmen, pseudoscorpions and the spider families Clubionidae, Salticidae, Scytodidae and Trachelidae. Although the levels of endemism presently appear low, numerous undescribed species from the region may potentially be endemic. Arachnids may be important for informing conservation management decisions in the region, once their distributions are better known and the many new taxa have been described.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

C.R.H. (University of the Free State) conducted field sampling, specimen identification, sourced literature and museum data for Araneae and Opiliones, prepared tables and figures, photographed specimens, and wrote and edited the manuscript. L.P. (American Museum of Natural History) sourced data for Amblypygi and Scorpiones, prepared the ecology table and wrote the Scorpiones section, and helped edit the manuscript. J.A.N. (National Museum) conducted field sampling, specimen identification, sourced literature and museum data for Pseudoscorpiones, prepared the ecology table and wrote the Pseudoscorpiones section, and helped edit the manuscript. A.S.D. (University of Venda) identified specimens, provided geographical data, and helped edit the manuscript.

Ethical considerations

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Data availability statement

Species-level data are available from the corresponding author or from each of the museums listed in the Data Mining section.

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Supplementary material

Supplementary Table 1. Checklist of the non-acarine arachnids of the Amathole District, Eastern Cape, South Africa (FB = Fort Beaufort; HB = Hogsback; KB = Katberg; MF = Mpofu Fort Fordyce Nature Reserve; QO = Qonce; ST = Stutterheim; OT = other Amathole localities; X = present; XT = type locality; † = new species)

	FB	HB	KB	MF	QO	ST	OT	References
AMBLYPYGI								
Phrynichidae								
Damon annulatipes (Wood, 1869)					X			Weygoldt (1999)
ARANEAE								
Agelenidae								
Agelena sp.		X						
Amaurobiidae								
Chresiona sp.		X						
Chumma foliata Jocqué & Alderweireldt, 2018		XT						Jocqué & Alderweireldt (2018)
Chumma subridens Jocqué & Alderweireldt, 2018				XT				Jocqué & Alderweireldt (2018)
Amaurobiidae sp. 1		X	X		X			
Amaurobiidae sp. 2		X		X				
Anapidae								
Pseudanapis sp.†		X						
Araneidae								
Acanthepeira sp.		X						
Acusilas africanus Simon, 1895					X			
Araneus nigroquadratus Lawrence, 1937	X	X	X	X				Dippenaar-Schoeman et al. (2010)
Araneus sp.		X						
Argiope australis (Walckenaer, 1805)		X						Dippenaar-Schoeman et al. (2010)
Argiope trifasciata (Forskal, 1775)		X						Dippenaar-Schoeman et al. (2010)
Bijoaraneus legonensis (Grasshoff & Edmunds 1979)			X					
Caerostris sexcuspidata Fabricius, 1793	X	X		X	X			Dippenaar-Schoeman et al. (2010); Gregorič et al. (2015)
Chorizopes sp.		X			X			
Cyclosa insulana (Costa, 1834)		X						Dippenaar-Schoeman et al. (2010)
Cyclosa sp. 2		X		X			X	
Cyphalonotus larvatus (Simon, 1881)		X						
Cyrtophora citricola (Forsskål, 1775)					X			Dippenaar-Schoeman et al. (2010)
Gasteracantha versicolor (Walckenaer, 1841)					X			

	FB	HB	KB	MF	QO	ST	OT	References
Araneidae (continued)								
Hypsosinga sp.		X			X			
Ideocaira transversa Simon, 1903		X						
Isoxya cicatricosa (C.L. Koch, 1844)		X			X			Dippenaar-Schoeman et al. (2010)
Isoxya stuhlmanni (Bösenberg & Lenz, 1885)					X			Dippenaar-Schoeman et al. (2010)
Isoxya tabulata (Thorell, 1859)					X			Dippenaar-Schoeman et al. (2010)
Nemoscolus elongatus Lawrence, 1947		X					X	
Neoscona hirta (C. L. Koch, 1844)		X			X			Dippenaar-Schoeman et al. (2010)
Neoscona subfusca (C. L. Koch, 1837)		X	X					Dippenaar-Schoeman et al. (2010)
Pararaneus cyrtoscapus (Pocock, 1898)		X						
Prasonica seriata Simon, 1895		X						Dippenaar-Schoeman et al. (2010)
Pycnacantha tribulus (Fabricius, 1781)					X			Dippenaar-Schoeman et al. (2010)
Trichonephila fenestrata (Thorell, 1859)		X			X			Dippenaar-Schoeman et al. (2010)
Bemmeridae								
Homostola abernethyi (Purcell, 1903)		X						
Spiroctenus flavopunctatus (Purcell, 1903)		XT						Purcell (1903); Dippenaar-Schoeman et al. (2010); Opatova et al. (2020); Montes de Oca et al. (2022)
Cheiracanthiidae								
Cheiramiona ansiae Lotz, 2002		X						Dippenaar-Schoeman et al. (2010)
Cheiramiona filipes (Simon, 1898)				X				
Cheiramiona hogsbackensis Lotz, 2015	X	XT		X				Lotz (2015)
Cheiramiona silvicola (Lawrence, 1938)		X			X			Dippenaar-Schoeman et al. (2010)
Cheiracanthium furculatum Karsch, 1879		X	X	X				
Clubionidae								
Clubiona biaculeata Simon, 1897		X		X				
Clubiona capensis Simon, 1897		X	X		X			
Clubiona pupillaris Lawrence, 1938		X			X			Dippenaar-Schoeman et al. (2010)
Clubiona sigillata Lawrence, 1952		X						Dippenaar-Schoeman et al. (2010)
Clubiona sp. 5†	X	X						
Clubiona sp. 6†		X	X	X				
Clubiona sp. 7†		X	X					

	FB	HB	KB	MF	QO	ST	OT	References
Clubionidae (continued)								
Clubiona sp. 8†	X	X	X					
Clubiona sp. 9†		X						
Clubiona sp. 10†		X						
Corinnidae								
Cambalida fulvipes (Simon, 1896)	X			X				Haddad (2012a)
Copa flavoplumosa Simon, 1885		X			X			Haddad (2013)
Copa kei Haddad, 2013		X	X					Haddad (2013)
Echinax sp. imm.		X						Haddad (2012b)
Pronophaea natalica Simon, 1897		X		X	X			Dippenaar-Schoeman et al. (2010)
Ctenidae								
Ctenus parvoculatus Benoit, 1979					X			Dippenaar-Schoeman et al. (2010)
Ctenus pulchriventris (Simon, 1896)					X			Dippenaar-Schoeman et al. (2010)
Cyatholipidae								
Cyatholipus sp.		X						
Isicabu sp.†		X						
Ulwembua sp.†		X						
Cyrtaucheniidae								
Ancylotrypa sororum (Hewitt, 1916)		X		X				
Homostola abernethyi (Purcell, 1903)		X						
Deinopidae								
Menneus camelus Pocock, 1902		X						Dippenaar-Schoeman et al. (2010)
Dictynidae								
Archaeodictyna sp.		X						
Dictyna sp.		X	X	X	X			
Mashimo leleupi Lehtinen, 1967							X	
Entypesidae								
Lepthercus mandelai Ríos-Tamayo & Lyle, 2020		XT						Ríos-Tamayo & Lyle (2020)
Euagridae								
Allothele australis (Purcell, 1903)				X				
Gallieniellidae								
Drassodella amatola Mbo & Haddad, 2019		XT						Mbo & Haddad (2019)
Drassodella tolkieni Mbo & Haddad, 2019		XT						Mbo & Haddad (2019)
Gnaphosidae								
Ammoxenus sp.				X				Dippenaar-Schoeman et al. (2010)

	FB	HB	KB	MF	QO	ST	OT	References
Gnaphosidae (continued)								
Aphantaulax signicollis Tucker, 1923				X	X			
Camillina capensis Platnick & Murphy, 1987		X						
Camillina cordifera (Tullgren, 1910)			X					Dippenaar-Schoeman et al. (2010)
Drassodes sp.		X				X		
Echemus sp.					X			
Micaria beaufortia (Tucker, 1923)				X				Booysen & Haddad (2021)
Nomisia sp.		X						
Poecilochroa sp.		X						
Scotophaeus sp.				X				
Trichothyse africana (Tucker, 1923)		X						
Xerophaeus aurariarum Purcell, 1907				X				
Xerophaeus communis Purcell, 1907	X	X						
Zelotes fuligineus (Purcell, 1907)		X						
Zelotes sp. 2		X						
Hahniidae								
Hahnia clathrata Simon, 1898				X	X			
Hahnia laticeps Simon, 1898				X				
Hahnia tabulicola Simon, 1898		X		X	X			
Hahnia sp.†		X	X					
Hersiliidae								
Neotama corticola (Lawrence, 1937)		X		X				
Linyphiidae								
Ceratinopsis sp.		X						
Limoneta sirimoni (Bosmans, 1979)		X						
Mecynidis sp.†		X	X	X	X			
Meioneta sp.		X						
Microlinyphia sterilis (Pavesi, 1883)		X	X					
Ostearius melanopygius (O. Pickard-Cambridge, 1879)		X						
Pelecopsis sp.		X		X	X			
Typhistes sp.		X						
Linyphiidae sp.1		X	X	X	X	X		
Linyphiidae sp. 2			X	X		X		
Linyphiidae sp. 3				X				
Linyphiidae sp. 4				X				
Lycosidae								
Allocosa sp.		X						
Arctosa promontorii (Pocock, 1900)		X						

	FB	HB	KB	MF	QO	ST	OT	References
Lycosidae (continued)								
Foveosa foveolata (Purcell, 1903)				X				
Hippasa australis Lawrence, 1927				X				
Hippasa funerea Lessert, 1925		X		X	X		X	
Hogna bimaculata (Purcell, 1903)				X				
Lycosa sp.		X		X				
Pardosa crassipalpis Purcell, 1903				X				
Pardosa sp.		X		X	X			
Proevippa bruneipes (Purcell, 1903)		X						
Pterartoria sp.		X						
Trabea nigriceps Purcell, 1903				X				
Trabea ornatipalpis Russell-Smith, 1982		X						
Trabea purcelli Roewer, 1951		X	X	X				
Trabea rubriceps Lawrence, 1952		X		X				
Lycosidae sp. 1						X		
Microstigmatidae								
Microstigmata amatola Griswold, 1985		XT				X		Griswold (1985); Dippenaar-Schoeman et al. (2010); Opatova et al. (2020); Montes de Oca et al. (2022)
Migidae								
Poecilomigas abrahami (O. Pickard-Cambridge, 1889)		X			X			Dippenaar-Schoeman et al. (2010); Opatova et al. (2020)
Mimetidae								
Anansi natalensis (Lawrence, 1938)		X			X			Dippenaar-Schoeman et al. (2010); Benavides et al. (2017)
Anansi sp. 2†	X	X	X	X				
Anansi sp. 3†		X						
Ero lawrencei Unzicker, 1966		X						
Miturgidae								
Parapostenus sp. 1†		X		X				Wheeler et al. (2017); Haddad (2022)
Parapostenus sp. 2†		X						
Nesticidae								
Nesticus sp.†		X						
Oecobiidae								
Oecobius navus Blackwall, 1859		X						Šťáhlavský et al. (2020)
Oonopidae								
Australoonops granulatus Hewitt, 1915		X						Dippenaar-Schoeman et al. (2010); Platnick & Dupérré (2010)

	FB	HB	KB	MF	QO	ST	OT	References
Oonopidae (continued)								
Australoonops skaife Platnick & Dupérré, 2010		X						Platnick & Dupérré (2010); Dippenaar-Schoeman et al. (2010)
Opopaea speciosa (Lawrence, 1952)		X			X			Dippenaar-Schoeman et al. (2010)
Orchestina sp.†		X						
Orsolobidae								
Afrilobus sp.†		X		X				
Azanialobus sp.†		X		X				
Oxyopidae								
Oxyopes bothai Lessert, 1915		x						
Oxyopes sp.		X		X	X			
Peucetia maculifera Pocock, 1900		X			X			Dippenaar-Schoeman et al. (2010)
Palpimanidae								
Palpimanus sp.				X				
Penestomidae								
Penestomus prendinii Miller, Griswold & Haddad, 2010				X				
Philodromidae								
Gephyrota glauca (Jézéquel, 1966)		X						
Philodromus brachycephalus Lawrence, 1952		x						
Philodromus spp.	X	X	X	X	X			
Thanatus sp.		x		X				
Tibellus minor Lessert, 1919		x		X				
Pholcidae								
Quamtana sp.†		X	X	X		X		
Smeringopus ubicki Huber, 2012				X				
Spermophora sp.†		X						
Phyxelididae								
Themacrys sp.†		X						
Vidole capensis (Pocock, 1900)		X				X		Dippenaar-Schoeman et al. (2010)
Xevioso sp.†		X						
Pisauridae								
Cispius kimbius Blandin, 1978		X						
Euprosthenopsis lamorali Blandin, 1977		X		X				
Nilus massajae (Pavesi, 1883)		X				X		Dippenaar-Schoeman et al. (2010)
Rothus auratus Pocock, 1900		X						

	FB	HB	KB	MF	QO	ST	OT	References
Salticidae								
Afraflacilla imitator (Wesołowska & Haddad, 2013)		XT						Wesolowska & Haddad (2013)
Asemonea amatola Wesołowska & Haddad, 2013		XT						Wesolowska & Haddad (2013, 2018)
Baryphas ahenus Simon, 1902		X						
Brancus mustelus (Simon, 1902)					X			
Dendryphantes purcelli Peckham & Peckham, 1903		X						Dippenaar-Schoeman et al. (2010)
Dendryphantes silvestris Wesołowska & Haddad, 2013		XT	X	X			X	Wesolowska & Haddad (2013)
Euophrys bifida Wesołowska, Azarkina & Russell-Smith, 2014		XT		X	X	X		Wesolowska et al. (2014)
Evarcha denticulata Wesołowska & Haddad, 2013		X						Haddad & Wesolowska (2013); Wesolowska & Haddad (2013)
Hasarius adansoni (Audouin, 1826)		X			X			Wesolowska & Haddad (2013)
Heliophanus aberdarensis Wesołowska, 1986		X						Wesolowska & Haddad (2018)
Heliophanus debilis Simon, 1901		X						
Heliophanus demonstrativus Wesołowska, 1986	X							Dippenaar-Schoeman et al. (2010)
Heliophanus deserticola Simon, 1901		X						Wesolowska & Haddad (2018)
Heliophanus gramineus Wesołowska & Haddad, 2013		XT	X	X				Wesolowska & Haddad (2013, 2018)
Heliophanus hastatus Wesołowska, 1986		X		X				Dippenaar-Schoeman et al. (2010)
Heliophanus nanus Wesołowska, 2003		X						Dippenaar-Schoeman et al. (2010)
Heliophanus orchesta Simon, 1886		X						Dippenaar-Schoeman et al. (2010)
Heliophanus sororius Wesołowska, 2003		X						Wesolowska & Haddad (2018)
Hispo georgius (Peckham & Peckham, 1892)		X						Haddad & Wesolowska (2013)
Langona sp.†		X						
Massagris honesta Wesołowska, 1993		X						Dippenaar-Schoeman et al. (2010); Wesolowska & Haddad (2013); Maddison (2015)
Massagris mirifica Peckham et Peckham, 1903		X		X				Haddad & Wesolowska (2013); Wesolowska & Haddad (2013, 2018)
Myrmarachne lesserti Lawrence, 1938				X	X			
Myrmarachne sp.		X		X	X		X	
Natta horizontalis Karsch, 1879		X			X			
Nigorella hirsuta Wesołowska, 2009					X			

	FB	HB	KB	MF	QO	ST	OT	References
Salticidae (continued)								
Oviballus vidae Azarkina & Haddad, 2020				X	X			Azarkina & Haddad (2020)
Phintella aequipes (Peckham & Peckham, 1903)					X			
Phlegra nuda Próchniewicz & Hęciak, 1994		X						
Planamarengo bimaculata (Peckham & Peckham, 1903)		X						Azarkina & Haddad (2020)
Pseudicius africanus Peckham & Peckham, 1903		X						
Pseudicius maculatus Haddad & Wesołowska, 2011		X						Wesolowska & Haddad (2013)
Rhene biguttata Peckham & Peckham, 1903					X			Wesolowska & Haddad (2018)
Rhene timidus Wesołowska & Haddad, 2013		XT						Wesolowska & Haddad (2013, 2018)
Rumburak hilaris Wesołowska, Azarkina & Russell-Smith, 2014		XT	X	X			X	Wesolowska et al. (2014)
Rumburak mirabilis Wesołowska, Azarkina & Russell-Smith, 2014		XT		X				Wesolowska et al. (2014)
Stenaelurillus sp.				X				
Thyene aperta (Peckham & Peckham, 1903)		X						Dippenaar-Schoeman et al. (2010)
Thyene natalii Peckham & Peckham, 1903					X			
Thyene ogdeni Peckham & Peckham, 1903		X						Dippenaar-Schoeman et al. (2010)
Thyene thyenioides (Lessert, 1925)				X				
Thyenula alotama Wesołowska, Azarkina & Russell-Smith, 2014		XT		X				Wesolowska et al. (2014)
Thyenula aurantiaca (Simon, 1902)				X				Dippenaar-Schoeman et al. (2010)
Thyenula juvenca Simon, 1902		X	X		X			
Thyenula leighi (Peckham & Peckham, 1903)					X			
Thyenula splendens Wesołowska & Haddad, 2018		XT						Wesolowska & Haddad (2018)
Tusitala barbata Peckham & Peckham, 1902	X	X						Azarkina & Foord (2015)
Wandawe australe Azarkina & Haddad, 2020			XT					Azarkina & Haddad (2020)
Wandawe benjamini (Wesołowska & Haddad, 2013)		XT	X					Wesolowska & Haddad (2013); Azarkina & Haddad (2020)
Scytodidae								
Scytodes triangulifera Purcell, 1904		X						

	FB	HB	KB	MF	QO	ST	OT	References
Scytodidae (continued)								
Scytodes sp. 2†		X						
Scytodes sp. 3†		X						
Scytodes sp. 4†		X						
Scytodes sp. 5†		X						
Scytodes sp. 6†				X				
Scytodes sp. 7†		X						
Scytodes sp. 8†		X						
Scytodes sp. 9†			X	X				
Scytodes sp. 10†		X		X				
Scytodes sp. 11†		X						
Scytodes sp. 12†		X	X					
Segestriidae								
Ariadna sp. 1		X			X			
Ariadna sp. 2		X						
Selenopidae								
Anyphops amatolae (Lawrence, 1940)		XT						Lawrence (1940); Dippenaar-Schoeman et al. (2010)
Anyphops gilli (Lawrence, 1940)		X						Dippenaar-Schoeman et al. (2010)
Anyphops whiteae (Pocock, 1902)					X			Lawrence (1940)
Anyphops sp.				X	X			
Selenops sp.	X							
Sparassidae								
Olios sp.		X					X	
Palystes perornatus Pocock, 1900		X						Dippenaar-Schoeman et al. (2010)
Palystes superciliosus L. Koch, 1875		X			X			Dippenaar-Schoeman et al. (2010)
Parapalystes lycosinus (Pocock, 1900)				X				
Stasimopidae								
Stasimopus insculptus Pocock, 1901					XT			Pocock (1901); Dippenaar-Schoeman et al. (2010)
Stasimopus schoenlandi Pocock, 1900					X			
Tetragnathidae								
Diphya simoni Kauri, 1950		X			X			Omelko et al. (2020)
Leucauge decorata (Blackwall, 1864)		X						
Leucauge festiva (Blackwall, 1866)		X						Dippenaar-Schoeman et al. (2010)
Leucauge levanderi (Kulczynski, 1901)		X						Dippenaar-Schoeman et al. (2010)
Leucognatha sp.		X						

	FB	HB	KB	MF	QO	ST	OT	References
Tetragnathidae (continued)								
Meta sp.		X						
Pachygnatha sp.†		X						
Tetragnatha keyserlingi Simon, 1890		X						Dippenaar-Schoeman et al. (2010)
Tetragnatha subsquamata Okuma, 1985			X	X	X			
Theraphosidae								
Harpactira tigrina Ausserer, 1875					X			
Theridiidae								
Achaearanea sp.†		X						
Anelosimus sp.†		X						
Argyrodes sp.†			X					
Chorizopella tragardhi Lawrence, 1947		X			X			
Coscinida sp.†		X						
Crustulina guttata (Wider, 1834)		X						Dippenaar-Schoeman et al. (2010)
Dipoenata sp.†			X					
Episinus bilineatus Simon, 1894		X	X	X	X			
Episinus sp. 2†		X						
Euryopis sp. †		X	X	X	X			
Latrodectus cinctus Blackwall, 1865	X							Dippenaar-Schoeman et al. (2010)
Latrodectus geometricus C.L. Koch, 1841		X			X			Dippenaar-Schoeman et al. (2010)
Parasteatoda sp.		X						
Phoroncidia sp.		X	X	X	X			
Ruborrhidion sp. 1†		X						
Ruborrhidion sp. 2†		X						
Ruborrhidion sp. 3†		X						
Ruborrhidion sp. 4†		X						
Steatoda capensis Hann, 1990			X					
Steatoda erigoniformis (O. Pickard-Cambridge, 1872)						X		
Steatoda foravae Dippenaar-Schoeman & Muller, 1992		X				X		
Theridion sp. 1	X	X	X		X			
Theridion sp. 2		X						
Theridula sp.		X						
Thymoites chopardi (Berland, 1920)		X						Dippenaar-Schoeman et al. (2010)
Theridiidae sp. 1	X	X	X	X	X		X	
Theridiidae sp. 2		X	X		X			

	FB	HB	KB	MF	QO	ST	OT	References
Theridiidae (continued)								
Theridiidae sp. 3			X					
Thomisidae								
Ansiea tuckeri (Lessert, 1919)				X				
Diaea puncta Karsch, 1884		X						Dippenaar-Schoeman et al. (2010)
Firmicus bipunctatus Caporiacco, 1941		X						
Geraesta congoensis (Lessert, 1943)		X						
Hewittia gracilis Lessert, 1928		X		X				
Misumenops rubrodecoratus Millot, 1941		X		X				
Monaeses austrinus Simon, 1910		X		X				
Monaeses paradoxus Lucas, 1864		X						Dippenaar-Schoeman et al. (2010)
Oxytate concolor (Caporiacco, 1947)		X						
Oxytate ribes (Jézéquel, 1964)		X	X					
Pactactes obesus Simon, 1895					X			
Pactactes trimaculatus Simon, 1895		X	X					
Phaenopoma nigropunctatum (O. Pickard-Cambridge, 1883)		X						
Pherecydes ionae Dippenaar-Schoeman, 1980		X	X	X				
Phrynarachne melloleitaoi Lessert, 1933		X	X		X			Dippenaar-Schoeman et al. (2010)
Runcinia erythrina Jézéquel, 1964		X		X				
Synema decens Karsch, 1878	X	X						
Synema langheldti Dahl, 1907			X					
Synema marlothi Dahl, 1907		X		X				
Synema imitatrix (Pavesi, 1883)					X			
Thomisops bullatus Simon, 1895		X			X			
Thomisus australis Comellini, 1957		X			X			Dippenaar-Schoeman et al. (2010)
Thomisus blandus Karsch, 1880		X				X	X	Dippenaar-Schoeman et al. (2010)
Thomisus stenningi Pocock, 1900		X		X	X		X	Lessert (1923); Dippenaar-Schoeman et al. (2010)
Tmarus comellinii Garcia-Neto, 1989		X						Dippenaar-Schoeman et al. (2010)
Tmarus cameliformis Millot, 1942	X		X	X	X			Dippenaar-Schoeman et al. (2010)
Xysticus mulleri Lawrence, 1952				X				
Xysticus sp. 2		X						
Trachelidae								
Afroceto africana (Simon, 1910)		X						Haddad (2019)

	FB	HB	KB	MF	QO	ST	OT	References
Trachelidae (continued)								
Afroceto martini (Simon, 1897)	X	X		X	X			Dippenaar-Schoeman et al. (2010); Lyle & Haddad (2010)
Capobula montana Haddad *et al.*, 2021		X						Haddad et al. (2021)
Fuchiba sp.†		X						
Jocquestus schenkeli (Lessert, 1923)		X		X				Dippenaar-Schoeman et al. (2010); Lyle & Haddad (2018)
Poachelas montanus Haddad & Lyle, 2008		X						
Thysanina transversa Lyle & Haddad, 2006		X		X	X			
Thysanina sp. 2†		X			X			
Thysanina sp. 3†		X						
Thysanina sp. 4†		X						
Trachelas sp. 1†	X	X		X				
Trachelas sp. 2†		X						
Trachelas sp. 3 †		X						
Trachelas sp. 4†		X						
Trachelas sp. 5†		X						
Trachelas sp. 6†	X	X		X	X			
Trochanteriidae								
Platyoides pusillus Pocock, 1898		X						Dippenaar-Schoeman et al. (2010)
Platyoides walteri (Karsch, 1886)		X			X			Dippenaar-Schoeman et al. (2010)
Uloboridae								
Hyptiotes akermani Wiehle, 1964		X	X		X			Dippenaar-Schoeman et al. (2010)
Miagrammopes sp.	X	X	X	X	X			
Philoponella angolensis (Lessert, 1933)					X			
Uloborus plumipes Lucas, 1846	X	X						
Uloborus sp.					X			
Zodariidae								
Chariobas lineatus Pocock, 1900		X			X			Dippenaar-Schoeman et al. (2010)
Cydrela sp.		X						
Diores annetteae Jocqué, 1990		X						
Zoropsidae								
Griswoldia sp.		X	X	X				
Phanotea xhosa Griswold, 1994		X			X			Griswold (1994)
Phanotea sp. 2†		X						

	FB	HB	KB	MF	QO	ST	OT	References
OPILIONES								
Biantidae								
Metabiantes hanstroemi Kauri, 1961			X					
Metabiantes pusulosus (Loman, 1898)		X				X		Staręga (1992); Lotz (2009, 2010)
Metabiantes urbanus Kauri, 1961				X				
Metabiantes zuurbergianus Kauri, 1961		X						Lotz (2009)
Metabiantes sp.					X	X		
Pettalidae								
Parapurcellia amatola De Bivort & Giribet, 2010		XT						De Bivort & Giribet (2010); Svojanovská et al. (2016)
Phalangiidae								
Rhampsinitus brevipes Kauri, 1961		X						
Rhampsinitus capensis (Loman, 1898)		X						Lotz (2009, 2010); Šťáhlavský et al. (2018)
Rhampsinitus crassus Loman, 1898		X						
Rhampsinitus fissidens Lawrence, 1933		X	X					
Rhampsinitus ingae Kauri, 1961		X						
Rhampsinitus lalandei Simon, 1879		X						
Rhampsinitus leighi (Pocock, 1902)		X						Lotz (2009)
Rhampsinitus silvaticus Lawrence, 1931		X						Lotz (2009)
Triaenonychidae								
Adaeulum brevidentatum Lawrence, 1934		XT						Lawrence (1934); Staręga (1992); Lotz (2009, 2010)
Adaeulum godfreyi Lawrence, 1931		X			X	X		Lotz (2009, 2010)
Adaeum squamatum Lawrence, 1931		X	X					
Amatola dentifrons Lawrence, 1931	X	XT			X	X		Lawrence (1931); Staręga (1992); Lotz (2009, 2010)
Biacumontia elata Kauri, 1961		X				X		
Biacumontia paucidens Lawrence, 1931				X				
Biacumontia truncatidens Lawrence, 1931		X						Staręga (1992); Lotz (2009, 2010)
Biacomontia sp.				X				
Ceratomontia irregularis Lawrence, 1931		X		X				Lotz (2010)
Ceratomontia pusilla Lawrence, 1934		X						
Ceratomontia reticulata Lawrence, 1934		XT		X		X		Lawrence (1934); Staręga (1992); Lotz (2009, 2010)
Ceratomontia rumpiana Lawrence, 1937		X	X					Lotz (2010)
Ceratomontia sanguinea Lawrence, 1934				X				
Ceratomontia setosa Lawrence, 1931		X						Lotz (2009, 2010)
Graemontia bifidens Lawrence, 1931		X	X			X		Lotz (2009)
Graemontia dentichelis Lawrence, 1931		XT	X		X	X		Lawrence (1931); Staręga (1992); Lotz (2009, 2010)

	FB	HB	KB	MF	QO	ST	OT	References
Triaenonychidae (continued)								
Larifuga mantonae Lawrence, 1934		XT						Lawrence (1934); Staręga (1992); Lotz (2009, 2010)
Larifugella afra Lawrence, 1933			X					
Larifugella sp.		X						
Mensamontia morulifera Lawrence, 1931					X			Lotz (2009)
Monomontia atra Lawrence, 1931		XT	X					Lawrence (1931); Staręga (1992); Lotz (2009, 2010)
Monomontia montensis Lawrence, 1938		X						
Monomontia rattrayi Lawrence, 1931		X			X			Lotz (2009, 2010)
Paramontia sp.†		X						Lotz (2010)
Roewerania guduana Kauri, 1961		X	X	X		X		Lotz (2010)
Roewerania lignicola Lawrence, 1934		XT						Lawrence (1934); Staręga (1992); Lotz (2009, 2010)
Paradaeum rattrayi Lawrence, 1931		XT				X		Lawrence (1931); Staręga (1992); Lotz (2009, 2010)
PSEUDOSCORPIONES								
Atemnidae								
Cyclatemnus globosus Beier, 1947		X			X			
Cheliferidae								
Aperittochelifer minusculus (Ellingsen, 1912)		X		X	XT			Ellingsen (1912); Hewitt & Godfrey (1929)
Beierius walliskewi (Ellingsen, 1912)					X	X		Ellingsen (1912); Hewitt & Godfrey (1929)
Ellingsenius sculpturatus (Lewis, 1903)					X			Hewitt & Godfrey (1929)
Hansenius torulosus (Tullgren, 1907)		X			X			Hewitt & Godfrey (1929)
Lophochernes mucronatus (Tullgren, 1907)					X	X		Ellingsen (1912); Hewitt & Godfrey (1929)
Microchelifer minusculoides (Ellingsen, 1912)					XT			Ellingsen (1912); Hewitt & Godfrey (1929)
Chthoniidae								
Anaulacodithella mordax (Tullgren, 1907)		X		X	X			Ellingsen (1912)
Tyrannochthonius contractus (Tullgren, 1907)		X	X	X	X	X		Ellingsen (1912)
Feaellidae								
Feaella mucronata Tullgren, 1907		X				X		
Garypinidae								
Garypinidius capensis (Ellingsen, 1912)		X			XT			Ellingsen (1912)
Geogarypidae								
Afrogarypus excelsus (Beier, 1964)				X	X	X		Neethling & Haddad (2016)
Afrogarypus impressus (Tullgren, 1907)					X			Ellingsen (1912)
Afrogarypus triangularis (Ellingsen, 1912)		X			XT			Ellingsen (1912); Neethling & Haddad (2016)

	FB	HB	KB	MF	QO	ST	OT	References
Gymnobisiidae								
Gymnobisium cuneatum Neethling & Neethling, 2023		X						Neethling & Neethling (2023)
Gymnobisium hogsbackense Neethling & Neethling, 2023		X						Neethling & Neethling (2023)
Gymnobisium prionotogladiatum Neethling & Neethling, 2023			X	X				Neethling & Neethling (2023)
Olpiidae								
Ectactolpium sp.		X						
Pseudochiridiidae								
Pseudochiridium lawrencei Beier, 1964		X			XT			Beier (1964)
Pseudotyrannochthoniidae								
Afrochthonius godfreyi (Ellingsen, 1912)		X	X	X	XT			Ellingsen (1912)
Selachochthonius serratidentatus (Ellingsen, 1912)					XT			Ellingsen (1912)
Withiidae								
Afrowithius paradoxus (Ellingsen, 1912)		X			XT			Ellingsen (1912)
Ectromachernes sp.		X						
Parallowithius sp.		X						
SCORPIONES								
Buthidae								
Parabuthus planicauda (Pocock, 1889)	X			X	X			
Uroplectes formosus Pocock, 1890		X	X			X		
Uroplectes triangulifer (Thorell, 1876)	X			X	X			
Hormuridae								
Cheloctonus crassimanus (Pocock, 1896)				X	X			
Cheloctonus glaber Kraepelin, 1896						X		
Hadogenes trichiurus (Gervais, 1843)	X							
Opisthacanthus validus Thorell, 1876		X			X	X		
Scorpionidae								
Opistophthalmus latimanus C.L. Koch, 1841	X			X	X			

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Urban intensity and flower community structure drive monkey beetle assemblage in Cape Town

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Background: Urban landscapes present an important opportunity for pollinator conservation, but little is known about the status and distribution of pollinator populations in urban habitats in Africa. This represents a major gap in the development of a global understanding of urban pollinators – particularly from the rapidly urbanising context. This study uses a speciose clade of flower-visiting beetles (Coleoptera: Scarabaeidae: Hopliini) to explore patterns of pollinator distribution in a major metropolitan area in South Africa.

Objectives: We investigated community composition across gradients of urban intensity (defined according to the percentage of soil-sealing within 1 km^2 of each sampling location) and socio-economic status to determine pollinator responses to these urban landscape effects.

Methods: A selection of 142 sites were surveyed twice in the austral spring seasons of 2018 and 2019. Data were collected on habitat structure, flower diversity, and pollinator diversity.

Results: The study found that different feeding guilds of monkey beetles favoured different levels of urban intensity and that beetle richness significantly correlated with flower richness. It did not, however, correlate with diversity indicating that abundance is less impacted than the number of species present. Monkey beetles with moderate sensitivity to urban intensity benefitted from the presence of preferred species of flowers.

Conclusion: Overall, the findings demonstrate the importance of plant community assemblage in supporting urban monkey beetles. We recommend landscaping with preferred flower species in urban parks to support urban pollinators.

Keywords: Hopliini; Coleoptera; community assemblage; pollinators; urban ecology; South Africa.

Introduction

Urban environments are important for the conservation and stewardship of healthy pollinator populations (Hall et al. 2017). Globally, studies that investigated the response of pollinators to urban gradients found that certain guilds and taxa can take advantage of the resources and habitat conditions available in cities (Glaum et al. 2017; Theodorou 2020a; Wenzel et al. 2020). Others can do well when particular conditions are met, such as the provision of small scale floral patches or the introduction of preferred plants (Leveau 2013; Simao et al. 2018). Another group is negatively impacted by urban intensity (soil-sealing) and is intolerant of urban environments (Theodorou 2020b; Wenzel et al. 2020). A recent global systematic review found a taxonomic and geographic bias. Most studies conducted on urban pollinators reported findings for Hymenoptera, temperate regions, and the global north (n = 99, 117 and 120 studies, respectively). A smaller number reported findings from tropical

regions and the global south (n = 24 and 21, respectively) (Wenzel et al. 2020). There is still much to be learned about the responses of other pollinating taxa; cities in the global south; and in under-studied climatic regions, such as tropical, arid and Mediterranean ecosystems (Wenzel et al. 2020). Africa is the most poorly represented continent and offers an opportunity to add to the body of knowledge from a rapidly urbanising perspective (United Nations, Department of Economic and Social Affairs 2018).

Monkey beetles (Scarabaeidae: Hopliini) are diverse in southern Africa and therefore provide adequate richness to observe patterns of change in community compositions. South Africa is a centre of diversity for monkey beetles, where 65% of the world's species and 40% of the genera are concentrated (Colville et al. 2018). The greatest density of monkey beetle species is found in the winter-rainfall region of Namaqualand and the Cape Floristic Region (CFR), where they are important pollinators of several plant families (Bernhardt 2000; Goldblatt & Manning 2011; Mayer et al. 2006). This includes many of the popular flowers that attract local and international tourists during the austral spring season (Kruger et al. 2015). Monkey beetles are especially suitable for study in urban environments because there is existing evidence, which shows that they respond with shifts in feeding guild structure and host plant use in response to human-driven disturbance (Colville et al. 2002, 2018). Additionally, they are easy to collect due to their use of flowers as breeding platforms (Bernhardt 2000; Dafni et al. 1990).

Existing studies have established that biodiversity in urban landscapes is driven by urbanisation (measured by percentage of soil sealing in the surrounding landscape, where soil sealing is the amount of soil under buildings, roads and paving) (McDonnell & Hahs 2008), local habitat characteristics such as dominant land-use or local habitat structure (Theodorou et al. 2017; Theodorou et al. 2020b); and social factors such as socio-economic status and societal norms (Aronson et al. 2016; Lepczyk et al. 2017).

Cape Town is a rapidly urbanising city with a long history of spatially planned social segregation, and land dispossession (Cilliers & Siebert 2012; Lubbe et al. 2010; Rebelo et al. 2011). Until recently, tracts of land remained as natural islands due to their use as buffers between racially divided historical neighbourhoods under Apartheid urban planning (although many of these patches now face encroachment from both planned and unplanned urban expansion), in which neighbourhoods were spatially divided according to race and economic standing (Turok 2011). This legacy remains spatially entrenched.

Economic equality is typically measured by the Gini-coefficient. The closer it is to 1, the more unequal the society, the closer it is to 0, the smaller the difference between the individuals with the highest income and the lowest income. In 2016, Cape Town's Gini-coefficient was estimated at 0.62 (Western Cape Government 2020), slightly more equal than the national value of 0.63, which ranked the country as the most unequal society in the world (World Bank 2014). As such, Cape Town presents steep socio-economic gradients across the city. Cape Town is an important city in which to consider the social and spatial drivers of urban biodiversity because of the mega-diversity of the Cape Floristic Region (CFR) (Rebelo et al. 2011), rapid urbanisation (United Nations, Department of Economic and Social Affairs 2018), and socio-economic inequality.

This study investigates the community composition and distribution of monkey beetles across several environmental gradients in Cape Town, namely: income, urban intensity, and historical soil and vegetation. It compares these data with monkey beetle assemblage response to local habitat and flower community structure. It tests three hypotheses: 1. Monkey beetle feeding guilds will change along environmental gradients (urban-agricultural, urban-natural, and socio-economic gradients); 2. They will associate with specific flower communities (preferred flowers); 3. Beetle and flower diversity are linked.

Study area

Cape Town is the largest city in the Western Cape, South Africa, and has a population of ± 4.5 million citizens (Statistics South Africa 2011). It falls within the CFR, a global biodiversity hotspot with two centres of endemicity (Mittermeier et al. 1999; Rebelo et al. 2011). Within the city metropole, there are 19 vegetation ecosystems (Rebelo et al. 2011). Neighbourhood dwelling density varies from 1 dwelling unit per hectare (du/ha), to 160 du/ha in informal settlements (Mittermeier et al. 1999; Rebelo et al. 2011).

Methods

An environmental gradient is a change in environmental condition across space. Examples of commonly studied gradients include moisture and altitude. Studying environmental gradients help us to understand habitat requirements and species and community responses to changes in abiotic conditions. Urban intensity gradients have been used extensively to monitor the impacts and drivers of biodiversity in cities (Blair 1996; Dubois & Cheptou 2017; Hirzel & Le Lay 2008; Jongman et al. 2006; Lizée et al. 2011).

Sampling locations were thus selected across a range of conditions along an urban intensity gradient at ± 1 km intervals. The largest proportion of sites were

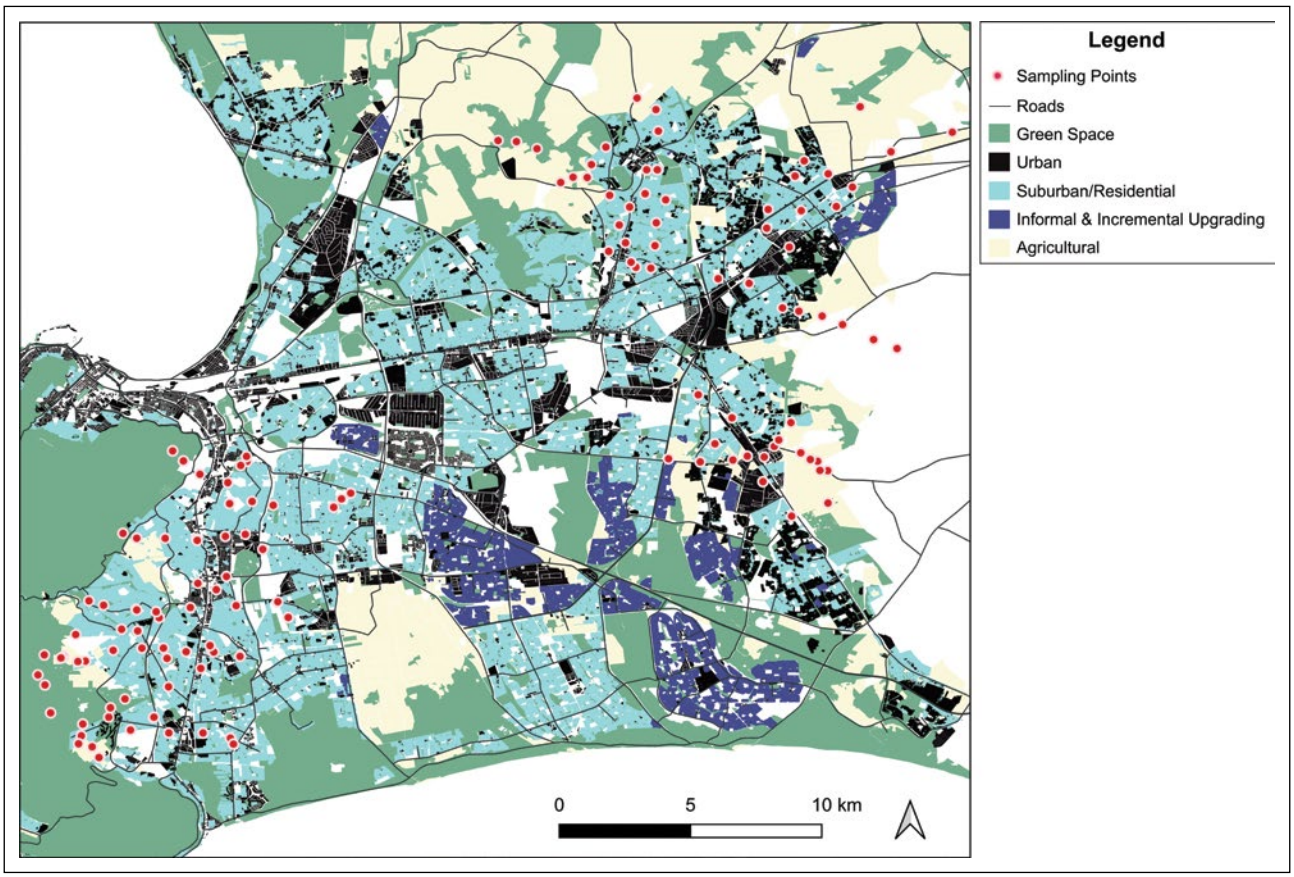

Figure 1. Monkey beetle sampling locations (n = 142) in Cape Town's city parks, vacant lots, farmland and islands of natural patches from which data were collected during the austral springs of 2018 and 2019.

community or district recreational parks, followed by vacant lots and road verges (Figure 1). Protected areas and cultivated farmland were included to represent unurbanised landscapes. To mitigate the effects of potentially conflicting environmental gradients and underlying heterogeneity, the sampling locations were stratified by historical vegetation type, income, and urban intensity in the second year of sampling. In 2018, 72 sites were visited. In 2019, the location of observation sites was adjusted to reduce the impact of variations in underlying vegetation and soil types across the city (a contextual adjustment that was required due to the underlying heterogeneity), resulting in 70 sites in 2019. The sites in the second year remained in similar locations to the first year. The second year's sampling had greater representative evenness across the urban intensity and economic gradients and corrected oversampling that had occurred in the suburban part of the gradients. In 2018, there were eight urbanisation gradients (five short gradients on the west of the city, two in the north and one to the east); in 2019, there were five gradients (two longer, rationalised gradients to the west, one in the east, one in northeast, one in the north of the city). Each blocked part of the gradient (urban, rural, natural, peri-urban, suburban) had at least six sampling locations within the corresponding block. Each block was sampled at least four times across the duration of the two years. Even though there were minor adjustments to the locations, there were very similar conditions between the years and sites included in each blocked category. Counted without any statistical blocking, the total number of sites was 142. Each of these was independently visited at least twice during 2018–2019 (August–December).

Five sets of blue, yellow and white fluorescent-painted pan traps of 75 mm diameter × 40 mm depth were laid out at 1.5–3.0 m distance from each other in a linear or zig-zag pattern depending on the layout of the site. Pan trap colours were selected for Coleoptera in accordance with the findings of Shrestha et al. (2019) and Vrdoljak and Samways (2012). Each trap received ± 75 ml of 2:1 dilute propylene glycol. Because of permit conditions, traps placed at sites in the South African National Parks (SANParks) properties contained only soapy water. The traps were left in place for 48 hours, allowing at least one clear, warm, sunny day. On collection, the contents of like-coloured pan traps were pooled rendering one sample per trap colour (three samples) per sampling event at each site. Each pooled sample was labelled with the collection date, the location code and the pan trap colour.

Local habitat data were sampled in ten quadrats of 1 m^2 in a transect across each site at 2 m intervals (or in a randomised grid if the area of the site was too narrow for a

linear transect). Counts of open flowers were recorded within each quadrat. Capitulate flowers were counted as one to the first joint with the main stem. Notes were made of species that were uncommon but present outside of the sampled quadrats on the site. Flowers in the surrounding 30 m radius of the traps were searched for monkey beetles for 15 minutes. As far as possible, three pairs of each species in copula were collected to provide a reference for the identification of sexual dimorphism. The flowers from which the monkey beetles were collected were identified to genus or species level, to provide evidence of the preferences of monkey beetles for colours and for flower species.

Laboratory and desk-top processing

Monkey beetles were separated from the by-catch and were identified to morphospecies level. Representatives of each morphospecies at each site were pinned and an overall representative sample was pinned for cross-checking identification. Where possible, individuals were identified to species level and cross-checked using reference samples in the Iziko Museum of Natural History, Cape Town.

Soil-sealing is the quantity of ground that is covered with buildings and hard surfaces, thereby preventing infiltration and soil penetration by living organisms. The amount of soil-sealing in the surrounding area was used as a proxy for urban intensity. Landscape level patterns were quantified from aerial photographs. Photographs of 1 km^2 were extracted from the Google Earth Engine by loading the GPS co-ordinates of sampling locations into a semi-automated urban intensity scoring tool that runs on machine learning and was developed by Seress et al. (2014). The GPS points entered into the database were centralised to the position where pan traps were placed at each observation site. The semi-automated urban scoring tool requires a minimum of three training points per image (captured by the researchers). It quantifies the area under building, vegetation and roads; it next conducts Principal Components Analysis (PCA) to generate an urban index score. The output file contains data on vegetative cover, buildings, roads and a total PCA urbanisation score (Seress et al. 2014).

Historical vegetation information was extracted by importing the national vegetation map (Mucina & Rutherford 2006) into QGIS (version 3.6.1) and using the point-picker tool to select the data that correspond with the GPS co-ordinates at each sampling location. The shapefile used for this was the South African Vegmap (Mucina & Rutherford 2006) downloaded from the South African National Biodiversity Institute's (SANBI) biodiversity GIS web portal (www.bgis.sanbi.org). Site size was extracted in QGIS (version 3.6.1) from the Cape Town City Council's 2016 Integrated Zoning Map (City of Cape Town 2017). Sites were post-coded to small (< 0.5 ha), medium (0.5–40 ha) and large (> 40 ha). Income data was sourced from the 2011 census (Statistics South Africa 2011), which reported mean household income by suburb. Values were post-classified into low = R0–R17 246, medium = R17 246–34 492, and high > R34 492, using the equal interval method.

Two separate data matrices consisting of counts of monkey beetle and flowers at each site were constructed. The matrix for the monkey beetles had 142 rows (sites) and 30 columns (Hopliini morphospecies) and the flowers contained 140 rows (sites) and 81 columns (flower species). Two sites had no flowers in bloom due to recent mowing. The elements of the matrices were square-root transformed and two dissimilarity matrices constructed using the Bray-Curtis index in Primer v 6.1.16 (Bray & Curtis 1957; Clarke & Gorley 2006). The one dissimilarity matrix contained dissimilarities between flower species (81 × 81), and the other one between beetle species (30 × 30). Hierarchical cluster analysis (group averages algorithm) were performed on both dissimilarity matrices (Kruskal 1964). Then non-metric multi-dimensional scaling (NMDS) was performed for both. The cluster analysis was overlain over the two-dimensional configuration generated by the NMDS (Everitt et al. 2011). Monkey beetle and flower species that were seen to be outliers in the NMDS and cluster analysis were mostly species which were seldom recorded (< 5 locations) and these rare species were removed from the dataset, so the dimensions of the dissimilarity matrices became smaller.

The NMDS and cluster analyses identified communities of co-occurring monkey beetles in the one matrix, and communities of co-occurring flowers in the other matrix. The groups generated by the hierarchical cluster analysis were added to the NMDS configuration as sets and subsets. Species-sets were grouped into a data vector (i.e., abundance was summed into one column) for each set and subset. Thus analysis was performed on groups of co-occurring monkey beetles and co-occurring flowers. Tests were run to determine what caused the beetle communities to cluster at different locations (i.e., was it the presence of specific flower communities or environmental factors?).

For economic and environmental factors, Kruskal-Wallis tests (Kruskal & Wallis 1952) were performed to test the null hypothesis that the species were randomly distributed across the categorical levels. Spearman's rank correlations (Glasser & Winter 1961) were calculated between the ranked sets and aggregated by each of the environmental factors to understand the direction of those relationships for which the null hypothesis could be rejected.

To examine the relationship between flowers and monkey beetles, the null hypothesis that monkey beetle

species would be randomly distributed across flower species was tested. A contingency table was generated for each monkey beetle species with cells containing the count of the number of sites at which each monkey beetle species was present or absent for each of the flower species. The chi-squared test of independence was performed on each table (Fisher 1922). If the test for a particular monkey beetle was significant, the frequency of co-occurrence with each flower species was ranked; this provided an indication of which flower species are associated with each monkey beetle. Because the data collection was done at the site level, there is a risk of producing misleading results in the context in which two flower species co-occur, but only one is attractive to monkey beetles. Hence, results were corroborated with field collections of monkey beetles collected off flowers and by examining the clustering of flower communities in the non-metric multi-dimensional scaling plots.

To investigate the relationship between the flower diversity and beetle diversity, two diversity indices (Shannon H-index, Inverse Simpson) (Hill 1973) were calculated at each of the 140 sites for both flowers and beetles, using the 'vegan' package (Oksanen et al. 2020) in R (RStudio version 3.6.0). Additionally, species richness (number of species) was calculated. Spearman's correlation was calculated for each diversity index and for species richness. In all statistical hypothesis testing, Bonferroni corrections were made if appropriate (Bonferroni 1936).

Results

A total of 19 387 individuals of monkey beetles were collected across 30 morpho-species. Non-metric multi-dimensional scaling and clustering produced two sets with 20% similarity (Set A and Set B) of commonly co-occurring monkey beetles (Figure 2). Set A included *Lepithrix ornatella* and *Heterochelus rufimanus*. The abundance of the species in Set A decreased with urban intensity and increased with site area. Set B incorporated eight species of monkey beetles. The abundance of individuals of each species in Set B increased with soil-sealing and decreased with site size, however when the subsets of Set B were assessed (40% similarity), only Subset 1 incorporating *Heterochelus* cf. *sexlineatus* and *Heterochelus hybridus* showed any relationship with soil-sealing. The underlying ecosystem, (soil and historical vegetation) was the main driver for the clustering of Subsets 2 and 3 (Table 1; Figure 3).

Flowers and beetles

Monkey beetle species composition was influenced by flower species assemblage at the local habitat scale for most species. The null hypothesis that beetles were randomly distributed across flower communities was rejected for all species except for *Lepithrix ornatella, Heterochelus gonager* and *Heterochelus rufimanus*. (Table 2). These species clustered in the NMDS results in Set A and were found in peri-urban areas and larger green areas, and indicate that the monkey beetle species *L. ornatella, H. gonager* and *H. rufimanus* have a generalist preference for flowers but require a greater percentage of green cover. For all other species, the flower species assemblage attracted specific monkey beetles to the site.

In the cluster analysis, the flower communities formed four co-occurring sets (10% similarity) and a further four subsets (20% similarity). Their clustering is attributable both to growth form and to location (Figure 4). For flowers, Set A consisted of ruderal species including

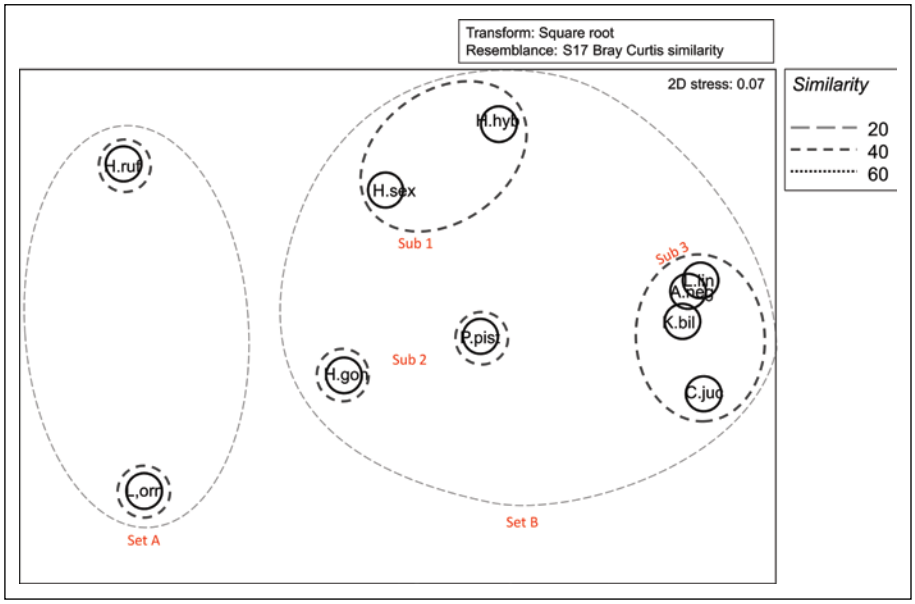

Figure 2. Non-metric multidimensional scaling with clustering overlaid showing the sets and subsets of monkey beetle communities in Cape Town. Set A (20% similarity): *Lepithrix ornatella* and *Heterochelus rufimanus*. Set B incorporated three subsets that were 40% similar, including: Subset 1: *Heterochelus* cf. *sexlineatus* and *Heterochelus hybridus*; Subset 2: *Heterochelus gonager* and *Peritrichia pistinaria*; Subset 3: *Khoina bilateralis*, *Chasme jucunda*, *Anisochelus neglectus* and *Lepithrix ornatella*.

Table 1. Summary of the tests for relationships between environmental variables and the species communities identified using NMDS and cluster analysis. The cluster analysis was 10% and 20% similarity between communities for sets, and 20% and 40% similarity for subsets of flowers and monkey beetles respectively. The p-values were determined using Kruskal-Wallis-tests against ranks of the abundance counts in each set and subset against four environmental factors. Soil-sealing is aggregated by the groups ex-urban (Ex), peri-urban (P), suburban (S) and urban (U). Site size is aggregated to small (S < 0.5 ha), medium (M = 0.5–40 ha) and large (L > 40 ha) and income is aggregated to low (Lo), medium (Me) and high (Hi). Historical vegetation type is assessed for Cape Flats Sand Fynbos (CFSF), Peninsula Granite Fynbos (PGF) and Swartland Shale Renosterveld (SSR). Bonferoni correction has been applied to the first level of significance (*=Bonferroni corrected significance threshold, ** = p<0.01, *** p <0.001)

Hopliini (monkey beetles)

	Soil Sealing						Site Size					Historical vegetation					Income				
	Ex	P	S	U	p-value		S	M	L	p-value		CFSF	PGF	SSR	p-value		Lo	Me	Hi	p-value	
Set A	88.2	77.9	68.8	52.4	<0.001	***	61.9	79.7	84.3	0.0030	**	58.0	86.7	67.6	<0.001	***	57.7	61.5	83.8	<0.001	***
Set B	52.3	58.2	85.8	87.9	<0.001	***	85.7	58.4	53.5	<0.001	***	83.7	36.4	36.5	<0.001	***	96.9	78.0	54.8	<0.001	***
Sub 1	49.1	54.0	90.3	90.6	<0.001	***	90.8	51.4	49.5	<0.001	***	83.0	32.8	42.5	<0.001	***	96.5	79.0	54.0	<0.001	***
Sub 2	72.3	72.2	70.8	70.8	0.990		70.3	73.1	72.5	0.9200		74.0	61.9	41.1	<0.001	***	82.2	75.9	63.7	0.0390	
Sub 3	69.0	67.0	76.0	73.0	0.600		73.1	65.5	74.3	0.4000		76.0	49.6	47.9	<0.001	***	96.0	70.2	59.2	<0.001	***

* Bonferroni adjusted significance threshold p-value = 0.01; ** p<0.005; *** p<0.001

Flowers

	Soil Sealing						Site Size					Historical vegetation					Income				
	Ex	P	S	U	p-value		S	M	L	p-value		CFSF	PGF	SSR	p-value		Lo	Me	Hi	p-value	
Set A	56.0	62.3	78.8	95.1	<0.001	***	86.7	62.2	56.5	<0.001	***	72.5	42.4	77.4	0.001	**	81.1	78.0	67.8	0.6146	
Set B	88.0	65.0	66.8	74.2	0.022		72.4	66.8	83.9	0.1114		68.8	70.2	63.8	0.723		72.5	75.8	73.1	0.2234	
Set C	74.4	76.9	70.5	72.8	0.864		74.9	68.6	75.8	0.5615		72.3	52.7	69.1	0.019		76.3	84.3	67.6	0.9147	
Set D	74.2	79.8	70.1	70.6	0.375		72.1	72.5	77.8	0.5619		71.0	60.3	66.2	0.149		69.7	77.6	73.8	0.0455	
Sub 1	65.4	81.3	74.5	73.1	0.072		74.3	74.9	70.0	0.6233		65.0	69.4	75.1	0.138		67.3	70.6	77.8	0.0836	
Sub 2	54.0	60.7	82.6	94.6	<0.001	***	88.5	58.4	56.9	<0.001	***	73.5	39.9	76.7	<0.001	***	80.1	80.9	67.1	0.1581	
Sub 3	88.3	68.4	68.0	69.6	0.010		71.1	66.6	86.9	0.0130		68.1	70.2	65.8	0.857		73.2	71.6	74.4	0.9045	
Sub 4	73.0	74.7	70.3	76.2	0.842		75.9	70.8	71.3	0.6146		70.9	53.5	72.5	0.016		80.0	82.6	66.5	0.0114	

* Bonferroni adjusted significance threshold = 0.00625; ** p<0.005; ***p<0.001

exotic and invasive weeds and the most common indigenous annuals. Set B was a combination of annuals and indigenous perennials. Apart from *Conicosia pugioniformis*, which is a disturbance tolerant species that resprouts from its taproot, Set C contained indigenous annuals. Set D contained indigenous geophytes. Monkey beetle collections made from flowers provided insights into which of the flower species within subcommunities were most preferred by each of the monkey beetles species (Table 3).

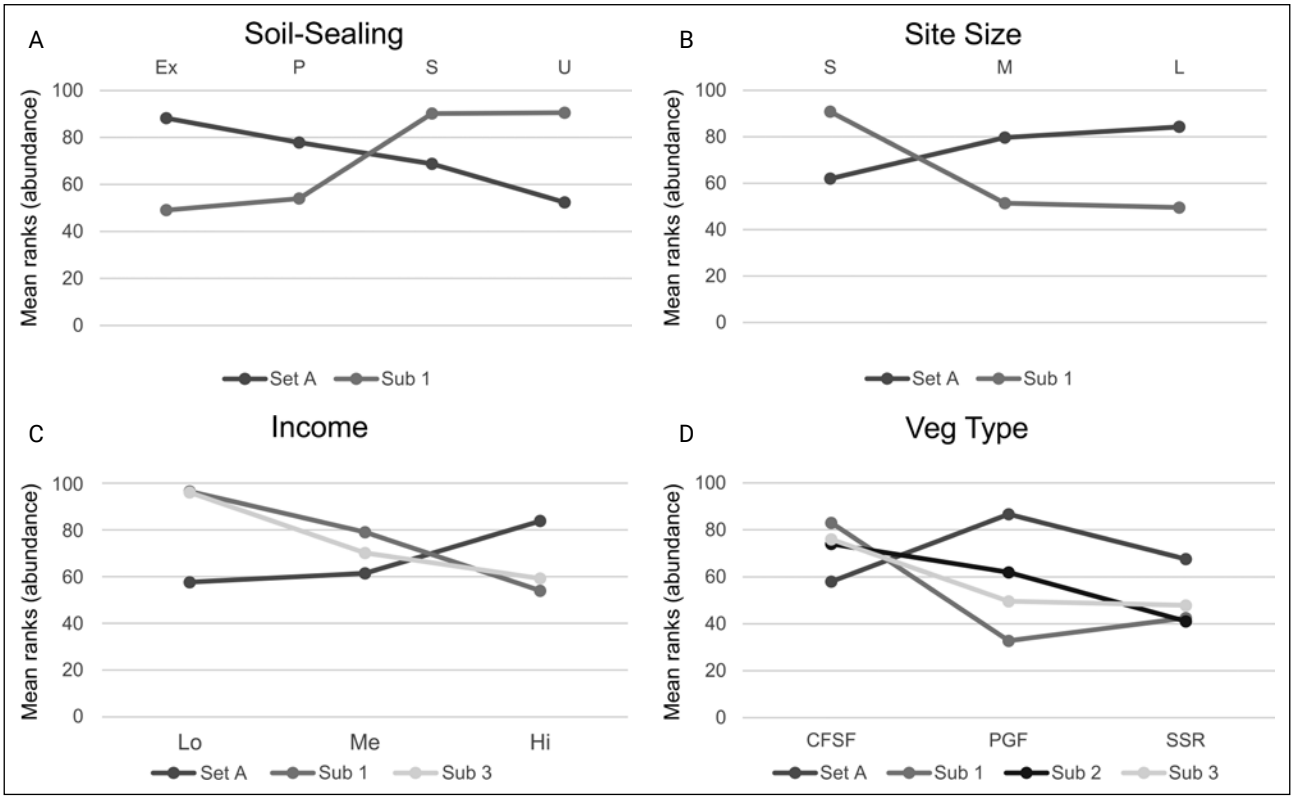

Figure 3. Plots of the means of ranked abundance of co-occurring monkey beetle species grouped by environmental gradients for which statistically significant relationships were observed. The plots are made to infer the direction of the relationships established in Table 1. Set A (20% similarity) contained *Lepithrix ornatella* and *Heterochelus rufimanus*. Set B incorporated three subsets that were at least 40% similar, including Subset 1: *Heterochelus* cf. *sexlineatus* and *Heterochelus hybridus*; Subset 2: *Heterochelus gonager* and *Peritrichia pistinaria*; Subset 3: *Khoina bilateralis*, *Chasme jucunda*, *Anisochelus neglectus* and *Lepithrix ornatella*. For the environmental factors, soil-sealing is aggregated by the groups: ex-urban (Ex), peri-urban (P), suburban (S) and urban (U). Site size is aggregated to small (S < 0.5 ha), medium (M = 0.5–40 ha) and large (L > 40 ha). Income is aggregated to low (Lo), medium (Me) and high (Hi) using the equal interval method from the 2011 national census data; and historical vegetation type is assessed for Cape Flats Sand Fynbos (CFSF), Peninsula Granite Fynbos (PGF) and Swartland Shale Renosterveld (SSR).

Table 2. Results of chi-squared tests on the contingency of Hopliini presence against flower presence. The null hypothesis was that each of 17 species of monkey beetle was distributed at random across 19 species of flowers. To counteract multiple hypothesis testing concerns, the Bonferroni correction was applied, so that the p-value for each individual test needs to be 0.05/17=0.0029 to achieve overall significance at the 5% level

	Chi-square	p-value
Lepithrix ornatella	21.02	0.0034
Heterochelus cf. *sexlineatus*	224.84	<0.0001
Heterochelus hybridus	42.12	<0.0001
Khoina bilateralis	57.11	<0.0001
Heterochelus gonager	22.70	0.0139
Anisochelus neglectus	52.94	<0.0001
Lepithrix lineata	64.52	<0.0001
Peritrichia pistinaria	49.98	<0.0001
Dolichiomicroscelis gracilis	107.62	<0.0001

	Chi-square	p-value
Lepithrix lineata (dark morph)	113.66	<0.0001
Heterochelus gonager (dark morph)	88.03	<0.0001
Heterochelus rufimanus	24.74	0.0062
Pachycnema crassipes	90.38	<0.0001
Dicranocnemus sp.	85.67	<0.0001
Heterochelus sp. 1	72.09	<0.0001
Heterochelus sp. 2	79.29	<0.0001
Chasme jucunda	82.61	<0.0001

Table 3. Number of monkey beetle morphospecies collected off each species of flower in Cape Town

Flower species	Flower guild	No. of monkey beetle species
Arctotheca calendula	Indigenous, annual	5
Cotula turbinate	Indigenous, annual	2
Dimorphotheca pluvialis	Indigenous, annual	3
Senacio sp.	Indigenous, annual	4
Ursinia nana	Indigenous, annual	3
Pauridia capensis	Indigenous, geophyte	1
Moraea miniate	Indigenous, geophyte	3
Heliophila sp.	Indigenous, annual	2
Echium sp.	Invasive, annual	1
Conicosia pugioniformis	Indigenous, perennial	4
Carpobrotus edulis	Indigenous, perennial	2
Pelargonium sp.	Indigenous, perennial	1

There was no relationship between overall floral diversity and beetle diversity ($p>0.05$). Monkey beetle and flower species richness (number of species), however, showed a positive correlation ($rho=0.31$, $p<0.001$).

Discussion

This study revealed patterns in pollinator assemblage in response to urban landscape environmental gradients. It also identified species of flowers favoured by monkey beetles. Monkey beetle guilds, environmental gradients and flower preference are discussed below.

Divergent responses according to guilds

In response to urban intensity, the findings of this study were consistent with those done in other parts of the world for Hymenoptera. Some species exploit urban conditions, some species avoid them, and others depended on connected flower communities to move through the urban landscape (Wenzel et al. 2020). The divergence in bees is associated with differences in nesting requirements, body size and life histories (e.g., ground-nesting bees decline with an increase in urban intensity, and cavity nesters increase) (Brom et al. 2022; Cane et al. 2006; Merckx et al. 2018; Shwartz et al. 2014).

Not much is known about the life histories of monkey beetles, but some understanding of their behaviour and feeding differences is known (Colville et al. 2018; Karolyi et al. 2016; Mayer et al. 2006; Picker & Midgley 1996). Existing studies have demonstrated a shift in feeding guilds along disturbance gradients towards the embedding guild, which favours ruderal species (Colville et al. 2002). Monkey beetles utilise flowers as a breeding platform during the austral spring floral flush (Goldblatt & Manning 2011). The mechanism for pollination is the presence of hair-like setae on the backs and bodies of the beetles, which collect and deposit pollen loads during their movement across and between flowers (Bernhardt 2000; Mayer et al. 2006; Mayer & Pufal 2007). Three feeding and breeding guilds have been identified according to feeding and behaviour (Colville et al., 2002; Karolyi et al. 2016; Picker & Midgley 1996). Monkey beetle behaviour is classified either as embedding or non-embedding, where females in the embedding guild bury their heads into the centre of the flower while males compete over them to mate. Thus the males are predominantly responsible for the pollination because they fly between flowers looking for females (Bernhardt 2000). Embedding guilds eat pollen and nectar and are attracted to flowers with long-wave colours (red, orange and yellow). The non-embedding guilds are split into two groups, by colour attraction. One non-embedding guild is attracted to short-wave (blue, pink and violet) coloured flowers, and the other is attracted to long-wave colours (Colville et al. 2018; Picker & Midgley 1996). *Lepithrix*, *Anisonyx* and *Peritrichia* are recorded as feeding on pollen and do not embed themselves, whereas species in the genus *Heterochelus* tend to embed themselves (Picker & Midgley 1996). The non-embedding group are characterised by being hairy and mobile, moving around between flowers more frequently than their embedding counterparts. For this reason, they are considered to be more effective pollinators (Goldblatt & Manning 2011).

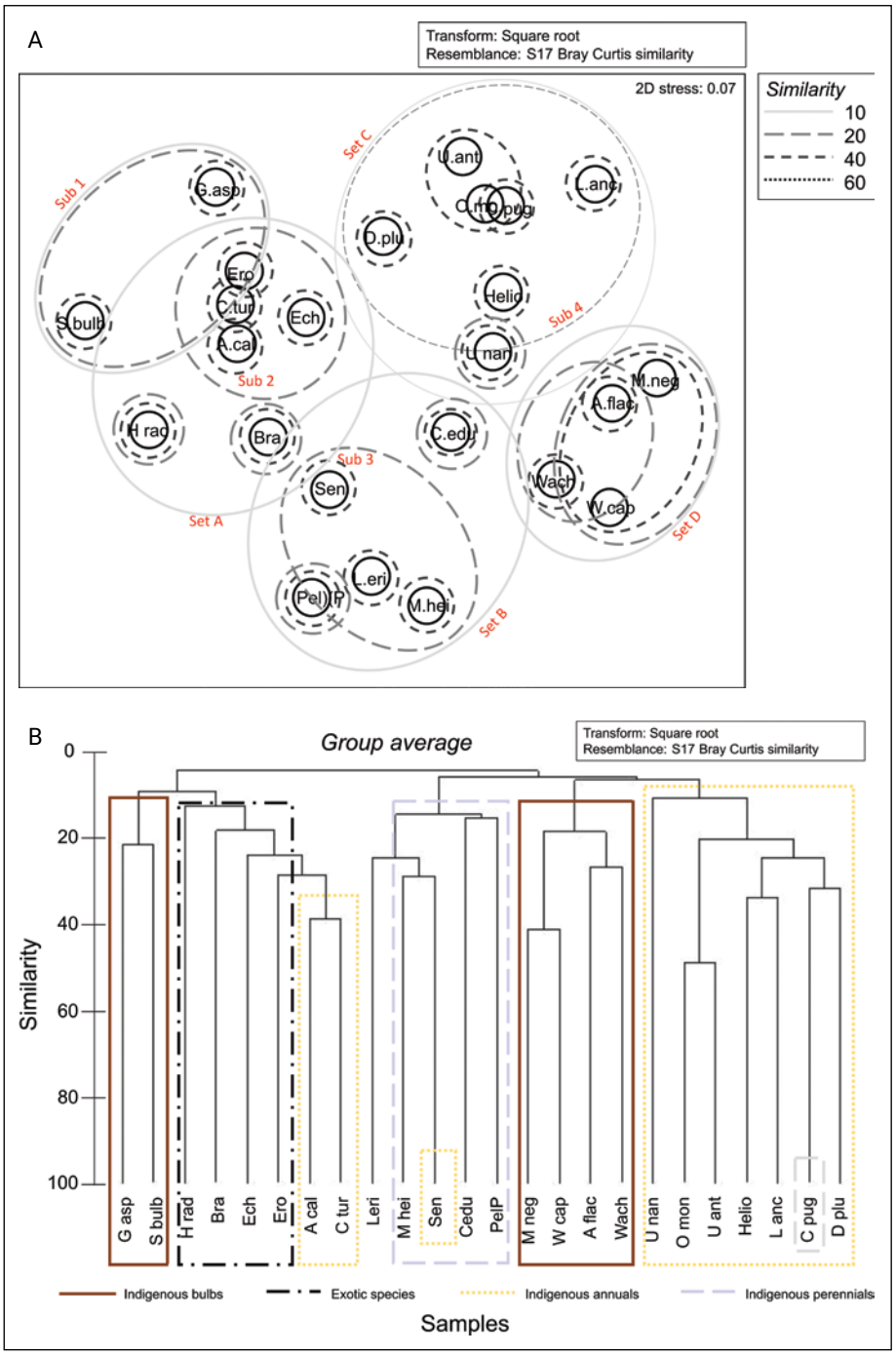

Figure 4. A, Clustering of flower abundance counts collected in Cape Town during spring season in 2018 and 2019; the plot represents the non-metric multi-dimensional scaling of the most commonly occurring species in the sample set including Set A: *Erodium* spp. *Cotula turbinata*, *Echium* spp. *Arctotheca calendula*, yellow Brassicaceae, *Hypochaeris radicata*; Set B: *Pelargonium* spp. (pink)., *Lobelia erinus*, *Senecio* spp., *Muraltia heisteria* and *Carpobrotus edulis*; Set C: *Ursinia anthemoides*, *Dimorphotheca pluvialis*, *Osteospermum monstrosum*, *Conicosia pugioniformis*, *Lapeirousia anceps*, *Heliophila* spp., *Ursinia nana*; and Set D: *Wachendorfia multiflora*, *Wahlenbergia capensis*, *Moraea neglecta*, *Albuca flaccida*; B, Group average clustering of flower species arranged according to growth form.

Response to urban environmental gradients

Comparisons between natural and agricultural landscapes revealed that monkey beetle species composition changes along gradients of disturbance where a 'shift away from perennial and bulb pollinator guilds towards those favouring weedy annuals' has been observed (Colville et al. 2002). The findings of this study were consistent with those earlier studies in that there was a shift towards species favouring ruderal annuals as soil-sealing (disturbance) increased. Specifically, the embedding *Heterochelus* cf. *sexlineatus* and *Heterochelus hybridis* were associated with ephemeral plants, including ruderal asters and indigenous annuals, and were abundant in urban landscapes in suburbs and close to commercial centres. *Chasme jucunda*, *Khoina bilateralis*, *Anisochelus neglectus*, *Lepithrix liniata* and *Peritrichia pistinaria* are species of the 'non-embedding' guild (Picker & Midgley 1996). They are typically sensitive to disturbance, associate with somewhat established floral communities containing perennials and geophytes (Colville et al. 2002), and, in this study, were found in large (> 30 ha), relatively undisturbed islands in the city. These findings are consistent with earlier studies along disturbance gradients in agricultural landscapes (Colville et al. 2002).

The phenomenon of an increase in biodiversity with wealth is known as the 'luxury effect' (Wu et al. 2014) and has been demonstrated across several studies and taxonomic groups (Aronson et al. 2016; Lubbe et al. 2010; Ossola & Hopton 2018; Venter et al. 2020). This can be attributed to the fact that the number and size of community parks decreases as income decreases (Venter et al. 2020), but poverty is also associated with an increase in population density and urban cover, thereby representing a particularly dense form of urbanism (Turok 2011; Wilkinson 2000), particularly in areas of unplanned expansion and informality (Gómez-Baggethun et al. 2013). It is therefore unsurprising that the guilds of monkey beetles that respond negatively to urban densification, also respond negatively to a decrease in income, and vice-versa, and the correlation with income is not necessarily causative, but rather can be seen as a driver of other intervening processes (e.g., loss of green infrastructure). A study conducted on weedy species in okra cultivation in West Africa found, for example, that morphological plant traits were most affected by agricultural practices that were influenced by farmers' socio-economic background and market orientation (i.e., for sale vs for personal consumption), however more critical changes in functional guilds were observed due to a decrease in animal grazing along the rural to urban gradient: weed species reliant on animal dispersal were more present in rural and peri-urban study sites (Stenchly et al. 2017). In this study, parks in poorer communities were either benignly neglected or over-used, while those in wealthier neighbourhoods appeared to have larger, more frequently maintained lawns, unless there was an active strategic spring mowing suspension during the sampling period. Furthermore, the density of poorer neighbourhoods means that there is less available private green infrastructure in the form of gardens. Additional research on the effects of socio-economic status on factors such as actual maintenance frequency, foot traffic volumes and grass length during sampling would likely provide a more detailed and meaningful interpretation of the habitat dynamics driving plant and pollinator assemblages across the socio-economic gradient.

The role of flower preference

Monkey beetles are generalist pollinators and most species will visit at least two species of flowers (Mayer et al. 2006; Steiner 1998). Records of flower visitations by monkey beetles to date have peaked at a maximum of five monkey beetle species per flower species (Mayer et al. 2006). Determining the relative preference and attractiveness of different flowers to monkey beetles was outside of the scope and objectives of this study, however the results of collections from flowers and field observations provide preliminary indications of the ways in which flowers are being used by monkey beetles in urban habitats within Cape Town. Further research investigating visitation rates is needed to confirm and clarify the relative importance of these species, however, when Table 3 and Supplementary Tables 1 and 2 are read together, co-occurrence data and the number of monkey beetle species collected off flower species point to the relative preferences for certain flowers. Of those observed, the most popular species were *Heliophila africana* and *Heliophila coronopifolia*, which were preferred by non-embedding monkey beetles who favour short-wave colours (visited by two species); *Conicosia pugioniformis* was preferred by the non-embedding guilds who favour long-wave colours (visited by four species); *Dimorphotheca pluvialis,* and *Ursinia nana* were visited by the embedding guild (three species); and *Arctotheca calendula* and *Senecio* sp., which are both mass-flowering spring annuals, and which can cover entire parks, were favoured by the embedding guild (three and four species respectively), but were also visited by the more sensitive non-embedding guild that were limited to peri-urban areas. These rates of 'most popular' are similar to other studies conducted in natural landscape settings (Mayer et al. 2006), indicating the viability of supporting monkey beetles through the introduction (or preservation) of targeted species of preferred flowers.

The occurrence of short-wave, non-embedding guilds was less common and associated with fewer flower species, which were relatively isolated across the city. Sites where floral communities included the most popularly visited species from all guilds, including *D. pluvialis, C. pugioniformis* and species of *Heliophila*, supported greater monkey beetle richness as reflected in the correlation between monkey beetle and flower species richness. It is therefore not only floral richness, but the presence of species preferred by the various guilds that drives monkey beetle richness.

Worth noting is that there was a relationship between richness but not abundance (diversity indices include calculations based on abundance), and that beetle species have preferred flowers; they are found where their preferred flowers occur. Some of the natural islands supported groups of flowers that were preferred by different species of beetles, but the abundance counts were hugely variable so we could not reject the null hypothesis that monkey beetle abundance was driven by flower abundance. One explanation could be based on temperatures. Weather in spring time is highly variable. Possibly, abundance was more directly affected by temperature on a given day.

Several studies conducted on urban pollinators have previously discussed the role of supplementary planting in urban landscapes and investigated the relative attractiveness of different species of plants in order to compile lists of recommended 'pollinator friendly' plants, or to assess the availability of foraging resources (Baldock et al. 2015; Garbuzov & Ratnieks 2014, 2015; Lowenstein et al. 2019; Martins et al. 2017; Michołap et al. 2018).

Garbuzov et al. (2017) tested if plants being advertised as 'pollinator friendly' by garden centres were accurately reflecting pollinator preferences and compared relative attractiveness of garden plants to generate a list of preferred species for their city (Garbuzov & Ratnieks 2014, 2015). Pauw and Louw (2012) considered the distribution of nectarivorous birds in Cape Town and suggested that the functional diversity of this bird guild could be restored across the city with strategic garden planting and the introduction of favoured plants. Introducing preferred species at regular intervals throughout the city can aid monkey beetle mobility and provide stepping stones between larger fragments. This could potentially be achieved by strategically identifying suitable pathways and implementing targeted landscaping along those routes (Cranmer et al. 2012; Simao et al. 2018). In addition to benefitting monkey beetles, other pollinators would be able to make use of the flower resources for foraging and as stepping stones (Brom et al. 2022). Collections from *Conicosia pugioniformis* consistently hosted the greatest number of monkey beetle species at any one site and was often found growing together with *Carpobrotus edulis*, a popular road verge plant for its hardiness, ease of propagation and brightly coloured flowers. *Conicosia pugioniformis* can easily be introduced as a co-plant to *C. edulis* along road verges and city parks. Further research is needed to determine the extent to which the introduction of favoured short-wave plants (e.g., *Heliophila* spp.), could restore the connectivity of landscapes for the non-embedding guild favouring blue and pink coloured flowers.

Conclusion

In this study, the distribution of monkey beetles was sampled in a metropolitan city in the Cape Floristic Region of southern Africa. It aimed to determine how monkey beetles were responding to urban environmental gradients and local habitat conditions. It found an established community of monkey beetles was responding in different ways to urban gradients. Embedding species were more tolerant of soil-sealing, congregating on ephemeral species of Asteraceae and geophytes. Non-embedding species were associated with preferred flower species and reduced abundance was recorded as soil-sealing increased in the surrounding landscape. Non-embedding species demonstrated a need for a greater percentage of green space in the surrounding landscapes, but this sensitivity can be mitigated with the introduction of preferred species of flowers. *Conicosia pugioniformis* in particular, hosted the greatest richness of monkey beetles at within-site scale, but the sites containing combinations of *Heliophila africana, Dimorphotheca pluvialis, Ursinia nana* and *Senecio littoreus*, together as dominant species, provided the scaffolding for supporting healthy populations of monkey beetles. The findings suggest that widespread introduction of a community of beetle-preferred flowers, including *C. pugioniformis* as a cornerstone species, to provide stepping-stones through the urban matrix, will aid monkey beetle mobility.

This study found that there is an intact community of monkey beetles in the city, and that there is a reliance on particular plant communities for breeding and foraging. This finding is promising for the conservation of rare bulbs within the urban landscape, which rely on a healthy population of monkey beetles for pollination services (Barraclough & Slotow 2010; Goldblatt et al. 2013; Goldblatt & Manning 2006; Johnson & Steiner 2003; Steiner 1998).

Acknowledgements

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Supplementary Material

Supplementary Table 1. Sites at which flower species were present and the percentage of those where each species of monkey beetle co-occurred. Percentages in the column are the percentage of flower presence sites where the monkey beetle species co-occurs. The dark grey highlights are the three largest percentages in each column; the light grey highlights are the remaining two values in the top five of each column

	No. of sites where flower occurred	*Lepithrix ornatella*	*Heterochelus sexlineatus*	*Heterochelus hybridus*	*Khoina bilateralis*	*Heterochelus gonager*	*Anisochelus neglectus*	*Lepithrix lineata*	*Peritrichia pistinaria*	*Dolichiomicroscelis gracilis*	*Lepithrix lineata* (dark morph)	*Heterochelus gonager* (dark morph)	*Heterochelus rufimanus*	*Pachycnema crassipes*	*Dicranocnemus*	*Heterochelus* sp. 3	*Heterochelus* sp. 2	*Chasme jucunda*	In Top 3	In Top 5	
Arctotheca calendula	71	30%	82%	38%	8%	30%	18%	14%	32%	6%	4%	8%	23%	11%	8%	11%	11%	10%	0	1	*
Carpobrotus edulus	16	31%	75%	38%	31%	31%	31%	25%	50%	19%	0%	13%	25%	19%	13%	31%	0%	38%	2	4	*
Conicosia pugioniformis	14	21%	93%	64%	50%	36%	71%	57%	93%	36%	29%	7%	0%	0%	29%	7%	7%	43%	11	11	*
Cotula turbinata	64	33%	69%	41%	14%	25%	16%	13%	30%	3%	6%	9%	30%	8%	9%	14%	9%	8%	0	0	*
Dimorphotheca pluvialis	19	32%	68%	63%	32%	32%	47%	32%	63%	16%	21%	5%	32%	5%	21%	11%	21%	37%	3	9	*
Echium sp.	47	34%	68%	40%	19%	32%	21%	19%	47%	6%	2%	9%	28%	9%	9%	13%	13%	13%	0	0	
Erodium sp.	51	22%	71%	31%	8%	16%	16%	12%	24%	4%	6%	8%	25%	10%	8%	10%	20%	8%	0	1	
Geissorhiza aspera	12	58%	33%	33%	8%	33%	0%	8%	8%	0%	0%	17%	25%	0%	17%	17%	25%	8%	2	4	
Heliophila sp.	9	11%	78%	78%	44%	56%	33%	33%	56%	33%	22%	0%	11%	0%	33%	22%	0%	56%	9	11	*
Lobelia erinus	9	44%	33%	11%	33%	11%	0%	11%	22%	0%	0%	22%	33%	0%	11%	11%	33%	11%	2	4	
Osteospermum monstrosum	10	30%	70%	60%	50%	60%	50%	40%	80%	30%	20%	0%	20%	10%	30%	10%	10%	50%	8	11	
Oxalis pes-caprae	21	19%	67%	52%	10%	24%	19%	5%	52%	10%	5%	0%	38%	14%	5%	5%	14%	14%	0	3	
Pelargonium sp. (pink)	14	50%	50%	29%	29%	29%	21%	14%	29%	14%	7%	14%	50%	0%	7%	14%	7%	14%	2	2	*
Romulea rosea	17	29%	82%	35%	6%	29%	6%	0%	41%	6%	0%	18%	24%	6%	18%	12%	0%	0%	0	3	
Senecio sp. (yellow)	25	36%	60%	56%	28%	24%	20%	12%	48%	4%	8%	12%	20%	12%	8%	8%	8%	16%	0	1	*
Trachyandra ciliata	10	10%	90%	80%	10%	10%	40%	30%	50%	0%	20%	0%	20%	0%	10%	10%	10%	0%	2	7	
Ursinia nana	14	21%	86%	50%	36%	36%	21%	21%	29%	14%	14%	7%	21%	7%	14%	21%	7%	29%	2	4	*
Vicia sp.	13	31%	38%	15%	15%	15%	8%	8%	23%	8%	0%	23%	38%	8%	0%	8%	0%	0%	2	2	
Brassicaceae spp. (yellow)	26	46%	62%	38%	23%	35%	15%	19%	42%	0%	0%	15%	35%	15%	8%	23%	4%	4%	2	5	*
Hypochaeris radicata	13	15%	69%	23%	8%	31%	15%	0%	23%	0%	0%	0%	15%	0%	0%	0%	8%	0%	0	0	
Lysimachia monelli	11	55%	9%	0%	18%	0%	0%	0%	36%	0%	0%	18%	64%	18%	0%	0%	27%	0%	5	5	

Supplementary Table 2. List of monkey beetle species collected from flowers across the City of Cape Town in 2018 and 2019

Hopliini	Flower	Flower colours
Non-embedding guild (short-wave colours)		
Dolichiomicroscelis gracilis	*Heliophila africana*	blue/violet
Peritrichia pistinaria	*Pelargonium, Heliophila africana, Echium* sp.	pink, blue/violet
Non-embedding guild (long-wave colours)		
Anisochelus neglectus	*Conicosia pugioniformis, Carpobrotus edulis*	yellow
Bizanus sp.	*Moraea miniata*	salmon-pink
Chasme jucunda	*Conicosia pugioniformis, Ursinia* sp.	yellow
Heterochelus rufimanus	*Bolusafra bituminosa, Arctotheca calendula*	yellow
Dicranocnemus	*Ursinia* sp.	yellow
Khoina bilateralis	*Conicosia pugioniformis*	yellow
Lepithrix lineata	*Arctotheca calendula, Conicosia pugioniformis, Senecio* sp.	yellow
Lepithrix ornatella	*Senecio* sp.	yellow
Pachycnema crassipes	*Carpobrotus edulis*	yellow
Platychelus lupinus	*Moraea miniata*	salmon-pink
Embedding guild (long-wave colours)		
Heterochelus sexlineatus sp.	*Arctotheca calendula, Cotula turbinata, Dimorphotheca pluvialis*	yellow, white
Heterochelus sp. 2	n/a	
Heterochelus gonager	*Dimorphotheca pluvialis, Senecio* sp., *Arctotheca calendula, Echium* sp.	white, yellow, blue/violet
Heterochelus hybridus	*Cotula turbinata, Arctotheca calendula, Ursinia nana, Dimorphotheca pluvialis, Pauridia capensis (yellow), Senacio* sp.	white, yellow
Ishnochelus sp.	*Hypochaeris radicata, Moraea miniata*	yellow, salmon-pink/yellow

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A Critically Endangered Proteaceae in the Cape Florisitic Region threatened by an invasive pathogen

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Background: *Sorocephalus imbricatus* (Thunb.) R.Br. is a range-restricted species endemic to the Cape Floristic Region (CFR), South Africa. It is currently classified as Critically Endangered in accordance with the IUCN criteria. Like many other species endemic to the CFR, *S. imbricatus* is subjected to several major threats including habitat loss, habitat degradation and the impacts of invasive alien species. *Sorocephalus imbricatus* was recently identified as a species requiring improved representation in *ex-situ* collections. During field work undertaken to collect germplasm for this purpose, a concerning number of dead and dying plants were observed.

Objectives: To determine the cause of rapid death of individuals in a remnant subpopulation of *S. imbricatus*.

Method: A field visit to a subpopulation of the only extant population, Elandskloof, was conducted to examine the symptoms associated with *S. imbricatus* mortality, and to collect samples for isolation and identification of putative pathogens.

Results: Dead and dying plants showed clear symptoms of root and collar rot, with *Phytophthora cinnamomi* Rands recovered from all samples. The collections highlighted the severe impact of *P. cinnamomi* on *S. imbricatus*, with the size of the subpopulation being reduced from 62 to 37 individuals (a 40% reduction) between October 2021 and May 2022.

Conclusion: This study describes, for the first time, rapid mortality of the Critically Endangered Proteaceae species, *S. imbricatus,* likely caused by the invasive pathogen *P. cinnamomi*. This concerning discovery highlights the urgent need for greater recognition of the threat *P. cinnamomi* poses not only to *S. imbricatus,* but to the broader floristic diversity of the CFR. Importantly, it illustrates a need for a substantial body of work to be undertaken to address a significant lack of knowledge regarding the relative threat that *P. cinnamomi* poses to species of the CFR.

Keywords: Proteaceae; *Phytophthora cinnamomi*; root rot; susceptibility continuum; threatened species.

Introduction

The Cape Floristic Region (CFR) contains more than 9 300 species of vascular plants, 68% of which are endemic to the region (Manning & Goldblatt 2012). The exceptionally high level of species diversity and endemism, together with having one of the highest concentrations of threatened plants in the world, has led to the recognition of the CFR as one of the 'hottest hotspots' of biodiversity globally (https://whc.unesco.org/en/list/1007/). Habitat loss (due to infrastructure development, urban expansion, crop cultivation, timber plantations and mines), habitat degradation (by overgrazing and inappropriate fire

management) and the impacts of invasive alien plant species have all been identified as major threats to the biodiversity of the CFR (Richardson et al. 1996; Bomhard et al. 2005; Manning & Goldblatt 2012). Importantly, climate change is also likely to be a major future threat (Bomhard et al. 2005).

Target 8 of the Global Strategy for Plant Conservation (GSPC; a programme adopted under the Convention on Biological Diversity) sets a goal for 'at least 75 per cent of threatened plant species in *ex-situ* collections, preferably in the country of origin, and at least 20 per cent available for recovery and restoration programmes'. In 2017, a full gap analysis of South African Proteaceae held in documented *ex-situ* collections (both living and seed), was undertaken by institutional members of Botanic Gardens Conservation International (BGCI), guided by Target 8 of the GSPC and the IUCN/SSC (2014) Guidelines on the Management of *Ex-situ* Populations for Conservation. Members of the genus *Sorocephalus* R.Br. were among those that stood out as horticulturally difficult to maintain (Blackhall-Miles 2020). *Sorocephalus* is a small genus, containing 11 species, five of which are Endangered and four that are Critically Endangered (IUCN 2022). At the time of the gap analysis, only two species of *Sorocephalus* were being conserved in *ex-situ* collections (Blackhall-Miles 2020).

One of the species for which the development of conservation measures was prioritised was *Sorocephalus imbricatus* (Thunb.) R.Br. Commonly known as the tile-leaf powder puff, *S. imbricatus* is a slender, single stemmed, sparsely branched shrub growing to 1.5 m, flowering from spring to midsummer (September to December) (Goldblatt & Manning 2000). Endemic to the CFR, with a limited distribution in mountains of the Western Cape, it occurs on sandstone fynbos and montane shale bands at 330–860 m altitude (Rebelo & Raimondo 2020). Historically, *S. imbricatus* occurred at three localities: Piketberg, Groot Winterhoek and Elandskloof mountains. Today, however, only the Elandskloof population remains extant, with a declining number of patches of plants occurring over a 14 km range (Rebelo & Raimondo 2020). Now Critically Endangered, several threats to the survival of this species have been identified. These include habitat loss due to dam construction and forestry plantations, and ongoing habitat degradation due to inordinately frequent fires and invasion by alien plants (Rebelo & Raimondo 2020).

In October 2021, one of the remaining Elandskloof patches of *S. imbricatus* was visted by members of the South African National Biodiversity Institute (SANBI), Cape Nature and the Millennium Seed Bank (MSB) (Figure 1B). The team monitored size and status of the population, collected cuttings for vegetative propagation trials, and hand pollinated and bagged flowers of several plants. Sixty-two individual plants were counted at the site. A follow-up visit to collect seed took place in February 2022. During this visit, it was noted that several *S. imbricatus* plants were dead, and others were dying, with the number of surviving plants reduced to 51 individuals. In May 2022, a site visit was conducted to determine the cause of death of individuals in this population. Symptoms of root and collar rot were immediately obvious, with plants appearing to have died rapidly. The observed symptoms appeared typical of *Phytophthora* infection. The aim of this study was to determine the cause of the root disease seriously threatening *S. imbricatus*.

Material and methods

Disease description, sample collection and isolations

The assessed Elandskloof *S. imbricatus* subpopulation was restricted to an area of approximately 2 500 square metres of the Witzenberg Local Municipality, Western Cape, South Africa (-33.389801, 19.100955). The area where the subpopulation occurs is surrounded by a plantation of *Pinus* (Figure 1A). The plants appeared to have died rapidly, with leaves still attached to stems and minimal decomposition of plant parts observed (Figure 1E).

Three dead plants were carefully removed from the ground and there was evidence of root and collar rot in all individuals. The diseased plants were collected together with rhizosphere soil, to determine the cause of their death in the laboratory. An additional two living but symptomatic plants were inspected (Figure 1C). Bark tissue of these two individuals was scraped back, revealing necrotic lesions in the collar and stem of both plants, typical of *Phytophthora* infection (Figure 1D). Root and soil samples were collected from these two plants.

Sorocephalus imbricatus root pieces were washed in distilled water, blotted dry and plated onto *Phytophthora* selective media, NARPH (50 mg nystatin, 200 mg ampicillin, 10 mg rifampicin, 25 mg pentachloronitrobenzene, and 50 mg hymexazol per 1 L deionised water and 17 g cornmeal agar, as described by Hüberli et al. 2000). Plates were kept in the dark at 22°C and examined daily. Preliminary identifications were made using microscopy. After three to five days, coralloid hyphae with hyphal swellings, typically characteristic of *P. cinnamomi,* emerged from root tissues of three of the five samples. Single hyphal tip isolations were made and transferred onto fresh half strength potato dextrose agar (½ PDA; 19.5 g PDA powder, Merck, South Africa, 7 g Difco™ agar, 1 L deionised water).

The soil and root samples from which *Phytophthora* was not recovered by direct plating were flooded with distilled water and 'baited' to further determine

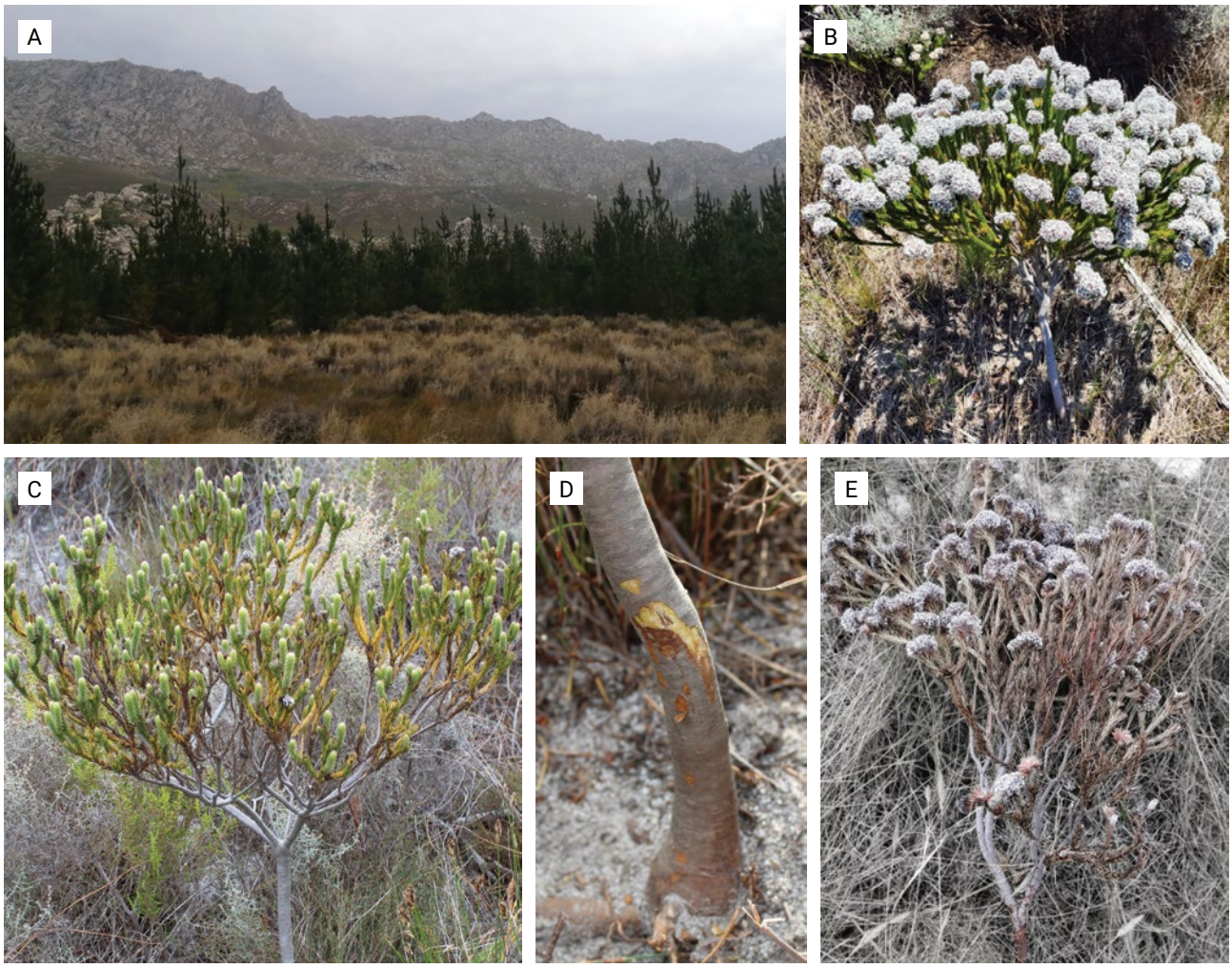

Figure 1. A, Study site – Elandskloof subpopulation of *Sorocephalus imbricatus,* surrounded by a *Pinus* plantation; B, healthy *S. imbricatus* in full flower, October 2021; C, symptomatic *S. imbricatus*; D, bark of plant in C removed to expose collar lesion; E, dead *S. imbricatus,* May 2022.

the presence of *Phytophthora*. Approximately 300 g of soil and roots was placed in a plastic tray containing 1 L of non-sterile distilled water. The floating plant litter was discarded and leaves of *Bauhinia galpinii* N.E.Br., *Hedera helix* L. and *Quercus suber* L., and petals of *Rosa hybrida* L. cv. 'Iceberg' were placed on the water surface as baits. The baits were maintained at room temperature and monitored regularly for lesion development over five days. Symptomatic baits with lesions were plated onto NARPH. Plates were stored, inspected and subcultured as described for the direct plated roots. Purified cultures were maintained on 10% clarified V8-agar (V8A; 0.1 L clarified V8 juice, Campbell Soup Company USA, 0.1 g $CaCO_3$, 15 g Difco™ Agar, Becton, Dickinson and Company, Sparks, USA, 0.900 L deionized water), and ½ PDA, at 22°C.

DNA extraction, sequencing and phylogenetic analysis

DNA was extracted from seven-day-old isolates grown on ½ PDA using Prepman® Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's protocols. The internal transcribed spacer regions 1 and 2 (ITS), including the 5.8S rRNA region, were amplified using primers ITS6 (Cooke et al. 2000) and ITS4 (White et al. 1990). PCR reactions were prepared following the protocols described by Bose et al. (2021). Amplified fragments were treated with ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA). The forward and reverse sequences were separately sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Thermo Fisher) following the manufacturer's protocol. The obtained products were cleaned and sequencing of the products was carried out at the Bioinformatics and Computational Biology Unit, University of Pretoria. CLC Main Workbench v. 8.0.1 (https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analy sis-and-visualization/qiagen-clc-main-workbench/) was used to assemble and trim the raw sequences, which were deposited in GenBank (Table 1). Consensus sequences were aligned to *P. cinnamomi* strain Ex-type CPHST BL 12 internal

transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence (length 866 bp) MG865473.

Permitting

Permission to collect samples was provided by the Western Cape Nature Conservation Board. Collections were made under permit number CN35-28-14709.

Results

Disease description and isolations

During the May 2022 visit, the number of surviving plants was further reduced from 51 in February 2022 to 37 individuals. *Phytophthora* was isolated from direct plated roots of the two living plants, and roots of one of the dead plants. Baiting confirmed the presence of *Phytophthora* in the remaining two samples. The morphology of the *Phytophthora* isolates recovered from the *S. imbricatus* root and rhizosphere samples was consistent with that known for *P. cinnamomi* Rands. Five isolates, one originating from each of the sampled *S. imbricatus* plants, were purified and have been preserved in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table 1). The five isolates were further identified by DNA sequencing.

Pathogen identification

For all five *Phytophthora* isolates, amplicons of approximately 840 bp were generated for the ITS region. The five isolates had identical sequences, and were identical to the sequence of the ex-type isolate of *P. cinnamomi* (CPHST BL 12). The molecular identification thus supported the morphological identification of *P. cinnamomi*.

Discussion

A site visit to investigate rapid death in a subpopulation of *S. imbricatus* identified root and collar rot as the cause of mortalility. The symptoms were typical of *Phytophthora* infection. Laboratory isolation and subsequent DNA sequencing identified isolates as *P. cinnamomi*. It was not possible to undertake pathogenicity tests due to the Critically Endangered status of *S. imbricatus* in its natural habitat and a lack of plants currently growing in *ex-situ* sites. However, despite the inability to conduct Koch's postulates, the consistent recovery of the invasive soilborne pathogen *P. cinnamomi* from dead and dying individuals, together with the observation of symptoms typical of those known to be caused by this pathogen, provide convincing evidence that it was the causal agent of the observed deaths.

The Global Invasive Species Database lists *P. cinnamomi* as one of the 100 worst invasive alien species (GISD 2022). It is a globally important plant pathogen, causing root and crown rot, cankers, dieback and mortality of approximately 5 000 woody plant species (Hardham & Blackman 2018). The association of *P. cinnamomi* with death of a species in the Proteaceae is not surprising. The first report of mortality in natural ecosystems of South Africa was by Van Wyk (1973), who provided an account of *P. cinnamomi* causing 'quick decline' of *Leucadendron argenteum* (L.) R.Br. in the Western Cape. Subsequent studies highlighted the importance of *P. cinnamomi* as a root rot pathogen of numerous native species (Von Broembsen 1984; Von Broembsen & Brits 1985; Von Broembsen & Kruger 1985; Hulbert et al. 2019). Despite this initial evidence for *P. cinnamomi* being a notable threat to the flora of the CFR, particularly members of the Proteaceae, very few studies have investigated the relative susceptibilities of the Cape flora to this pathogen by artificial inoculation. Much of the limited pathogenicity work that has been conducted dates back to the 1970s and 1980s (Van Wyk 1973; Von Broembsen 1984; Von Broembsen & Brits 1985).

The identification of *P. cinnamomi* as a disease-causing agent is an important component regarding the

Table 1. GenBank accession numbers of *Phytophthora cinnamomi* isolates obtained from *Sorocephalus imbricatus*

Species	Isolate[a]	Host	Substrate	ITS
Phytophthora cinnamomi	CMW58750	*S. imbricatus* dead	rhizosphere soil & roots (baited)	OP748937
Phytophthora cinnamomi	CMW58751	*S. imbricatus* dead	rhizosphere soil & roots (baited)	OP748938
Phytophthora cinnamomi	CMW58752	*S. imbricatus* living	roots (direct plated)	OP748939
Phytophthora cinnamomi	CMW58753	*S. imbricatus* living	roots (direct plated)	OP748940
Phytophthora cinnamomi	CMW58754	*S. imbricatus* dead	roots (direct plated)	OP748941

[a] CMW: Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa

management of threatened species such as *S. imbricatus*. Like South Africa's CFR, the Southwest Australian Floristic Region (SWAFR) is a global biodiversity hotspot, characterised by a Mediterranean climate and old, weathered, nutrient-deficient landscapes (Hopper & Gioia 2004). Similar to the CFR, the biodiversity of the region is threatened by habitat loss, habitat degradation, the impacts of invasive alien plant species and climate change (Monks et al. 2019). In addition, *P. cinnamomi* is considered to be one of the most significant threats to the conservation of the floral diversity of the SWAFR (Shearer et al. 2007; Cahill et al. 2008; Barrett & Rathbone 2018). Several of the plant families most severely impacted by *P. cinnamomi* in the SWAFR (e.g. Ericaceae, Fabaceae and Proteaceae), are families that are important components of the CFR (Goldblatt 1997; Barrett & Yates 2015). There is a pressing need to assess the risk *P. cinnamomi* poses to conservation of the Cape flora.

Rebelo et al. (2019) noted high mortality of immature and mature individuals in remaining subpopulations of *S. imbricatus*. They highlighted the threats posed by afforestation, alien plant invasion, too frequent fire and dam construction. It seems plausible, however, that *P. cinnamomi* has been overlooked as the cause of the observed mortality. Similarly, while the IUCN assessment of Rebelo and Raimondo (2020) lists threats by invasive species, this is limited to the impacts of invasive plants, and disease threats by invasive pathogens (including *P. cinnamomi*) are not currently considered. The presence of this pathogen represents a very concerning threat to the viability of this subpopulation, as demonstrated by the rapid and substantial (40%) reduction in population size observed between October 2021 (n=62) and May 2022 (n=37). Left unchecked, *P. cinnamomi* will likely continue to cause mortality of *S. imbricatus* individuals within this subpopulation. A hygiene plan should be developed to minimise the risk of further spread of *P. cinnamomi* within the area where the population occurs. Ideally, the health status of additional *S. imbricatus* subpopulations should be determined.

The use of the systemic biodegradable fungicide, phosphorous acid (phosphite), may assist in maintaining the health of remaining *S. imbricatus* individuals. While this treatment cannot eradicate *Phytophthora* from the soil, it induces a defence response in *Phytophthora*-challenged plants and has been shown to increase the resistance of susceptible plant species to infection by *P. cinnamomi* (Hardy et al. 2001; Shearer et al. 2012; Eshraghi et al. 2014). The application of phosphite could buy time to allow the collection of additional *S. imbricatus* germplasm to be stored in seed banks, or propagated and grown in botanical gardens for future translocation programs.

In 1975, Knox-Davies warned of the potential susceptibility of many components of the fynbos vegetation to *P. cinnamomi*. Importantly, he emphasised the need for an intensive local research programme to, among other things, determine the relative susceptibilities of fynbos species to *P. cinnamomi*. Today, near 50 years on, very little progress has been made in this regard. Considering the alarmingly high number of rare and threatened taxa occurring in the CFR, and with many of these taxa such as *S. imbricatus* belonging to families known to be susceptible to *P. cinnamomi,* it is imperative that a research programme similar to that proposed by Knox-Davies (1975), be established. Methods to position taxa on a *P. cinnamomi* resistance-susceptibility continuum have been well developed by researchers working with threatened flora of the SWAFR (Shearer et al. 2013). Understanding the relative susceptibility of taxa can inform strategies for *in-situ* conservation, as well as informing where conservation priorities and recovery efforts should be directed. Substantial efforts have been made to identify and address many of the threats to the CFR. However, the rapid decline in population numbers of the Critically Endangered *S. imbricatus* highlights the urgent need to better understand the role of the invasive pathogen, *P. cinnamomi,* as a key threatening process to the flora of this region.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

MN raised the alarm after observing mortality in the *Sorocephalus imbricatus* population. MN and TP collected samples, TP conducted the diagnostics and wrote the first draft of the manuscript. All authors contributed to manuscript revision and approved the submitted version.

Ethical considerations

This article followed all ethical standards for research without direct contact with human or animal subjects.

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Evolutionary patterns in South African brambles (*Rubus* L.) – new insights from molecular markers

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Background: South African brambles (*Rubus* L., Rosaceae) represent a complex group of six native species and at least 12 introduced taxa with different ploidy levels and varying tendencies to hybridisation. The role of hybridisation, introgression and apomixis in the ongoing evolution has been hypothesised based on morphological observations, but it has not been rigorously studied to date, and nor has the phylogeny of the group.

Objectives and methods: This paper aims to reveal the evolutionary patterns and mechanisms in South African brambles by employing three types of molecular markers: plastid and nuclear ribosomal DNA sequences, and nuclear microsatellites.

Results: The data confirmed the tetraploid *R. thaumasius* A.Beek and diploid *R. ludwigii* Eckl. & Zeyh. as distinct native species, while the other four native species are shown to be closely related and likely derived from three ancestors.

Conclusion: Ancient hybridisation and limited gene flow between regions (particularly between winter- and summer-rainfall zones) appear to be the main drivers of current patterns in the tetraploid *R. pinnatus* Willd. and hexaploid *R. rigidus* Sm. Current hybridisation is also likely, although rare. The mechanism of 'octoploid bridge' is proposed, which overcomes the ploidy reproduction barrier between *R. pinnatus* (or other tetraploids) and *R. rigidus*. No gene flow was detected between native and alien taxa, but clonal duplications were discovered in the *R. bergii* × *pinnatus* hybrid, which implies the possibility of apomictic spread of homoploid hybrids formed between native and introduced brambles and the potential for a new invasion. On the other hand, heteroploid hybrids (*R. bergii* × *rigidus*) are formed recurrently and spread only vegetatively.

Keywords: apomixis, clonal spread, hybridisation, introgression, reticulate evolution

Introduction

Rubus L. (Rosaceae: Rosoideae), commonly known as brambles, blackberries, raspberries, dewberries etc., is a complex genus due to its thousands of species and diverse evolutionary mechanisms, of which hybridisation, polyploidisation and apomixis are among the most important (Sochor et al. 2015), and often exhibiting strong phylogeographic patterns (Sochor & Trávníček 2016; Sochor et al. 2017). The genus has been relatively intensively studied in some parts of the world, e.g., in central and northwestern Europe, where an elaborate morphology-based system of a few sexual species and > 750 recognised apomictic microspecies (i.e., asexual genotypes of certain distribution areas and distinct morphology) organised in series, subsections and sections is in use

(Weber 1996; Kurtto et al. 2010). On the other hand, the genus has been relatively neglected in other regions (e.g., North America or the Caucasus: Alice et al. 2015; Sochor &Trávníček 2016). This uneven distribution of knowledge is reflected not only in the taxonomy but also in the phylogenetics, phylogeography and evolutionary biology of the group (biosystematics in a wide sense).

The African continent belongs among the understudied regions of the world. None of the native African *Rubus* taxa (disregarding North Africa, which is home to a few species of predominantly European distribution) were included in the two worldwide phylogenetic studies (Alice & Campbell 1999; Carter et al. 2019), and just a single DNA sequence from a native sub-Saharan African *Rubus* species is present in the NCBI GenBank nucleotide database (*R. rigidus* Sm., accession number U95229). Genetic diversity in African accessions has been analysed only among selected Kenyan specimens (unfortunately mostly undetermined and thus of unknown primary origin) using morphological and microsatellite markers with the aim of characterising potential breeding material (Ochieng et al. 2018, 2019). The evolution and phylogeny of African brambles are therefore unexplored.

Within the African continent, the taxonomy and fundamental biological properties (reproduction mode, polyploidy) are best explored in the South African *Rubus* taxa owing to recent advances in our understanding of the species in the region (Sochor et al. 2018, 2022; Van de Beek 2021). Applying a very narrow morphology-based (micro-)species concept, inspired by the one used for European *Rubus* apomicts, Van de Beek (2021) distinguished 16 native species in six series in the Cape Floristic Region alone and commented on the existence of a number of other 'species', so far insufficiently studied. On the other hand, Sochor et al. (2022) incorporated ploidy level and reproductive mode data, and identified six native South African *Rubus* species in total (see Table 1 for overview), all of them sexual di-, tetra- or hexaploids (2n = 14, 28, 42, respectively), and some of them phenotypically highly variable. In addition, 12 introduced taxa and 12 hybrids were identified, which indicated potential ongoing evolution in South African brambles via hybridisation, introgression and apomixis. However, the real effect of these processes on natural populations could not be evaluated properly based on phenotypic and cytometric data only.

In this work, we used the *Rubus* material that was studied in recent biosystematic/taxonomic investigations (Van de Beek 2021; Sochor et al. 2022) and analysed the sampled individuals by employing three types of DNA markers to address South African *Rubus* evolution from two perspectives. First, plastid and ribosomal nuclear sequence data were used for phylogenetic and phylogeographic reconstructions, and for identifying/confirming the identity of hybrids and potential introgressants. Specifically, we aimed at revealing not only phylogenetic relationships among taxa, but also at detecting any signatures of potential ancient or ongoing gene flow between native species or between native and introduced taxa. Second, simple sequence repeats (SSR, microsatellites) were used for primary evaluation of genotypic diversity and microevolutionary processes in a model group of *R. bergii* (Cham. & Schltdl.) Eckl. & Zeyh., *R. rigidus*, *R. pinnatus* Willd. and their hybrids. In particular, we aimed at detecting clonal duplications and quantifying the degree of apomixis at a regional scale, and thus evaluating the evolutionary and invasive potential of the hybrids. The new DNA data helped us understand the evolutionary history and phenotypic patterns in this relatively young and species-poor (in the context of the Cape flora) but evolutionary complex plant group.

Table 1. Overview of *Rubus* taxa occuring in South Africa with their distribution, ploidy level and reproductive mode (all from Sochor et al. 2022) and plastid haplotypes (detected in this study); LP = Limpopo; MP = Mpumalanga; G = Gauteng; FS = Free State; KZN = KwaZulu-Natal; EC = Eastern Cape; WC = Western Cape

Taxon/hybrid	Distribution in South Africa (provinces)[1]	DNA ploidy level	Reproduction	Plastid haplotype
Native taxa				
R. apetalus Poir.	LP, MP, KZN, EC	4x	sexual	*Ape1*, *Ape2*
R. ludwigii Eckl. & Zeyh.	MP, FS, KZN, EC, WC	2x	sexual	*Lud1*
R. pinnatus subsp. *pinnatus* Willd.	WC	4x	sexual	*Pin1*
R. pinnatus subsp. *pappei* (Eckl. & Zeyh.) Sochor	MP, KZN, EC	4x	sexual	*Pin3*, *Rig6*
R. rigidus Sm.	All except Northern Cape	6x	sexual	*Rig1–11*
R. thaumasius A.Beek	EC	4x	sexual	*Tha1*
R. transvaalensis Gust.	MP, KZN	6x	sexual	*Rig5*

Table 1. Overview of *Rubus* taxa occuring in South Africa with their distribution, ploidy level and reproductive mode (all from Sochor et al. 2022) and plastid haplotypes (detected in this study); LP = Limpopo; MP = Mpumalanga; G = Gauteng; FS = Free State; KZN = KwaZulu-Natal; EC = Eastern Cape; WC = Western Cape (continued)

Taxon/hybrid	Distribution in South Africa (provinces)[1]	DNA ploidy level	Reproduction	Plastid haplotype
North American taxa				
R. sect. *Arguti* (Rydb.) L.H.Bailey	LP, MP, G, FS, KZN, EC	4x	apomictic	*Arg1*
R. sect. *Cuneifolii* (L.H.Bailey) L.H.Bailey	KZN, EC	4x	apomictic	*Cun2*
R. titanus L.H.Bailey	WC	6x	sexual	*Urs1*
R. trichogynus A.Beek	LP, MP, FS, KZN, EC, WC	7x	sexual	*Urs1*
European/Caucasian taxa				
R. armeniacus Focke	EC, WC	4x	apomictic	*Dol1*
R. bergii (Cham. & Schltdl.) Eckl. & Zeyh.	EC, WC	4x	apomictic	*Ulm1*
R. aff. *bergii*	KZN	4x	apomictic	*Ulm2*
R. ulmifolius Schott	MP, KZN	2x	sexual	*Ulm1*, *Ulm2*
Asian taxa				
R. ellipticus Sm.	KZN	2x	sexual	*Eli1*
R. niveus Thunb.	LP, MP, KZN, EC	2x	sexual	*Niv1*
R. phoenicolasius Maxim.	KZN, EC	2x	sexual	*Phe1*
R. rosifolius Sm.	KZN, (WC)	2x	sexual	*Ros1*
Hybrids				
R. apetalus × *R. ludwigii*	MP	3x	not analysed	*Lud1*
R. apetalus × *R. pinnatus*	MP	4x	sexual	*Ape2*
R. sect. Arguti × *R. pinnatus*	MP	4x	apomictic	*Rig6*
R. sect. Arguti × *R. rigidus*	M, KZN, EC	5x, 8x	sexual	*Rig1*, *Rig4*, *Rig5*, *Rig9*
R. bergii × *R. pinnatus*	WC	4x		*Pin1*, *Rig6*
R. bergii × *R. rigidus*	EC, WC	5x	sexual	*Rig2*, *Rig3*, *Rig4*, *Rig6*, *Rig9*
R. bergii × *R. thaumasius*	EC	4x	apomictic	*Tha1*
R. ludwigii × *R. pinnatus*	KZN	3x	not analysed	*Lud1*
R. niveus × *R. transvaalensis*	MP	4x	not analysed	*Niv1*
R. pinnatus × *R. rigidus*	WC, EC	5x, 8x	sexual	*Rig3*
R. rigidus × *R. ulmifolius*	MP	4x	not analysed	*Rig5*
R. pinnatus × *R. thaumasius*	EC	not analysed	not analysed	*Tha1*

Materials and methods

Sampling and DNA extraction

DNA samples (see Supplementary Table S1) were collected during fieldwork for a biosystematic treatment of South African *Rubus* (see Sochor et al. 2022 for details). The specimens were simultaneously thoroughly studied morphologically and mostly also analysed for ploidy level and reproduction mode. Of the available collections, 224 specimens (particularly from non-apomictic taxa and hybrids) were used for sequencing, whereas only a selection of 45 specimens of *R. bergii*, *R. rigidus*, *R. pinnatus* and their hybrids, mostly from Western Cape (see Supplementary Table S1), was used for SSR analysis for primary evaluation of genotypic and allelic diversity, confirmation of phenotypic determinations, as well as assessment of the suitability of the markers for further studies. Six specimens of *R. bergii* from its native range in Western Europe were included as well. DNA was extracted from silica gel-dried leaves using the CTAB method (Doyle & Doyle 1987). Eight specimens, four of them being the type specimens, were provided by A. van de Beek, which represented his new species or his conception of old species (Supplementary Table S1; Van de Beek 2021; see also Sochor et al. 2022 for further discussion and revised taxonomic concepts); their DNA was extracted from two seeds per specimen using GenElute™ Plant Genomic DNA Miniprep kit (Sigma-Aldrich, USA).

Sequencing

Two plastid regions were analysed: the *matK* intron was amplified and sequenced with primers XFA and AST_R (Dunning & Savolainen 2010) and the *trnL-trnF* intergenic spacer with primers c and f (Taberlet et al. 1991). The ribosomal nuclear locus ITS (internal transcribed spacer) was amplified and sequenced with primers ITS1 and ITS4 (White et al. 1990). Polymerase chain reactions (PCRs) were performed using EliZyme FAST Taq mix (Elisabeth Pharmacon, Czechia) according to the manufacturer's protocol in reaction volume of 15 μL. PCR products were checked on agarose gel electrophoresis, purified by precipitation with polyethylene glycol (10% PEG 6000 and 1.25 M NaCl in the precipitation mixture) and sequenced using the Sanger method at Macrogen Europe (the Netherlands). In selected specimens, the ITS amplicon was cloned into a bacterial vector to obtain sequences of different ITS alleles (ribotypes) within one individual. In these cases, PCR was performed using EliZyme HIFI polymerase (Elisabeth Pharmacon) with proofreading activity. The PCR product was purified, its concentration estimated by Nanodrop 2000, and 18 ng of the PCR product was ligated in the total volume of 10 μL ligation mixture into pJET1.2/blunt cloning vector using CloneJET PCR Cloning Kit (Thermo Scientific, USA). The plasmid was further used for transformation of *Escherichia coli* strain DH5α using TransformAid Bacterial Transformation Kit (Thermo Scientific) following the overnight bacterial culture protocol, with a modification that the initial cultivation in C-medium was not longer than six hours and the colony used for its inoculation was not older than one day. Transformed bacterial colonies were used as a template in a colony PCR with primers pJET1.2 forward and reverse (supplied with the cloning kit). PCR products were checked, purified and sequenced with the amplification primers similarly to direct sequencing as described above.

SSR analysis

Ten microsatellite loci (Graham et al. 2004, 2006; Woodhead et al. 2008) were selected based on amplification efficiency and variability in a selection of samples, and amplified using the EliZyme FAST Taq (Elisabeth Pharmacon) in 10μL reaction volume with 7.5 ng template DNA following the standard manufacturer's protocol (see Supplementary Table S2 for further details). Fluorescent labelling was performed using a nested PCR containing three primers: a template-complementary forward primer with M13 tail at its 5′ end (final concentration 0.1 μM), a template-complementary reverse primer (concentration 0.4 μM), and a fluorescently 5′-modified M13 primer (5′-TGTAAAACGACGGCCAGT; NED™, PET®, VIC™ or FAM™ modification; concentration 0.4 μM). To facilitate annealing of the universal M13 primer the annealing temperature was lowered to 53°C in the last nine PCR cycles. Such labelled PCR products were separated together with the GeneScan 600LIZ® size standard on an ABI 3730XL capillary sequencer at Macrogen Europe.

Data analysis

DNA sequence editing, alignments and haplotype/ribotype identification were performed in Geneious 8 (Biomatters, New Zealand). Plastid haplotypes were compared with the sequences of Sochor et al. (2015) and Sochor and Trávníček (2016), and their codes assigned accordingly. A median-joining algorithm was used to create a phylogenetic haplotype network in Network 10.1.0.0 (Bandelt et al. 1999). All sequences were deposited in NCBI GenBank (accession numbers OL899048–OL899299 [ITS], OL954095–OL954503 [*matK* and *trnL-trnF*]). ITS data were checked for the presence of contaminations by microorganisms, pseudogenes and PCR recombinants as in Sochor et al. (2015). The filtered alignment was analysed in Network using star contraction (number of mutations set to three) and median-joining algorithms, and in SplitsTree 4 (Huson & Bryant 2006) using NeighbourNet algorithm with uncorrected P character transformation.

SSR chromatograms were analysed and scored manually in Peak Scanner 1.0 (Applied Biosystems). Alleles were coded according to their length in bp and saved as both codominant and binary data. Shannon information index was computed and principal coordinate analysis (PCoA) using the distance method with standardisation was performed in GenAlEx 6.5 (Peakall & Smouse 2012) based on the binary data matrix. Histogram of genetic distances and genotype identification were performed in Genotype 2.0b23 (Meirmans & Van Tienderen 2004).

Results

Patterns in plastid DNA variation

Plastid DNA data were obtained for 219 specimens. Among native South African *Rubus* taxa, 18 plastid haplotypes were distinguished when both single nucleotide polymorphisms (SNPs) and indels were considered, and 14 haplotypes when indels were rejected (Figure 1). Two haplotypes characterised *R. ludwigii* and *R. thaumasius*, respectively, and grouped separately from other native species. The other haplotypes formed two mutually related groups shared mainly by *R. pinnatus* and *R. rigidus*. The haplotype of *R. transvaalensis* (haplotype *Rig5*) was shared with *R. rigidus*, and two haplotypes (*Ape1, Ape2*) were found only in *R. apetalus* and its hybrid. Each of the Asian species bore a single unique haplotype. Tetraploid Euro-Caucasian taxa were also characterised by their haplotypes, but two haplotypes were detected in the diploid *R. ulmifolius* – one shared with *R. bergii* and one with *R.* aff. *bergii*. Four haplotypes were distinguished among North American taxa, one borne by at least three morphotypes of *R.* sect. *Arguti*, one by *R.* sect. *Cuneifolii*, one by two undetermined morphotypes (one of them possibly belonging to *R.* sect. *Alleghenienses*), and one haplotype was shared by *R. titanus* and *R. trichogynus* (*Urs1*; presumably derived from the western North American *R.* sect. *Ursini*).

The haplotypes of *Rubus rigidus* and *R. pinnatus* exhibited clear patterns in geographic distribution. *Rubus rigidus* bore only three haplotypes in the westernmost part of the range, all from the A group (Figure 1D), whereas only haplotypes of the C group (with one exception of the *Rig6* haplotype detected once near Alexandria, EC) were detected in eastern regions outside of the Cape Floristic Region, and the highest diversity was discovered in KwaZulu-Natal (KZN). A roughly similar pattern (Figure 1C) was detected in *R. pinnatus* and corresponded to its subspecific classification, in which western *R. pinnatus* subsp. *pinnatus* bore only the *Pin1* haplotype or its derivative *Pin4*, whereas *R. pinnatus* subsp. *pappei* had mostly the *Rig6* haplotype shared with western *R. rigidus*, or one haplotype from the C group differing from the eastern *R. rigidus* haplotypes only in one indel (*Pin3*).

All of the studied hybrids of *R. rigidus* [*R. bergii* × *rigidus* – 22 individuals (ind.); *R.* sect. *Arguti* × *rigidus* – 11 ind.; *R. rigidus* × *ulmifolius* – 3 ind.; *R. rigidus* × *pinnatus* – 1 ind.] exhibited haplotypes derived from that species. Similarly, *R. thaumasius* served as the pistillate parent of all of its studied hybrids (with *R. bergii* – 3 ind.; and with *R. pinnatus* – 1 ind.), as did *R. ludwigii* (with *R. apetalus* – 1 ind.; and *R. pinnatus* – 1 ind.). *Rubus pinnatus* served as pistillate parent in all of the studied hybrids with *R. bergii* (6 ind. representing 4 genotypes) but not in the hybrids with *R. apetalus*, whose pistillate parent was the latter species (1 ind.). The hybrid *R. niveus* × *transvaalensis* shared the haplotype with the first species.

Variation in ITS

ITS data were generated from 118 individuals in total, of which 90 were sequenced directly (individuals without length variation in the amplicon) and 28 were cloned (Supplementary Table S1). One to eight cloned sequences (185 in total, 6.6 on average) were obtained per individual after the exclusion of contaminants (10 sequences in total) and recombinants (14 sequences). 275 sequences were included in the final analyses. ITS exhibited more variation than plastid DNA, but part of it was not shared among individuals and was thus uninformative. The cloned sequences from hybrids always confirmed their hybrid origin. Similarly, two or more divergent orthologous ITS alleles were detected in alien apomictic polyploids. Except for the (putatively primary) hybrids, no gene flow/introgression was detected between native and introduced taxa.

Among native taxa, *R. thaumasius* and *R. ludwigii* formed distinct phylogenetic lineages, while the other species formed three groups (A, B, C, corresponding to the plastid haplotype groups according to their presumed origin), two of which could be subdivided into three subgroups each (Figure 2A). *Rubus apetalus* formed a separate distinct branch diverging from the base of the A group. *Rubus transvaalensis* was restricted to the C1 subgroup, which was not shared by any other species, but was placed at the split of C2 and C3 subgroups belonging to *R. rigidus*. Specimens of *R. pinnatus* subsp. *pinnatus* had only B2 ribotypes, but specimens from the transitional zone in the eastern parts of WC, as well as *R. pinnatus* subsp. *pappei* from MP bore mostly B1 ribotypes, and the remaining eastern populations had B3 ribotypes (Figure 2B). The B1 subgroup was the only one shared with *R. rigidus*, although only rarely in KZN and MP. *Rubus rigidus* was otherwise represented in A and C groups (Figure 2C): A was detected almost throughout the studied area, C3 dominated the lowlands of KZN and C2 was detected in MP always as an ortholog together with B1.

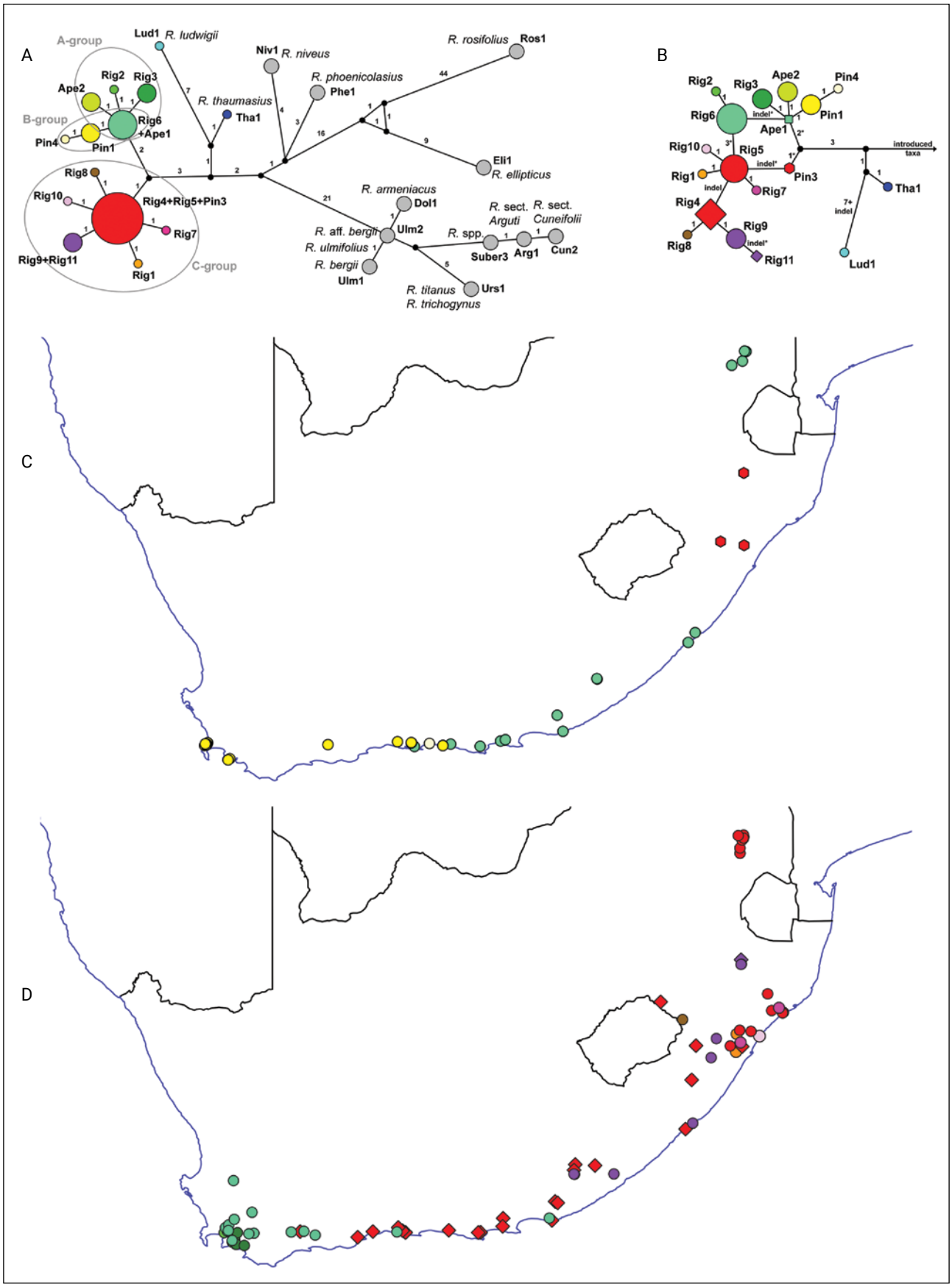

Figure 1. Plastid haplotype diversity patterns in South African *Rubus* taxa. A, haplotype network based on SNPs; B, haplotype network based on SNPs and indels (number of SNP mutations or indels shown above branches, forward and reverse mutations at the same position indicated by asterisks; symbol size corresponds with the frequency of the haplotype in the dataset); C and D, geographic distribution of haplotypes in *R. pinnatus* and *R. rigidus* (including their hybrids), respectively, in South Africa (symbol shapes and colours correspond with A and B). *Rubus apetalus* (haplotypes *Ape1*, *Ape2*) and *R. transvaalensis* (*Rig5*) are not included in the maps.

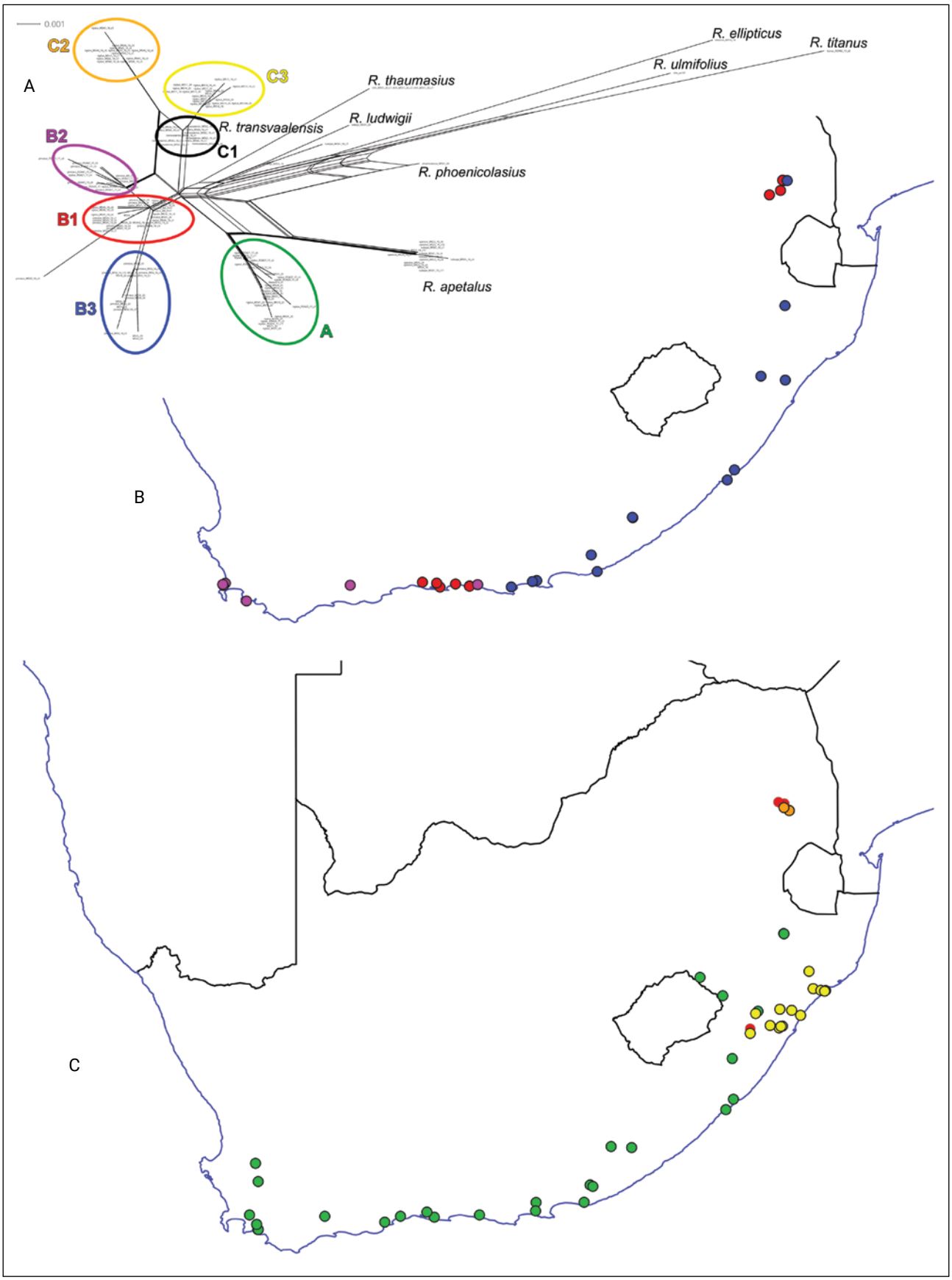

Figure 2. ITS ribotype diversity patterns in South African *Rubus* taxa. A, SplitsTree phylogenetic network based on cloned and directly sequenced ITS amplicons. B and C, geographic distribution of ribotypes in *R. pinnatus* and *R. rigidus* (including their hybrids), respectively, in South Africa (symbol colours correspond with A). Note that the C1 group is exclusive for *R. transvaalensis* and is not included in the maps.

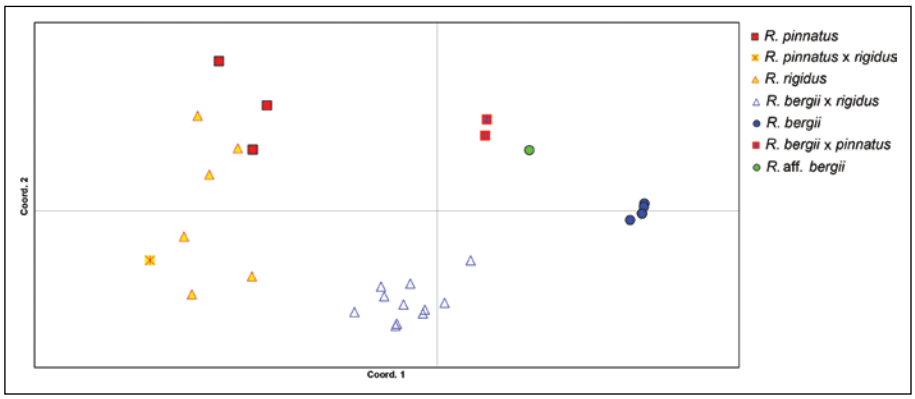

Figure 3. PCoA analysis of SSR data; the two axes explain 43% and 18% of the whole variation, respectively.

Variation in microsatellites

Ten SSR loci were selected following our previous work (e.g., Király et al. 2017), but Rubus123a was excluded due to poor amplification efficiency. In total, 99 alleles were detected in the studied sample set of 45 individuals across the nine loci (3–21 alleles per locus, mean ± standard deviation 11.0 ± 5.8; Supplementary Table S3). However, only null alleles (no PCR products) were detected at two loci (Rubus26a and ERubLR_SQ01_G16) in *R. bergii* and relatives. Distribution of genetic distances among individuals indicated the threshold between within-genotype and among-genotype variation to be set at three mutations (not shown). Applying this threshold, genotype assignment was almost identical to analysis with the threshold of zero (i.e., no mutation within a genotype allowed; Table 2); only *R. bergii* exhibited three different mutations in three individuals (one per individual; Supplementary Table S3). Despite that, this species was clearly monoclonal in both its native and secondary range (Table 2). Besides *R. bergii*, clonality was detected in the hybrid *R. bergii* × *pinnatus*. In contrast, *R. rigidus*, *R. pinnatus* and *R. bergii* × *rigidus* exhibited no clonal duplication. PCoA analysis supported identification of the parents in all of the presumed hybrids (Figure 3).

Discussion

Evolutionary history is complex in native species

Both the ITS and cpDNA confirm that *R. ludwigii* and *R. thaumasius* are distinct native species that diverged from the common ancestor of all South African *Rubus* taxa. This finding is contrary to previous interpretations of the origin of *R. thaumasius*, which was originally presumed to be a hybrid of *R. rigidus* and some other taxon (Gustafsson 1934) or even of purely European origin (Stirton 1981; Henderson 2011). However, its presumed relationship with tropical African species, such as *R. runssorensis* Engl. and *R. friesiorum* Gust. (Van de Beek 2021; Sochor et al. 2022), needs to be confirmed, as no material from tropical Africa was available for this study.

A different pattern was observed in the other four native species. *Rubus apetalus* is well differentiated for both ITS and cpDNA data and is not participating in the current evolution of the other species. It is undoubtedly closely related to both *R. pinnatus* and *R. rigidus*. *Rubus transvaalensis* is even more closely related to

Table 2. Summary statistics of SSR data; N = number of individuals; G = number of genotypes identified at different mutation thresholds (th = 0 or 3 allowing no or up to three mutations within a genotype, respectively); Alleles = average number of observed alleles per individual across all nine loci (± standard deviation); Shannon index = Shannon information index computed in GenAlEx from a binary matrix (± standard error)

Taxon	N	G (th = 0)	G (th = 3)	Alleles (± S.D.)	Shannon index (± S.E.)
R. pinnatus	3	3	3	15 (± 3.454)	0.092 (± 0.022)
R. rigidus	6	6	6	19.5 (± 2.950)	0.285 (± 0.026)
R. bergii	17	4	1	17 (0)	0.012 (± 0.005)
R. bergii × *rigidus*	11	11	11	22.8 (± 1.940)	0.195 (± 0.025)
R. bergii × *pinnatus*	5	2	2	23.8 (± 0.448)	0.082 (± 0.018)
R. pinnatus × *rigidus*	1	1	1	20 (NA)	NA
R. aff. bergii	1	1	1	18 (NA)	NA

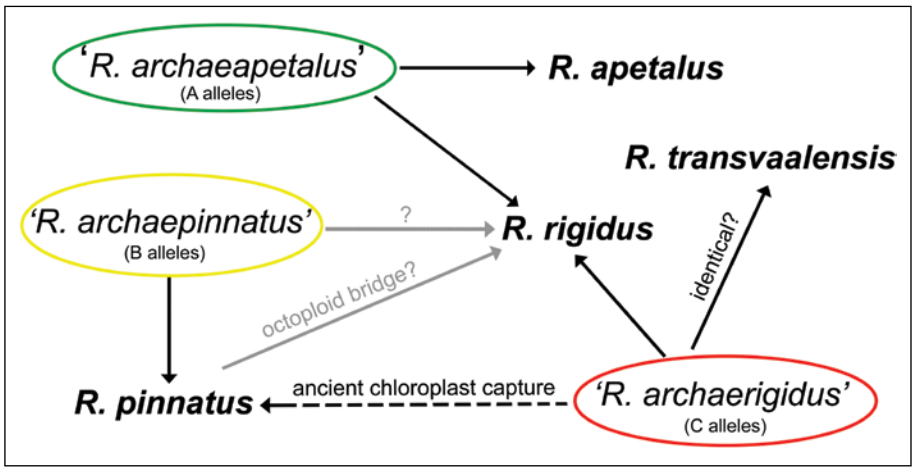

Figure 4. Scheme of proposed evolutionary relationships between modern species of the *R. rigidus–pinnatus–apetalus* group and their hypothetical ancestors; see text for explanation.

R. rigidus as inferred from phenotype (see Sochor et al. 2022) and DNA sequences (Figures 1, 2). Relationships between *R. pinnatus* and *R. rigidus* appear to be complex due to shared haplotypes and ribotypes, but in relation to the geographic distributions, this pattern cannot be explained simply by free recurrent gene flow. Taking into account the phylogenetic relationships among haplotypes and ribotypes and taxonomic and geographic distribution patterns, the following scenario can be hypothesised (Figure 4).

Three ancestral species, possibly already tetraploid or even hexaploid, migrated through the coastal regions from northeast to southwest, occasionally hybridised and further evolved into the species as currently recognised, although the ancestral species themselves disappeared. The first ancestor, '*R. archaeapetalus*', is represented in our data as the basal ribotypes and haplotypes of the A group (Figures 1A & 2A). This ancestor evolved directly into *R. apetalus* but must have contributed to the formation of *R. rigidus*, as implied from the A ribotypes throughout its range and the A haplotypes in the west (which, however, may have been derived also from the second ancestor despite the fact that the current geographic patterns rather contradict this possibility; Figures 1C & 1D). A second ancestor, '*R. archaepinnatus*' (B alleles) gave rise to *R. pinnatus* with considerable geographic genetic variation between the winter-rainfall and summer-rainfall zones but also contributed to the genome of *R. rigidus* to some extent, at least in the east (see ITS; Figure 2). A third ancestor, '*R. archaerigidus*' (C groups), must have had an identical ribotype (C1) and haplotype (*Rig5*) as *R. transvaalensis* and may have therefore also been very similar to this modern species in other respects (e.g., in hexaploidy?). This ancestor probably did not spread to westernmost South Africa as no traces of it have been detected in any modern taxon there. It must, however, have contributed to the formation of *R. rigidus* (mainly in eastern regions), of *R. transvaalensis*, and to a lesser degree also *R. pinnatus*. However, as far as we know, *R. pinnatus* only bears one haplotype derived from '*R. archaerigidus*' (*Pin3*). This haplotype differs from *Rig5* in the absence of one 6-bp repetition, which makes *Pin3* the basal-most haplotype within the C group. Therefore, the *Pin3* haplotype can only be a result of an ancient chloroplast capture, rather than a continuous gene flow from '*R. archaerigidus*' to *R. pinnatus*.

Similar reticulate evolution pathways are often observed in polyploid complexes. For example, Fehrer et al. (2009) revealed very complex evolutionary patterns in both diploid and polyploid accessions of European *Hieracium* s.str. (Asteraceae). Highly reticulate evolution associated with late Quaternary phylogeography of sexual ancestors was reconstructed in European blackberries, among which more than 750 species are recognised, but these originate in just around six ancestral diploids, some of them extinct (Sochor et al. 2015, 2017). However, hybridisation has long been recognised as an important process in plant evolution and speciation in general, not only in apomictic genera (Rieseberg 1995; Nolte & Tautz 2010).

Current gene flow among taxa seems to be limited

In our previous paper (Sochor et al. 2022), we reported on the occurrence of 12 hybrid combinations in South African brambles, some of which are locally even more frequent than their parents (e.g. *R. bergii* × *R. rigidus*). The hybrid origins of all of these taxa were supported by the molecular data presented here (Figure 3; see also Supplementary Table S1 for plastid haplotypes). The frequent occurrence of hybrids and the successful production of seeds and even the occurrence of facultative apomixis in some of them made us consider the evolutionary potential of hybridisation in South African brambles. Furthermore, two octoploid fertile sexual hybrids derived from *R. rigidus* (with *R. pinnatus* or *R.* sect. *Arguti*) were also discovered, which implies that such hybrids are not rare (the two specimens represented 4.5% of the 44 hybrid individuals with known

ploidy in our dataset). Hypothetically, these octoploids could backcross with the tetraploid parent (2x gamete) due to the formation of regular reduced 4x gametes (see Sochor et al. 2022). The offspring (6x) would then share ploidy level and ± 50% of the genome with the first parent. Therefore, only two generations can be sufficient to overcome the ploidy reproduction barrier between tetra- and hexaploids.

Although potentially very effective and explanatory for the extraordinary phenotypic variability of *R. rigidus* (see Sochor et al. 2022), this 'octoploid bridge' (parallelism of triploid bridge sensu Ramsey & Schemske 1998) does not appear to be a common evolutionary mechanism, because no shared alleles have so far been detected between native and introduced taxa (except for the apparent hybrids), and only a few shared alleles were detected among native species. An example is the *Rig6* haplotype in *R. rigidus* near Alexandria, EC, where this haplotype is shared with *R. pinnatus*, but *R. rigidus* bears it in regions much further west (because of shared ancestry) and a transition zone was only documented in the eastern parts of WC (Figure 1D). Another possible example are the B1 ribotypes, which seem to originate from the *R. pinnatus*/'*archaepinnatus*' lineage but were found also in *R. rigidus* in KZN and MP, in all cases together with the C ribotypes in each individual. This last fact could imply that the five *R. rigidus* individuals (all confirmed hexaploids) can actually be early-generation introgressants, because the ribosomal cistron has not yet been homogenised. However, due to the rather limited sample set, we cannot rule out the possibility of ancient gene flow between the two species and the local preservation of genes of *R. pinnatus*/'*archaepinnatus*' in *R. rigidus*.

Genetic diversity is geographically structured in *R. pinnatus* and *R. rigidus*

Genotypic and allelic diversity and its structuring are crucial information for the management of both introduced and native taxa (especially those of conservation concern), and for understanding their evolutionary behaviour. Our data provide two perspectives. While DNA sequences from the conservative markers provide a wide and superficial overview, the population-genetic data from microsatellites enable much finer and deeper insights, but were restricted in this study to a single model system of *R. bergii*, *R. rigidus*, *R. pinnatus* and their hybrids.

From the wider, phylogeographic perspective, our sequence data imply relatively low genetic diversity in *R. thaumasius*, *R. apetalus*, *R. transvaalensis,* and also *R. ludwigii*. The latter was, however, included only marginally in this study and its geographic variation may not have been sampled. In contrast, *R. pinnatus* and particularly *R. rigidus* exhibit high diversity in plastid DNA and ITS, which is clearly geographically structured. This structuring appears to reflect not only the reticulate evolution discussed above, but also a long-term isolation of populations and limited gene flow among regions. The most conspicuous genetic differences are between the summer-rainfall and winter-rainfall zones (Figures 1 & 2), implying that the haplotypic/ribotypic geographical differentiation may have been accompanied by niche shift, which in turn may be associated with a slight phenotypic shift in *R. pinnatus*. These geographically linked differences justify its subdivision into two subspecies (Sochor et al. 2022).

In *R. rigidus*, on the other hand, major phenotypic traits (e.g. structure of leaves, fruit colour, leaf indumentum) do not correspond with haplotypes or ribotypes. Consequently, putatively distinct morphotypes (or species sensu Van de Beek 2021) are widespread across South Africa, but are obviously composed of diverse genotypes of different phylogenetic/genealogical history. In other words, taxonomic treatment of such morphotypes on the species level is contradicted not only by their obligate sexuality (Sochor et al. 2022), but also their diverse polytopic origin. A narrow species concept, such as that used in Europe for apomictic genotypes, is, therefore, clearly inapplicable in South African native taxa.

Clonality implies apomictic spread in *R. bergii* and *R. bergii* × *pinnatus*

Originally, we suspected the *R. bergii* × *R. rigidus* hybrids to be partly apomictic and able to persist and spread without recurrent formation of new genotypes via hybridisation (Sochor et al. 2018). This would result in the presence of the same genotype at different locations, and later in the dominance of one or a few successful hybrid genotypes within each region. However, no clonal (i.e., apomictic) duplication was detected among the 11 hybrid individuals in our dataset, despite the fact that the sampling was focused on a small area in westernmost WC (see Supplementary Table S3). This fact supports our later conclusion (Sochor et al. 2022) that these pentaploid hybrids are possibly exclusively sterile and can persist and spread only via vegetative means. On the other hand, the high frequency of occurrence of the hybrid in some regions implies its easy and common recurrent formation.

Surprisingly, clonal duplications were identified in the hybrid *R. bergii* × *pinnatus*, although this was not in our primary focus and was therefore represented by only five individuals in our SSR data set. Four of the individuals turned out to belong to a single genotype (Table 2; Supplementary Table S3). The sampled area was very small with distances between the individuals of the clone being 0.33–1.36 km. Such distances, however,

seem to be too long to be explained by the spontaneous vegetative spread. As human-mediated propagation can be most likely excluded, the most probable explanation for our finding is asexual dispersal via seeds – apomixis. We have reported on apomictic seeds in two other homoploid hybrids between native and introduced *Rubus* taxa (*R. bergii* × *thaumasius* and *R. pinnatus* × sect. *Arguti*; Sochor et al. 2022) but it was not clear whether these seeds were viable and able to secure dispersal.

Similarly, Clark and Jasieniuk (2012) detected (rare) hybridisation among native and introduced *Rubus* taxa in western United States, as well as apomixis at the level of the embryo. However, seedlings derived from the hybrids exhibited higher allelic variation than would be expected for apomictic offspring, and apomixis, therefore, was not confirmed on the level of seedlings. In contrast, the frequent occurrence of hybrids between *Taraxacum officinale* (alien) and *T. japonicum* (native) (Asteraceae) was reported in western Japan despite a very low hybridisation rate (Matsuyama et al. 2018). The number of hybrid genotypes detected in that study in natural populations was surprisingly high but still indicated their apomictic spread. A combination of apomixis, high genotypic diversity, and hybrid origin from a native species seemed to promote effective natural selection and propagation of well-adapted genotypes, and thus enhanced invasiveness.

Rubus bergii × *pinnatus*, as well as the other two hybrids with apomictic seeds, is only locally common and of rather low importance as an invader at this moment. However, these hybrids may potentially pose an initial phase of new invasion that can take advantage of local adaptations of the native parent (Pfennig et al. 2016), clonal multiplication of a superior genotype (Parepa et al. 2014), potential hybrid vigour (Ayres 2004) or simply of being an evolutionary novelty (Ellstrand & Schierenbeck 2006). Targeted sampling of the tetraploid hybrids with subsequent assessment of genotypic diversity and invasive potential is required to evaluate this hypothesis.

High genotypic and allelic diversity were detected in *R. rigidus* (Table 2), three or four alleles per locus and individual being no exception, which is consistent with its sexual mode of reproduction and allopolyploid origin. In contrast, *R. bergii* was confirmed to be monoclonal with no signs of recombination or introgression from other taxa, yet with relatively high allelic diversity (reflecting its allopolyploid origin; Table 2). Monoclonality in our dataset also confirmed the identity of South African *R. bergii* and European plants usually treated under the name *R. vigorosus* P.J.Müll. & Wirtg. (Kurtto et al. 2010; Van de Beek 2014). Such extremely low genotypic diversity is consistent with data from other apomictic *Rubus* microspecies (Király et al. 2017; Šarhanová et al. 2017). Although the monoclonality is contradictory to the relatively high proportion of sexually derived embryos as detected by flow cytometric seed screen in the demonstrably monoclonal microspecies (cf. Šarhanová et al. 2012; Sochor et al. 2022), this paradox appears to be a common phenomenon in *Rubus*, so far without explanation (see also Šarhanová et al. 2017). Similar patterns are therefore presumed to occur in other South African alien apomictic blackberries such as *R. armeniacus* and *R.* sect. *Cuneifolii* (both likely monoclonal in South Africa), and *R.* sect. *Arguti* with two widespread clones and several genotypes of local occurrence (Sochor et al., 2022).

Conclusion

South Africa is not a hotspot for *Rubus* diversity, but the genus is taxonomically challenging and has been rather overlooked in this region (Van de Beek 2021; Sochor et al. 2022). A combination of traditional phenotype-based, molecular, and cytometric methods have improved our understanding of its diversity and evolutionary behaviour.

Contrary to previous concerns and notions that the group (or at least some of the taxa) is a hardly intelligible tangle (Sochor et al. 2018; Van de Beek 2021), the biosystematics of South African *Rubus* is not intractable. Despite frequent hybridisation, gene flow among modern species appears to be weak, as the hybrids mostly do not contribute to further evolution via hybridogenesis or introgression. However, clonal duplications and asexually derived seeds detected in tetraploid hybrids of native and introduced taxa may indicate incipient new plant invasions, and this process deserves further attention.

High phenotypic variability in some species, which has caused much confusion, can readily be explained by their allopolyploid origin and phylogeographic patterns. For example, the extreme variability in *R. rigidus* seems to be caused by: 1) its hexaploidy; 2) its origin in (at least) three ancestral species (Figure 4); 3) among-population isolation and subsequent differentiation particularly between winter-rainfall and summer-rainfall zones but also within the zones to some extent; and 4) probably weak but possibly continuous gene flow from other species, such as *R. pinnatus* and *R. transvaalensis*.

The data presented here and in our previous papers are not exhaustive and should be regarded rather as a foundation for further studies. Besides the invasive potential of the tetraploid hybrids, the most challenging task for the future is to unearth evolutionary links between the South African and tropical African *Rubus* flora, as well as better characterise the diversity of alien, particularly North American taxa, which seem to be quite rich, yet underexplored in the eastern regions of South Africa. However, our experiences show that new and often surprising discoveries can be expected around every corner of (not only) South African batology.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

MS performed the sampling, laboratory work, data analyses and wrote the first draft of the manuscript, JCM contributed to the fieldwork and data interpretations, and edited the manuscript.

Ethical considerations

This article followed all ethical standards for research without direct contact with human or animal subjects.

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Data availability statement

All sequences were deposited in NCBI GenBank (accession numbers OL899048–OL899299 [ITS], OL954095–OL954503 [*matK* and *trnL-trnF*]). SSR data matrix is available in Supplementary table S3. Herbarium vouchers are deposited in public herbaria OL, NBG, PRE, NU and L.

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Supplementary Material

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
RSA01/17	*R. bergii*	SA, WC, Table Mt., Cape Town, Valley of Isolation, ca. 800 m NW of Waterworks Museum, at S oriented rock wall at a tourist path	890	-33.970	18.402	3/11/2017	4×*	OL, NBG	Ulm1			dir. seq.	
RSA02/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Table Mt., Cape Town, Skeleton Gorge, 300 m SE of the beginning of Hely-Hutchinson dam	660	-33.980	18.417	3/11/2017	4×	OL, NBG	Pin1	B2		dir. seq.	
RSA04/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Table Mt., Cape Town, Woodhead Dam, 60 m W of Waterworks Museum	740	-33.975	18.407	3/12/2017	4×	OL	Pin1		1		
RSA05/17	*R. bergii* × *pinnatus*	SA, WC, Cape Town, Newlands, Riverside Road	38	-33.985	18.445	3/15/2017	4×	OL, NBG	Pin1		1		
RSA06/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Cape Town, Rondebosch, 300 m NW of the Rhodes Memorial, at the road	209	-33.951	18.456	3/16/2017		OL, NBG	Pin1	B2	1	dir. seq.	
RSA07/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Cape Town, Rondebosch, 750 m W of the Rhodes Memorial	351	-33.953	18.451	3/16/2017			Pin1				
RSA08/17	*R. bergii*	SA, WC, Cape Town, Rondebosch, 750 m W of the Rhodes Memorial	351	-33.953	18.451	3/16/2017		NBG	Ulm1		1	dir. seq.	
RSA09/17	*R. bergii* × *pinnatus*	SA, WC, Cape Town, Kirstenbosch arboretum, SW margin at the stream	94	-33.989	18.437	3/16/2017	4×	NBG	Pin1		1		
RSA10/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Cape Town, Kirstenbosch arboretum, S margin at the stream	84	-33.989	18.439	3/16/2017	4×*	OL	Pin1				

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
RSA11/17	*R. bergii* × *pinnatus*	SA, WC, Cape Town, Kirstenbosch arboretum, central part	90	-33.988	18.440	3/16/2017		n/a			1		
RSA12/17	*R. titanus*	SA, WC, Cape Town, at the road Kirstenbosch - Hout Bay, behind the crossroads to Alphen	215	-34.008	18.413	3/18/2017	6×	OL, NBG	Urs1			dir. seq.	
RSA13/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Hout Bay, NE end of the town, at the road to Constantia	48	-34.015	18.384	3/18/2017		NBG	Pin1		1		
RSA14/17	*R. armeniacus*	SA, WC, Hout Bay, at the road Kirstenbosch - Hout Bay, 0.5 km W of Constantia Neck	176	-34.012	18.400	3/18/2017	4×	OL, NBG	Dol1				
RSA15/17	*R. bergii* × *pinnatus*	SA, WC, Cape Town, at the road Kirstenbosch - Hout Bay, at the crossroads to Alphen	182	-34.007	18.418	3/18/2017	4×	OL, NBG	Pin1		1		
RSA16/17	*R. bergii*	SA, WC, Stellenbosch, Devonvallei, at the main road to Cape Town	87	-33.948	18.819	3/21/2017		NBG	Ulm1		1	dir. seq.	
RSA17/17	*R. bergii*	SA, WC, Stellenbosch, base of Pappegaaiberg, S of the top	98	-33.940	18.845	3/21/2017	4×	OL, NBG	Ulm1		1	cloned	
RSA18/17	*R. bergii* × *rigidus*	SA, WC, Stellenbosch, Idasvallei, 0.6 km SW of Idasvallei Dam	186	-33.923	18.896	3/21/2017	5×	OL, NBG	Rig6		1		
RSA19/17	*R. bergii*	SA, WC, Kylemore, at the road Stellenbosch - Pniel	264	-33.912	18.944	3/21/2017	4×	NBG	Ulm1		1		
RSA20/17	*R. bergii* × *rigidus*	SA, WC, Kylemore, at the road Stellenbosch - Pniel	264	-33.912	18.944	3/21/2017	5×	OL, NBG	Rig2		1	dir. seq.	
RSA21/17	*R. bergii* × *rigidus*	SA, WC, at the road R45 between Simondium and Drakenstein	164	-33.858	18.972	3/21/2017		OL, NBG	Rig6		1	dir. seq.	
RSA22/17	*R. bergii*	SA, WC, at the road R45 between Simondium and Cillie	127	-33.816	18.951	3/21/2017		NBG	Ulm1		1		

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
RSA23/17	*R. bergii*	SA, WC, Paarlsberg, at the picnic place 0.9 km NE of the top	379	-33.735	18.947	3/21/2017	4×*	OL	Ulm1		1		
RSA24/17	*R. bergii*	SA, WC, Paarlsberg, 2 km S of the top, under Victoria Dam	241	-33.759	18.948	3/21/2017		NBG	Ulm1		1		
RSA25/17	*R.* sp. *(North American)*	SA, WC, Kogelberg Nature Reserve, 300 m W of Visitors Centre	41	-34.323	18.964	3/22/2017	7×	OL, NBG	Urs1			cloned	
RSA26/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Kogelberg Nature Reserve, 500 m NW of Visitors Centre	24	-34.319	18.964	3/22/2017	4×	OL, NBG	Pin1				
RSA27/17	*R. pinnatus* × *rigidus*	SA, WC, at the road R44 (Kleinmond - Bot River), N of Arabella Country Estate	31	-34.304	19.135	3/22/2017	5×	OL, NBG	Rig3	A+B2	1	cloned	
RSA28/17	*R. bergii* × *rigidus*	SA, WC, at the road R44 (Kleinmond - Bot River), N of Arabella Country Estate	31	-34.304	19.135	3/22/2017	5×	OL, NBG	Rig3	A	1	cloned	
RSA29/17	*R. bergii*	SA, WC, ca. 8.7 km NE of Kleinmond, Elgin Valley, among vineyards	411	-34.280	19.086	3/22/2017		OL	Ulm1		1	dir. seq.	
RSA30/17	*R. bergii* × *rigidus*	SA, WC, Elgin Valley,10 km NNE of Kleinmond, among orchards at the stream	138	-34.254	19.054	3/22/2017	5×	OL, NBG	Rig3		1	dir. seq.	
RSA31/17	*R. bergii* × *rigidus*	SA, WC, Grabouw, Highlands Road	235	-34.216	19.055	3/22/2017		n/a	Rig6		1		
RSA32/17	*R. bergii* × *pinnatus*	SA, WC, Kirstenbosch Botanical Garden, 200 m NW of the N entrance, in forest	170	-33.985	18.430	3/23/2017	4×	OL, NBG	Pin1		1	dir. seq.	
RSA33/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Constantia, between Spilhaus Ravine and Constantia Nek, at Contour Path	325	-34.002	18.416	3/26/2017		OL	Pin1	B2		dir. seq.	
RSA34/17	*R. armeniacus*	SA, WC, 450 m NNE of Constantia Nek	254	-34.008	18.408	3/26/2017	4×	OL, NBG	Dol1				

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
RSA35/17	*R. bergii*	SA, WC, Table Mt., Disa Gorge	668	-33.978	18.399	3/26/2017		n/a	Ulm1		1		
RSA36/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Table Mt., Disa Gorge	668	-33.978	18.399	3/26/2017		n/a	Pin1	B2		dir. seq.	
RSA37/17	*R. pinnatus* subsp. *pinnatus*	SA, WC, Table Mt., Disa Gorge	690	-33.978	18.401	3/26/2017		n/a	Pin1				
RSA38/17	*R. bergii*	SA, WC, Table Mt., Cape Town, Woodhead Dam, 60 m W of Waterworks Museum	740	-33.975	18.407	3/26/2017	4×	NBG	Ulm1		1		
RSA39/17	*R. bergii* × *rigidus*	SA, WC, at the road N2 between Patryslaagte and Houwhoek (E of Grabouw)	237	-34.192	19.110	3/29/2017	5×	OL, NBG	Rig3	A	1	cloned	
RSA40/17	*R. rigidus*	SA, WC, at the road R43, 6.2 km NNE of Fisherhaven	11	-34.306	19.146	3/29/2017	6×	OL, NBG	Rig3	A	1	dir. seq.	
RSA41/17	*R. bergii* × *rigidus*	SA, WC, at the road between Sandbaai and Caledon, Creation	244	-34.333	19.331	3/29/2017	5×	OL, NBG	Rig3		1	dir. seq.	
RSA42/17	*R. bergii* × *rigidus*	SA, WC, Genadendal, crossroads on SW margin of the town	231	-34.049	19.553	3/29/2017		OL, NBG	Rig6		1		
RSA43/17	*R. bergii*	SA, WC, Genadendal, bushes 240 m SW of the Moravian Church	257	-34.036	19.556	3/29/2017		OL, NBG	Ulm1		1		
RSA44/17	*R. bergii* × *rigidus*	SA, WC, at the road between Genadendal and Helderstroom (at the river in the middle of the distance)	237	-34.062	19.438	3/29/2017	5×	OL, NBG	Rig6		1	dir. seq.	
RSA45/17	*R. bergii* × *rigidus*	SA, WC, Helderstroom	253	-34.066	19.370	3/29/2017		n/a			1		
RSA47/17	*R. pinnatus* subsp. *pinnatus*	SA, Betty's Bay Botanical Garden, 500 m NW of the entrance	66	-34.348	18.925	4/4/2017	4×	NBG	Pin1	B2		cloned	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
RSA48/17	*R. titanus*	SA, Betty's Bay Botanical Garden, 170 m NW of the entrance	34	-34.351	18.926	4/4/2017	6×	NBG	Urs1			cloned	
MS02/18	*R.* sp. *(North American)*	SA, KZN, Royal Natal, Tugela Valley, 0.4 km SSE of Thendele Upper Camp, secondary/disturbed woodland	1490	-28.713	28.935	2/20/2018	6×	OL	Fla1			cloned	
MS03/18	*R. ludwigii*	SA, KZN, Royal Natal, Tugela Valley, 0.4 km SSE of Thendele Upper Camp, secondary/disturbed woodland	1490	-28.713	28.935	2/20/2018	2×	OL	Lud1			dir. seq.	
MS04/18	*R. rigidus*	SA, KZN, Royal Natal, Tugela Valley, 0.5 km S of Thendele Upper Camp, primary montane grassland/woodland	1530	-28.715	28.935	2/20/2018	6×	OL	Rig4	A	1	dir. seq.	
MS05/18	*R.* sect. *Arguti*	SA, KZN, Howick, 1.5 km W of the city centre, ruderal shrubland at the river	1021	-29.486	30.218	2/22/2018	4×	OL	Arg1				*R. originalis* sensu Beek
MS06/18	*R.* sect. *Arguti*	SA, KZN, top of Beacon Hill, edge of Eucalyptus plantation	1175	-29.471	30.212	2/22/2018	4×	OL	Arg1			cloned	*R. originalis* sensu Beek
MS07/18	*R.* sect. *Cuneifolii*	SA, KZN, top of Beacon Hill, edge of Eucalyptus plantation	1168	-29.471	30.211	2/22/2018	4×	OL	Cun2				
MS08/18	*R. apetalus*	SA, KZN, Karkloof, 0.4 km N of Canopy Tours, primary forest	1224	-29.319	30.261	2/22/2018	4×	OL	Ape2	D		dir. seq.	
MS09/18	*R. pinnatus* subsp. *pappei*	SA, KZN, Karkloof, 0.2 km NW of Canopy Tours, primary forest	1197	-29.321	30.262	2/22/2018	4×	OL	Pin3	B3		cloned	
MS10/18	*R.* sect. *Cuneifolii*	SA, KZN, Karkloof, 0.2 km S of Canopy Tours, edge of dirt road	1155	-29.324	30.263	2/22/2018	4×	OL	Cun2				

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS11/18	*R.* sect. *Cuneifolii*	SA, KZN, 1.25 km NNW of Karkloof Waterfall, edge of Eucalyptus plantation	1073	-29.390	30.273	2/22/2018	4×	OL	Cun2			cloned	
MS12/18	*R.* cf. sect. *Procumbentes* × *rigidus*	SA, KZN, Assagay, Assagay Crescent street, at a road	675	-29.784	30.740	2/23/2018	7×	OL	Rig1				
MS13/18	*R. rigidus*	SA, KZN, Assagay, Assagay Crescent street, at a road	675	-29.784	30.739	2/23/2018	6×	OL, PRE	Rig1	C3	1	cloned	
MS14/18	*R. ellipticus*	SA, KZN, Assagay, Assagay Crescent street, at a road	675	-29.785	30.738	2/23/2018	2×	OL	Eli1			dir. seq.	
MS15/18	*R. ellipticus*	SA, KZN, Assagay, N of Assagay Road, secondary shrubby vegetation near an ornamental garden	672	-29.769	30.750	2/23/2018	2×	OL	Eli1				
MS16/18	*R. rigidus*	SA, KZN, Waterfall town, Brackenhill Rd., roadside/ riverbank	544	-29.746	30.814	2/23/2018	6×	OL	Rig4	C3		dir. seq.	
MS17/18	*R. rigidus*	SA, KZN, N of Hillcrest, Ngwele Rd., roadside	643	-29.752	30.778	2/23/2018	6×	OL, PRE	Rig7	C3	1	dir. seq.	
MS18/18	*R. rigidus*	SA, KZN, E of Camperdown, side of highway	736	-29.731	30.540	2/23/2018	6×	OL, PRE	Rig5	C3		dir. seq.	
MS19/18	*R. ludwigii*	SA, KZN, at road R617, N of Ncwadi	1376	-29.717	29.957	2/24/2018	2×	OL, PRE	Lud1				
MS20/18	*R.* cf. sect. *Alleghenienses*	SA, KZN, at road R617, N of Ncwadi	1376	-29.717	29.957	2/24/2018	6×*	OL	Fla1			cloned	
MS21/18	*R. transvaalensis*	SA, KZN, at road R617, N of Ncwadi	1376	-29.717	29.957	2/24/2018	6×	OL, PRE	Rig5	C1		dir. seq.	
MS22/18	*R. apetalus*	SA, KZN, N part of Marutswa Nature Reserve E of Bulwer, at primary forest margin	1459	-29.808	29.787	2/24/2018	4×	OL	Ape2	D		cloned	
MS23/18	*R.* sect. *Cuneifolii*	SA, KZN, N part of Marutswa Nature Reserve E of Bulwer, at primary forest margin	1470	-29.808	29.786	2/24/2018		OL	Cun2				

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS24/18	*R.* aff. *bergii*	SA, KZN, Ingelabantwana Nature Reserve N of Bulwer, between forest and Eucalyptus plantation	1423	-29.725	29.744	2/24/2018	4×	OL	Ulm2		1		
MS25/18	*R. apetalus*	SA, KZN, Ingelabantwana Nature Reserve N of Bulwer, forest margin	1424	-29.726	29.745	2/24/2018	4×	OL, PRE	Ape2				
MS26/18	*R. rigidus* × sect. *Arguti*	SA, KZN, at the road NE of Ingelabantwana Nature Reserve, N of Bulwer	1409	-29.725	29.749	2/24/2018	5×	OL	Rig4				
MS27/18	*R. ludwigii* × *pinnatus*	SA, KZN, at the road E of Ingelabantwana Nature Reserve, N of Bulwer	1225	-29.734	29.760	2/24/2018	3×	OL	Lud1				
MS28/18	*R.* sect. *Arguti*	SA, KZN, Howick, below the waterfall, in forest gap	907	-29.485	30.239	2/25/2018	4×	OL	Arg1				*R. originalis* sensu Beek
MS29/18	*R. niveus*	SA, KZN, Howick West, junction of Main Rd. and Ogilvie Rd.	1046	-29.509	30.232	2/25/2018	2×	OL	Niv1				
MS30/18	*R. niveus*	SA, KZN, at former railway Merrivale - Howick	1043	-29.502	30.236	2/25/2018	2×	OL	Niv1				
MS31/18	*R.* sect. *Cuneifolii*	SA, KZN, Howick, pasture/meadow W of the town	1038	-29.485	30.204	2/26/2018		OL	Cun2				
MS32/18	*R.* sect. *Arguti*	SA, KZN, Howick, roadside/marsh W of the town	1024	-29.484	30.205	2/26/2018	4×	OL	Arg1				*R. originalis* sensu Beek
MS33/18	*R. trichogynus*	SA, KZN, Howick, N end of Park Rd., garden margin	1037	-29.483	30.226	2/26/2018	7×	OL	Urs1			cloned	
MS34/18	*R. ludwigii*	SA, KZN, Giant's Castle, Langalibalele Ridge, montane grassland	2162	-29.286	29.478	2/28/2018	2×	OL	Lud1				
MS35/18	*R.* sect. *Arguti*	SA, KZN, W of Estcourt, at White Mountain Resort, in secondary Acacia bushveld	1550	-29.107	29.614	3/1/2018	4×	OL	Arg1				*R. revealii* sensu Beek
MS36/18	*R.* sect. *Cuneifolii*	SA, KZN, W of Estcourt, at White Mountain Resort, in secondary Acacia bushveld	1550	-29.107	29.613	3/1/2018	4×	OL	Cun2				

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS37/18	*R. rigidus*	SA, KZN, Drakensberg, 0.5 km S of Injasuti Camp, roadside in montane grassland	1449	-29.123	29.440	3/4/2018	6×	OL	Rig8	A	1	dir. seq.	
MS38/18	*R. ulmifolius*	SA, KZN, in pass 5 km S of Charlestown (near Volksrust), in a small ravine at margin of pine plantation	1680	-27.461	29.873	3/4/2018	2×	OL, PRE	Ulm2			dir. seq.	
MS39/18	*R. ulmifolius*	SA, MP, Volksrust, behind houses in the 1st Ave, at a wall	1707	-27.373	29.867	3/4/2018	2×	OL	Ulm2				
MS40/18	*R. rigidus* × sect. *Arguti*	SA, MP, at road R37 near its junction with R539, pine plantation margin	1082	-25.288	30.766	3/5/2018	5×	OL, PRE	Rig5				
MS41/18	*R. rigidus* × sect. *Arguti*	SA, MP, forest fragment 2.5 km NNW of Hendriksdal	1448	-25.169	30.764	3/5/2018	5×	OL	Rig5				
MS42/18	*R. pinnatus* subsp. *pappei*	SA, MP, forest fragment 2.5 km NNW of Hendriksdal	1448	-25.169	30.764	3/5/2018	4×	OL	Rig6	B1		dir. seq.	
MS43/18	*R. rigidus* × sect. *Arguti*	SA, MP Graskop, forest margin S of Panorama Camp	1438	-24.949	30.846	3/6/2018	8×	OL, PRE	Rig5				
MS44/18	*R. niveus*	SA, MP, Graskop, forest margin S of Panorama Camp	1438	-24.949	30.846	3/6/2018	2×	OL	Niv1				
MS45/18	*R. apetalus* × *pinnatus*	SA, MP, Graskop, forest margin S of Panorama Camp	1435	-24.949	30.846	3/6/2018	4×	OL, PRE	Ape2	B3+D		dir. seq.	
MS45B/18	*R. pinnatus* subsp. *pappei*	SA, MP, Graskop, forest margin S of Panorama Camp	1435	-24.949	30.846	3/6/2018	4×	OL	Rig6	B1		dir. seq.	
MS46/18	*R. rigidus*	SA, MP, Graskop, forest margin on edge of the kloof W of the waterfall	1400	-24.944	30.841	3/6/2018	6×	OL, PRE	Rig5	B1+C2	1	cloned	
MS47/18	*R. rigidus*	SA, MP, Graskop, at road R533 in S part of the town	1421	-24.947	30.843	3/6/2018	6×	OL	Rig5	B1+C2		cloned	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS47B/18	*R. rigidus*	SA, MP, Graskop, at road R533 in S part of the town	1421	-24.947	30.843	3/6/2018	6×	OL	Rig5	B1+C2		dir. seq.	
MS48/18	*R.* sect. *Arguti* × *pinnatus*	SA, MP, Graskop, 300 m SE of Panorama Camp, overgrown pasture/ grassland	1428	-24.949	30.846	3/6/2018	4×	OL, PRE	Rig6	B1		cloned	
MS49/18	*R. rigidus* × sect. *Arguti*	SA, MP, Graskop, 250 m SSW of the waterfall, in secondary shruby vegetation	1414	-24.945	30.839	3/6/2018	5×	OL, PRE	Rig5				
MS50/18	*R. rigidus* × *ulmifolius*	SA, MP, Graskop, 250 m SSW of the waterfall, in secondary shruby vegetation	1414	-24.945	30.839	3/6/2018	4×	OL, PRE	Rig5				
MS51/18	*R. rigidus* × sect. *Arguti*	SA, MP, Graskop, edge of pine plantation at the Pinnacle rock	1443	-24.913	30.852	3/7/2018	5×	OL	Rig5				
MS52/18	*R. rigidus* × *ulmifolius*	SA, MP, Graskop, at the Pinnacle rock, grassland	1439	-24.912	30.853	3/7/2018	4×	OL	Rig5	B1		cloned	
MS53/18	*R. rigidus* × sect. *Arguti*	SA, MP, 7 km N of Graskop, junction of R532 and R534, roadside in pine plantations	1429	-24.868	30.847	3/7/2018	5×	OL	Rig5				
MS54/18	*R. pinnatus* subsp. *pappei*	SA, MP, 3.4 km W of Graskop, 0.9 km S of junction of R532 and R533, roadside in (secondary?) forest	1480	-24.939	30.811	3/7/2018	4×	OL	Rig6	B1		dir. seq.	
MS55/18	*R. rigidus* × sect. *Arguti*	SA, MP, 130 m NE of Mary Shire Falls SW of Graskop, roadside between forest and pine plantation	1261	-24.984	30.812	3/7/2018	5×	OL	Rig5				
MS58/18	*R. ulmifolius*	SA, MP, Graskop, pastures at electric transformer station	1431	-24.935	30.843	3/8/2018	2×	OL	Ulm1				

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS59/18	*R. rigidus* × *ulmifolius*	SA, MP, Graskop, pastures at electric transformer station	1431	-24.935	30.843	3/8/2018	4×	OL	Rig5				
MS60/18	*R. rigidus*	SA, MP, view point 3.4 WNW of Pilgrimsrest, montane grassland	1508	-24.881	30.725	3/8/2018	6×	OL	Rig5	B1+C2		cloned	
MS61/18	*R. apetalus* × *ludwigii*	SA, MP, roadside 14 km SSE of Lydenburg	1740	-25.222	30.478	3/8/2018	3×	OL	Lud1	D		cloned	
MS62/18	*R. transvaalensis*	SA, MP, Buffelskloof Nature Reserve, secondary shrubland 1.6 km S of headquarters	1692	-25.303	30.522	3/8/2018	6×	OL	Rig5	C1		cloned	
MS63/18	*R. apetalus*	SA, MP, Buffelskloof Nature Reserve, secondary shrubland 1.6 km S of headquarters	1692	-25.303	30.522	3/8/2018	4×	OL	Ape2	D		dir. seq.	
MS64/18	*R. pinnatus* subsp. *pappei*	SA, MP, Buffelskloof Nature Reserve, 2.5 km NNW of headquarters, forest	1861	-25.267	30.518	3/9/2018	4×	OL	Rig6				
MS65/18	*R. pinnatus* subsp. *pappei*	SA, MP, Buffelskloof Nature Reserve, 2.5 km NNW of headquarters, forest	1861	-25.267	30.518	3/9/2018	4×	OL	Rig6	B1		cloned	
MS66/18	*R. niveus* × *transvaalensis*	SA, MP, Buffelskloof Nature Reserve, 300 m W of headquarters, riverside	1752	-25.289	30.521	3/9/2018	4×	OL	Niv1	C1		cloned	
MS67/18	*R. apetalus*	SA, MP, Buffelskloof Nature Reserve, N margin of headquarters, grassland	1768	-25.288	30.524	3/9/2018	4×	OL	Ape2				
MS68/18	*R.* sect. *Arguti*	SA, MP, Buffelskloof Nature Reserve, 0.5 km N of headquarters, roadside/ pine plantation margin	1782	-25.284	30.524	3/10/2018	4×	OL	Arg1				*R. originalis* sensu Beek
MS01/20	*R. rigidus*	SA, KZN, Vryheid Hill, 500 m S of North Gun Point, margin between grassland and woodland	1403	-27.743	30.790	3/6/2020	6×	NBG	Rig11	A		dir. seq.	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS02/20	*R.* sect. *Cuneifolii*	SA, KZN, Vryheid Hill, 400 m S of North Gun Point, margin between grassland and woodland	1428	-27.742	30.789	3/6/2020		NBG	Cun2				
MS03/20	*R. pinnatus* subsp. *pappei*	SA, KZN, Vryheid Hill, 400 m S of transmitting tower, grassland/woodland	1443	-27.745	30.794	3/6/2020		OL	Pin3	B3		dir. seq.	
MS04/20	*R. rigidus*	SA, KZN, Vryheid Hill, 900 m SSE of transmitting tower, roadside in forest	1276	-27.749	30.796	3/6/2020	6×	OL	Rig9	A		dir. seq.	
MS05/20	*R. apetalus*	SA, KZN, Vryheid Hill, 900 m SSE of transmitting tower, roadside in forest	1366	-27.749	30.796	3/6/2020		NBG	Ape1	D		dir. seq.	
MS06/20	*R. rigidus* × sect. *Arguti*	SA, KZN, Vryheid, W end of Noord Street, abandoned garden/orchard	1215	-27.757	30.789	3/6/2020	5×	OL	Rig9				
MS07/20	*R. rigidus*	SA, KZN, 6 km N of Melmoth, roadside	743	-28.530	31.402	3/6/2020	6×	OL	Rig5	C3		dir. seq.	
MS09/20	*R.* sect. *Arguti*	SA, KZN, Eshowe, at Fort Nonquai, park margin, potentially originally planted	496	-28.904	31.446	3/7/2020		NBG	Arg1				*R. originalis* sensu Beek
MS100/20	*R. bergii* × *rigidus*	SA, WC, Heidelberg, VanRiebeeck Street, E margin og the town, roadside among pastures	77	-34.092	20.967	3/24/2020	5×	OL	Rig6				
MS101/20	*R. bergii* × *rigidus*	SA, WC, Suurbraak, Tradouw Pass road, bank of Gootvaderbosch River, roadside	129	-34.005	20.707	3/24/2020	5×	OL	Rig6				
MS102/20	*R. bergii* × *rigidus*	SA, WC, Swellendam, at R60, 3 km W of the city centre	119	-34.022	20.415	3/25/2020	5×	OL	Rig6				
MS103/20	*R. bergii* × *rigidus*	SA, WC, Worcester, N2 roadside 10 km NE of the city centre	318	33.567	19.511	3/25/2020	5×	OL	Rig6				

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS104/20	*R. rigidus*	SA, WC, Paarl, at R101, 4.4 km SSE of Du Toitskloof Pass, roadside in fynbos	551	-33.729	19.100	3/25/2020	6×	OL	Rig6			dir. seq.	
MS106/20	*R. thaumasius*	SA, EC, at a road R345, 20 km NNE of Hogsback, roadside/Eucalyptus plantation	1390	-32.438	27.036	3/13/2020	4×	OL	Tha1			dir. seq.	
MS10A/20	*R. rigidus*	SA, KZN, 4 km SE of Eshowe, roadside in rural ladscape	339	-28.916	31.494	3/7/2020	6×	OL		C3		dir. seq.	
MS10B/20	*R. rigidus*	SA, KZN, 4 km SE of Eshowe, roadside in rural ladscape	339	-28.916	31.494	3/7/2020		OL	Rig5	C3		dir. seq.	
MS11/20	*R. rigidus*	SA, KZN, near R102, at a dirt (W) road to Obanjeni, woodland	54	-28.952	31.664	3/7/2020		NBG	Rig7	C3		dir. seq.	
MS12/20	*R. rigidus*	SA, KZN, Mtunzini, uMlalazi, T-junction N of camping place, roadside/ reed bed	4	-28.956	31.768	3/7/2020	6×	OL	Rig5	C3		dir. seq.	
MS13/20	*R. rigidus*	SA, KZN, Mtunzini, Valley Drive, 1.5 km SW of former railway station, roadside between railway and Eucalyptus plantations	17	-28.971	31.747	3/8/2020	6×	OL	Rig5	C3		dir. seq.	
MS14/20	*R. rigidus*	SA, KZN, Shaka's Rock, roadside 600 m SE of N2 e×it 212	78	-29.506	31.215	3/8/2020	6×	OL	Rig10	C3		dir. seq.	
MS15/20	*R. rigidus*	SA, KZN, Shaka's Rock, roadside 500 m SE of N2 e×it 212	81	-29.505	31.214	3/8/2020	6×	OL	Rig10	C3		dir. seq.	
MS16/20	*R. pinnatus* subsp. *pappei*	SA, KZN, 21 km NNW of Tongaat, R614 roadside among fields	632	-29.409	30.801	3/8/2020		OL	Pin3	B3		dir. seq.	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS17/20	*R. rigidus*	SA, KZN, 21 km NNW of Tongaat, R614 roadside among fields	632	-29.395	31.018	3/8/2020	6×*	OL	Rig5	C3		dir. seq.	
MS18/20	*R. rigidus* × sect. *Arguti*	SA, KZN, 19 km ENE of Wartburg, R614 roadside among fields	1001	-29.371	30.757	3/8/2020		OL	Rig1				
MS19/20	*R. rigidus*	SA, KZN, 19 km ENE of Wartburg, R614 roadside among fields	1001	-29.371	30.759	3/8/2020	6×	NBG	Rig5	C3		dir. seq.	
MS20/20	*R. rigidus*	SA, KZN, 19 km ENE of Wartburg, R614 roadside among fields	1008	-29.371	30.756	3/8/2020	6×	OL	Rig5	C3		dir. seq.	
MS21/20	*R. rosifolius*	SA, KZN, Queen Elizabeth Park NW of Pietermaritzburg, N part of the reserve, bushes in abandoned plantations in a small valley	914	-29.562	30.317	3/9/2020	2×	OL	Ros1			dir. seq.	
MS23/20	*R.* aff. *bergii*	SA, KZN, Hilton, N end of Hayfields Road, heavily invaded anthropogenic habitats (R. aff. bergii, R. sect. Cuneifolii, R. sect. Arguti)	1119	-29.543	30.296	3/9/2020	4×*	NBG	Ulm2				
MS28/20	*R.* sect. *Arguti*	SA, KZN, 8 km WNW of Richmond, at a dirt road in Eucalyptus plantations	970	-29.854	30.198	3/10/2020		OL	Arg1				*R. originalis* sensu Beek
MS29/20	*R. rigidus*	SA, KZN, Mkomazi River Valley, 300 m N of Hela Hela camp	555	-29.906	30.098	3/10/2020	6×	OL	Rig9	B1+C3		dir. seq.	
MS30/20	*R. niveus*	SA, KZN, right bank of Mkomazi River, 400 m WNW of Hela Hela camp	553	-29.908	30.094	3/10/2020		NBG	Niv1			dir. seq.	
MS31/20	*R. ludwigii*	SA, KZN, E of Kokstad, at the road P570, 1 km from junction with N2, bushes among plantations	1286	-30.514	29.650	3/10/2020		OL	Lud1			dir. seq.	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS32/20	*R. rigidus*	SA, KZN, E of Kokstad, at the road P570, 1 km from junction with N2, bushes among plantations	1286	-30.514	29.650	3/10/2020	6×	NBG	Rig4	A		dir. seq.	
MS35/20	*R.* sect. *Cuneifolii*	SA, EC, Wild Coast, Mbizana, 1.2 km NNW of R394 and R61 junction	1008	-30.857	29.604	3/11/2020	4×*	OL	Cun2				
MS36/20	*R. pinnatus* subsp. *pappei*	SA, EC, Wild Coast, Lusikisiki area, 4.8 km NE of Magwa Falls, woodland	505	-31.416	29.676	3/11/2020		NBG	Rig6	B3		dir. seq.	
MS37/20	*R. rigidus*	SA, EC, Wild Coast, Lusikisiki area, 4.8 km NE of Magwa Falls, woodland	505	-31.416	29.676	3/11/2020	6×	NBG	Rig9	A		dir. seq.	
MS38/20	*R. pinnatus* subsp. *pappei*	SA, EC, Wild Coast, Port St John's area, right bank of the Bulolo River, 950 m N of its mouth	-10	-31.643	29.515	3/12/2020		NBG	Rig6	B3		dir. seq.	
MS39/20	*R. rigidus*	SA, EC, Wild Coast, Port St John's area, at a dirt road to Sileka Nature Reserve, 1 km NW of the Bulolo River Mouth	94	-31.648	29.508	3/12/2020	6×*	NBG	Rig4	A		dir. seq.	
MS40/20	*R. rigidus*	SA, EC, 3.5 km W of Komgha, R63 roadside	680	-32.587	27.856	3/13/2020	6×	OL	Rig9				
MS41/20	*R. rigidus*	SA, EC, Fort Cunynghame Forestry Station, 9 km N of Stutterheim, grasslands among tree plantations	973	-32.488	27.421	3/13/2020	6×	OL	Rig4	A		dir. seq.	
MS42/20	*R. pinnatus* subsp. *pappei*	SA, EC, Fort Cunynghame Forestry Station, 9 km N of Stutterheim, grasslands among tree plantations	971	-32.487	27.421	3/13/2020		NBG	Rig6	B3		dir. seq.	
MS43/20	*R. pinnatus* subsp. *pappei*	SA, EC, Fort Cunynghame, 10.5 km N of Stutterheim, roadside among tree plantations	1056	-32.475	27.413	3/13/2020		OL	Rig6	B3		dir. seq.	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS44/20	*R. bergii*	SA, EC, at a road R345, 15 km N of Hogsback, roadside/small ravine	1374	-32.466	26.965	3/13/2020	4×*	OL	Ulm1				
MS45/20	*R. rigidus*	SA, EC, at a road R345, 15 km N of Hogsback, roadside/small ravine	1371	-32.465	26.965	3/13/2020	6×*	OL	Rig4	A		dir. seq.	
MS46/20	*R. apetalus*	SA, EC, 1 km SE of Hogsback, forest gap	973	-32.602	26.947	3/14/2020	4×*	NBG	Ape2				
MS47/20	*R.* sect. *Arguti*	SA, EC, 900 m E of Hogsback, in tree plantations	1144	-32.598	26.948	3/14/2020	4×	OL	Arg1				*R. revealii* sensu Beek
MS48/20	*R. armeniacus*	SA, EC, 900 m E of Hogsback, stream bank in tree plantations	1137	-32.597	26.948	3/14/2020		NBG	Dol1			cloned	
MS49/20	*R. bergii* × *rigidus*	SA, EC, 900 m E of Hogsback, stream bank in tree plantations	1144	-32.595	26.947	3/14/2020	5×	OL	Rig9				
MS50/20	*R. thaumasius*	SA, EC, Hogsback, arboretum, stream bank	1230	-32.591	26.936	3/14/2020	4×	OL	Tha1			dir. seq.	
MS51/20	*R. trichogynus*	SA, EC, Hogsback, NW margin of the town, park/ garden	1275	32.592	26.930	3/14/2020	7×	OL	Urs1				
MS52/20	*R. bergii* × *thaumasius*	SA, EC, Hogsback, Hydrangea Lane, roadside	1178	-32.598	26.940	3/14/2020	4×	OL	Tha1				
MS53/20	*R. bergii* × *thaumasius*	SA, EC, Hogsback, Hydrangea Lane, roadside	1201	-32.599	26.941	3/14/2020	4×	OL	Tha1			cloned	
MS55/20	*R. armeniacus*	SA, EC, Hogsback, S margin of the town, Away With The Fairies camp garden	1163	-32.602	26.939	3/15/2020	4×*	NBG	Dol1				
MS57/20	*R. thaumasius*	SA, EC, Hogsback, arboretum, among bushes in tree plantations	1232	-32.590	26.937	3/15/2020	4×*	OL	Tha1			cloned	
MS58/20	*R. pinnatus* × *thaumasius*	SA, EC, Hogsback, arboretum, among bushes in tree plantations	1232	-32.590	26.937	3/15/2020		OL	Tha1				

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS59/20	*R. bergii* × *thaumasius*	SA, EC, Hogsback, arboretum, among bushes in tree plantations	1200	-32.590	26.933	3/15/2020	4×	OL	Tha1			cloned	
MS60/20	*R. rigidus* × sect. *Arguti*	SA, EC, Hogsback, arboretum, among bushes in a park	1208	-32.590	26.935	3/15/2020		OL	Rig9				
MS61/20	*R. phoenicolasius*	SA, EC, Hogsback, 200 m NW of the entrance to arboretum, roadside/ margin of plantation	1221	-32.592	26.934	3/15/2020	2×	OL	Phe1			dir. seq.	
MS62/20	*R. bergii* × *rigidus*	SA, EC, Hogsback, 130 m SE of the entrance to arboretum, roadside/ margin of plantation	1213	-32.594	26.937	3/15/2020		OL	Rig4				
MS63/20	*R.* sect. *Arguti (different* sp.*!)*	SA, EC, Hogsback, 350 m SE of the entrance to arboretum, garden hedge	1229	-32.595	26.938	3/15/2020		OL	Arg1				
MS64/20	*R. thaumasius*	SA, EC, Hogsback, S margin of the town, Away With The Fairies camp garden	1200	-32.603	26.939	3/16/2020	4×	OL	Tha1				
MS65/20	*R. rigidus*	SA, EC, Makhanda (Grahamstown), 2.1 km WSW of Albany Museum, grassland	654	-33.318	26.500	3/16/2020	6×*	OL	Rig4	A		dir. seq.	
MS66/20	*R. pinnatus* subsp. *pappei*	SA, EC, Makhanda (Grahamstown), 2.1 km WSW of Albany Museum, grassland	644	-33.318	26.500	3/16/2020		OL	Rig6	B3		dir. seq.	
MS67/20	*R. rigidus*	SA, EC, Makhanda (Grahamstown), 5.3 km SE of Albany Museum, roadside in thickets	346	-33.350	26.560	3/17/2020		OL	Rig4	A		dir. seq.	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS68/20	*R. pinnatus* subsp. *pappei*	SA, EC, Boesmanriviermond (Kenton on Sea area), 2.1 km WSW of the town cente, among bushes in pastures	27	-33.689	26.632	3/18/2020	4×	OL	Rig6	B3		dir. seq.	
MS69/20	*R. rigidus*	SA, EC, Cannon Rocks (Alexandria area), 15 km WSW of the Boknes River mouth, roadside in grassland/pastures	176	-33.754	26.428	3/18/2020	6×	NBG	Rig4				
MS70/20	*R. rigidus*	SA, EC, Alexandria, at the main facilities of Addo Woody Cape Section of Addo Elephant NP, grassland	176	-33.700	26.367	3/18/2020		OL	Rig6	A		dir. seq.	
MS71/20	*R. rigidus*	SA, EC, Groendal, Lower Blindekloof hiking trail, riverbank in forest	115	-33.704	25.302	3/19/2020	6×*	OL	Rig4	A		dir. seq.	
MS72/20	*R. pinnatus* subsp. *pappei*	SA, EC, Uitenhage area, 3.4 km NE of Witteklip Railway Station, R334 roadside	266	-33.892	25.292	3/20/2020		OL	Rig6	B3		dir. seq.	
MS73/20	*R. rigidus*	SA, EC, Uitenhage area, 3.4 km NE of Witteklip Railway Station, R334 roadside	266	-33.892	25.292	3/20/2020	6×*	OL	Rig4	A		dir. seq.	
MS74/20	*R. pinnatus* subsp. *pappei*	SA, EC, left bank of Van Stadens River at R102, park/forest	94	-33.912	25.196	3/20/2020		OL	Rig6	B3		dir. seq.	
MS75/20	*R. bergii* × *rigidus*	SA, EC, Humansdorp, Voortrekker Road, NE of hospital, roadside/park/pasture	127	-34.028	24.783	3/20/2020	5×	OL	Rig4				
MS76/20	*R. pinnatus* subsp. *pappei*	SA, EC, Humansdorp, at R102, 4 km WSW of the town centre	107	-34.034	24.728	3/20/2020		OL	Rig6	B3		dir. seq.	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS77/20	*R. bergii* × *rigidus*	SA, EC, Humansdorp, at R102, 4 km WSW of the town centre	107	-34.034	24.728	3/20/2020		OL	Rig4				
MS78/20	*R. bergii*	SA, EC, left bank of Elands River, at R102, roadside	229	-33.982	24.051	3/20/2020		OL	Ulm1				
MS79/20	*R. bergii* × *rigidus*	SA, EC, left bank of Elands River, at R102, roadside	229	-33.982	24.051	3/20/2020		NBG	Rig4				
MS80/20	*R. pinnatus (transitional)*	SA, EC, right bank of Elands River, at R102, roadside/plantation	230	-33.981	24.050	3/20/2020	4×*	OL		B2		dir. seq.	
MS81/20	*R. pinnatus (transitional)*	SA, EC, right bank of Elands River, at R102, 150 S of entrance to Wolf Sanctuary, roadside/pasture	237	-33.982	24.048	3/20/2020	4×*	OL	Rig6	B1		dir. seq.	
MS82/20	*R. rigidus*	SA, EC, right bank of Elands River, at R102, 150 S of entrance to Wolf Sanctuary, roadside/pasture	237	-33.982	24.048	3/20/2020		NBG	Rig4	A		dir. seq.	
MS83/20	*R. pinnatus (transitional)*	SA, EC, Storms River Mouth, at the main gate, forest/park	201	-34.011	23.869	3/21/2020	4×*	OL	Pin1	B1		dir. seq.	
MS84/20	*R. pinnatus (transitional)*	SA, WC, Nature's Valley, left bank of Groot River, at R102, roadside among forests	17	-33.967	23.560	3/21/2020		OL	Pin4	B1		dir. seq.	
MS85/20	*R. trichogynus*	SA, WC, rest place at N2 in the middle between Plettenberg Bay and Knysna, forest margin	265	-34.038	23.215	3/21/2020	7×	OL	Urs1				
MS86/20	*R. bergii* × *pinnatus*	SA, WC, Knysna Forest, 400 m W of King Edward VII Big Tree, roadside in forest	403	-33.957	23.148	3/22/2020		NBG	Rig6				
MS87/20	*R. pinnatus (transitional)*	SA, WC, Knysna Forest, 1.2 km W of King Edward VII Big Tree, roadside in forest	446	-33.958	23.140	3/22/2020	4×*	OL	Pin1	B1		dir. seq.	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS89/20	*R. bergii* × *pinnatus*	SA, WC, Knysna Forest, 800 m NNW of King Edward VII Big Tree, roadside in forest/old forest clearing	506	-33.950	23.148	3/22/2020		OL	Pin1				
MS90/20	*R. rigidus*	SA, WC, Knysna, S end of Gardiners Road, edge of plantation, stream bank	79	-34.031	23.047	3/22/2020	6×*	OL	Rig4	A		dir. seq.	
MS92/20	*R. bergii* × *rigidus*	SA, WC, Knysna, continuation of Lakeview Street, former plantation/ garden	50	-34.033	23.053	3/22/2020	5×	OL	Rig4				
MS93/20	*R. bergii* × *rigidus*	SA, WC, Knysna area, Rheenendal Road, 5 km NNE of junction with N2, roadside among plantations	186	-33.992	22.985	3/23/2020		OL	Rig4				
MS94/20	*R. rigidus*	SA, WC, Sedgefield area, Seven Passes Road, 4 km E of Karatara, roadside among pastures	235	-33.918	22.882	3/23/2020	6×*	OL	Rig4	A		dir. seq.	
MS95/20	*R. pinnatus* subsp. *pinnatus*	SA, WC, Sedgefield area, Seven Passes Road, T-junction 1.5 km SW of Karatara, roadside	213	-33.928	22.825	3/23/2020		OL	Pin1	B1		dir. seq.	
MS96/20	*R. bergii* × *rigidus*	SA, WC, Sedgefield area, Barrington Link Road, 500 m NNW of junction with N2	6	-34.020	22.852	3/23/2020		OL	Rig6				
MS97/20	*R. rigidus*	SA, WC, Mosselbay area, at R327, 7.7 km NW of its junction with N2, roadside among grasslands	224	-34.141	21.948	3/24/2020	6×	OL	Rig4	A		dir. seq.	
MS98/20	*R. pinnatus* subsp. *pinnatus*	SA, WC, Riversdale area, at R323, 11 km NWN of Riversdale, roadside among plantations	416	-33.995	21.225	3/24/2020	4×	OL	Pin1	B2		dir. seq.	
MS-Pin1	*R. pinnatus (transitional)*	SA, WC, E of Knysna, Bitou Municipality	264	-34.038	23.215	3/21/2020		n/a	Rig6	B1		dir. seq.	

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
MS-Pin2	*R. pinnatus (transitional)*	SA, WC, Storms River Mouth	106	-34.018	23.868	3/21/2020		n/a	Pin1	B1		dir. seq.	
MS-Pin3	*R. pinnatus (transitional)*	SA, WC, Knysna Forest	364	-33.947	23.142	3/22/2020		n/a	Pin1	B1		dir. seq.	
Young 2620	*R. rosifolius*	SA, KZN, road from Kranskop to Tugela River, 11.5 km from Kranskop				5/9/2017		NU	Ros1				
Beek2019.97	*R. rigidus*	SA, WC, Tulbagh, Winterhoek road	220	-33.241	19.144	11/21/2019	6×*	L		A		dir. seq.	*R. ecklonii* sensu Beek, epitype
Beek 2020.07	*R. rigidus*	SA, WC, along the R102 between Groot Brakrivier and George	160	-34.011	22.298	1/13/2020	6×*	L	Rig4	A		dir. seq.	*R. fertilis* A. Beek, holotype
Beek 2019.4	*R. rigidus*	SA, WC, Stellenbosch, Jonkershoek reserve, along the circular drive ± 1.5 or 2 km after the bridge at the right side	330	-33.987	18.964	1/23/2019	6×*	L	Rig6				*R.* cf. *chrysocarpus* "anthraco-carpus"
Beek 2019.39	*R. rigidus*	SA, WC, Stellenbosch, Jonkershoek Reserve, along the left turn off along the river from the circular drive, beginning short after the dam	290	-33.983	18.951	2/24/2019	6×*	L	Rig6	A		dir. seq.	*R. chrysocarpus* sensu Beek
Beek 2020.11	*R. pinnatus* subsp. *pappei*	SA, EC, Uitenhage, Van Stadenspas	275	-33.881	25.308	1/15/2020	4×*	L	Rig6				*R. anas* A.Beek, holotype
Beek 2019.100	*R. rigidus*	SA, WC, Grabouw, Vyeboom, along the R321	320	-34.036	19.174	11/28/2019	6×*	L	Rig3				*R. rigidus* sensu Beek, epitype
Beek 2019.94	*R. rigidus*	SA, WC, Citrusdal, south of Theerivier	240	-32.837	19.083	11/20/2019	6×*	L	Rig6	A		dir. seq.	*R. leptodytus* A.Beek
Beek 2020.05	*R. rigidus*	SA, WC, along the road from Swellendam	130	-34.012	20.620	1/13/2020	6×*	L	Rig4	A		dir. seq.	*R. mundtii* sensu Beek
Manning 3601	*R.* sect. *Arguti*	SA, KZN, Howick						NBG	Arg1				*R. originalis* sensu Beek

Supplementary Table S1. Details on the analysed material, including the assigned haplotypes and ribotypes (the latter for *R. rigidus* and *R. pinnatus* only) (continued)

Collection No.	Species	Locality	Elevation	GPS	GPS	Date	Ploidy	Voucher in	Haplotype	Ribotype (pinnatus–rigidus group)	Used for SSR	ITS analysis	Note
Manning 3602	*R. rigidus*	SA, KZN, Howick						NBG	Rig9	A+C3		dir. seq.	
Beek2018-15	*R. bergii* × *rigidus*	SA, WC, Stellenbosch, Helshoogte, entrance of Hillcrest	280	-33.912	18.942	2/24/2018		L	Rig2		1		
R151/11	*R. bergii*	Germany, Lower Sa×ony, Voltlage	50	52.430	7.753			OL	Ulm1		1		
VŽ-Vig1	*R. bergii*	Germany, Hessen, NE Jügesheim, Rodfeld umweit des Reitplatzes	135	50.035	8.903	8/27/2009		Žíla	Ulm1		1		
VŽ-Vig2	*R. bergii*	Germany, Hessen, SW Urberach, Thomashüttenschneise	195	49.956	8.773	8/26/2009		Žíla	Ulm1		1		
VŽ-Vig3	*R. bergii*	Germany, Hessen, S Mernes, grosses Feldkreuz und Scheune	290	50.225	9.488	10/1/2007		Žíla	Ulm1		1		
VŽ-Vig4	*R. bergii*	Germany, Hessen, Babenhausen, Staatstr.	125	49.980	8.929	9/3/2006		Žíla			1		
VŽ-Vig5	*R. bergii*	Germany, Lower Sa×ony, oppidum Recke, pagus Voltlage	40	52.430	7.753	8/16/2011		Žíla	Ulm1		1		

* analysed via FCSS from seed(s)

Supplementary Table S2. Primer sequences, PCR conditions and number of observed alleles for the used SSR loci

Working name	Original name	Sequence of F primer (5′-3′)	Sequence of R primer (5′-3′)	Ta [°C]	Cycles	Reference	No. of alleles
RUB4	Rubusr47a	(M13)-AAGCAGGACACCTCAGATGC	CAGCCAACCATCATCAGCTA	59	25+9	Graham et al. (2004)	12
RUB5	Rubusr76b	(M13)-CTCACCCGAAATGTTCAACC	GGCTAGGCCGAATGACTACA	63	24+9	Graham et al. (2004)	4
RUB6	Rubus105b	(M13)-GAAAATGCAAGGCGAATTGT	TCCATCACCAACACCACCTA	59	24+9	Graham et al. (2004)	16
SSR05	Rubus275a	(M13)-CACAACCAGTCCCGAGAAAT	CATTTCATCCAAATGCAACC	51	33	Graham et al. (2004)	18
SSR27	Rubus26a	(M13)-AACACCGGCTTCTAAGGTCT	GATCCTGGAAAGCGATGAAA	53	32	Graham et al. (2004)	11
SSR17	Rub236b	(M13)-TGTGCCAAGAACTCATCGTC	CCGAACGCCTCCCTACTT	62	25+9	Graham et al. (2006)	20
SSR13	ERubLR_SQ01_G16	(M13)-GCACCCTAATCTCCATGACC	CCGCTGTAGTTCCTGTAGGC	59	22+9	Woodhead et al. (2008)	6
SSR14	RubPara_SQ005_K23	(M13)-AGGTGAGGTGGGAGATGATG	ATCCTCGGTTCCTCCAAAAT	63	25+9	Woodhead et al. (2008)	3
SSR18	Rub238h	(M13)-GTGACCTCCGAGAGCTTGAG	CTTCACCGCCACTAGTACCC	63	25+9	Woodhead et al. (2008)	9
RUB7*	Rubus123a	(M13)-CAGCAGCTAGCATTTTACTGGA	GCACTCTCCACCCATTTCAT	62	26+10	Graham et al. (2004)	1

* Excluded from final analysis

Supplementary Table S3. Samples used for SSR analyses, allelic data and genotype assignment as determined by GENOTYPE

Taxon/hybrid	Collection code	Locality	Latitude	Longitude	Genotype assignment at different thresholds						Detected alleles	
					Th=0	Th=1	Th=2	Th=3	Th=4	Th=5	Rub5 = Rubusr76b	Rub6 = Rubus105b
R. bergii × *pinnatus*	RSA05/17	SA, WC, Newlands, Riverside Road	-33.985	18.445	1	1	1	1	1	1	169+209+221	163+167+181+193
R. bergii × *pinnatus*	RSA09/17	SA, WC, Kirstenbosch arboretum, SW margin	-33.989	18.437	1	1	1	1	1	1	169+209+221	163+167+181+193
R. bergii × *pinnatus*	RSA11/17	SA, WC, Kirstenbosch arboretum, central part	-33.988	18.440	1	1	1	1	1	1	169+209+221	163+167+181+193
R. bergii × *pinnatus*	RSA15/17	SA, WC, at the road Kirstenbosch - Hout Bay	-34.007	18.418	2	2	2	2	2	2	209	163+169+181+193
R. bergii × *pinnatus*	RSA32/17	SA, WC, Kirstenbosch Bot. Garden, 200 m NW of the N entrance	-33.985	18.430	1	1	1	1	1	1	169+209+221	163+167+181+193
R. pinnatus	RSA04/17	SA, WC, Table Mt., Woodhead Dam	-33.975	18.407	3	3	3	3	3	3	209	163+181
R. pinnatus	RSA06/17	SA, WC, Rondebosch, 300 m NW of the Rhodes Memorial	-33.951	18.456	4	4	4	4	4	4	209	163+181
R. pinnatus	RSA13/17	SA, WC, Hout Bay, NE end of the town	-34.015	18.384	5	5	5	5	5	5	209	163+181
R. rigidus × *pinnatus*	RSA27/17	SA, WC, at the road R44 (Kleinmond - Bot River)	-34.304	19.135	6	6	6	6	6	6	209	163+173+181+200
R. rigidus	RSA40/17	SA, WC, at the road R43, 6.2 km NNE of Fisherhaven	-34.306	19.146	7	7	7	7	7	7	209	163+173+202
R. rigidus	MS04/18	SA, KZN, Royal Natal, Tugela Valley	-28.715	28.935	8	8	8	8	8	8	209	163+173+196
R. rigidus	MS37/18	SA, KZN, Drakensberg, 0.5 km S of Injasuti Camp	-29.123	29.440	9	9	9	9	9	9	209	163+173+198
R. rigidus	MS46/18	SA, M, Graskop, edge of the kloof	-24.944	30.841	10	10	10	10	10	10	209+215	163+177+198
R. rigidus	MS13/18	SA, KZN, Assagay	-29.784	30.739	11	11	11	11	11	11	209+215	163+177+200+204
R. rigidus	MS17/18	SA, KZN, N of Hillcrest, Ngwele Rd.	-29.752	30.778	12	12	12	12	12	12	209	163+171+177+179+193
R. bergii × *rigidus*	RSA20/17	SA, WC, Kylemore	-33.912	18.944	13	13	13	13	13	13	209+221	163+167+185+198
R. bergii × *rigidus*	RSA21/17	SA, WC, between Simondium and Drakenstein	-33.858	18.972	14	14	14	14	14	14	209	163+173+185+198
R. bergii × *rigidus*	RSA28/17	SA, WC, at the road R44 (Kleinmond - Bot River)	-34.304	19.135	15	15	15	15	15	15	209+221	163+167+173+181+185
R. bergii × *rigidus*	RSA30/17	SA, WC, Elgin Valley,10 km NNE of Kleinmond	-34.254	19.054	16	16	16	16	16	16	209+221	163+173+193

Detected alleles						
SSR5 = Rubus275a	**SSR17 = Rub236b**	**SSR27 = Rubus26a**	**SSR18 = Rub238h**	**SSR14 = RubPa-ra_SQ005_K23**	**Rub4 = Rubusr47a**	**SSR13 = ERubLR_SQ01_G16**
140+160+248	165+199	176	145+154+156+163	215+244	233+237+244+250	222
140+160+248	165+199	176	145+154+156+163	215+244	233+237+244+250	222
140+160+248	165+199	176	145+154+156+163	215+244	233+237+244+250	222
144+160+178+248	161+180+197	162	145+154+156+163	215+244	223+233+244	222
140+160+248	165+199	176	145+154+156+163	215+244	233+237+244+250	222
140+164	161+199	160	145+154	215	244	222
140+144+168	161+165+197+201	160+176	145+151+154	215	233+244	222
144+162+164	201	158	145+154	215	244	222
134+152+162	167+188	158+182	145+151	215+242	233+244	222+231
134+162	167	158	145	215+242	233+244	231
138+148+156	165+174	148	145+151+156	215+242	221+235+244	231
134+148+158	167+178+180	148	145+151+156	215+242	221+235+244	231
146+152+158	163+184+188	156+178	145+149+156	244	235+241+246	233+235
128+238+144+152	187+189	158	145+156	215	233+235+244	233
128+140+152	165+191+203	154+158	145+148+156	215	235+239+244	233
148+160	167+176+180	158	145+156+163	215+242+244	231+235+237+244	231
148+162+178+248	167+172+180	158	145+156	215+242+244	223+233+235+237+244	231
134+160	167+180+186	158	145+163	215+242+244	231+233+244	231
136+160+178	167+186	158	145+156	242+244	231+233+244	231

Supplementary Table S3. Samples used for SSR analyses, allelic data and genotype assignment as determined by GENOTYPE (continued)

Taxon/hybrid	Collection code	Locality	Latitude	Longitude	Genotype assignment at different thresholds						Detected alleles	
					Th=0	Th=1	Th=2	Th=3	Th=4	Th=5	Rub5 = Rubusr76b	Rub6 = Rubus105b
R. bergii × *rigidus*	RSA31/17	SA, WC, Grabouw, Highlands Road	-34.216	19.055	17	17	17	17	15	15	209+221	163+167+173+181+185
R. bergii × *rigidus*	RSA39/17	SA, WC, between Patryslaagte and Houhoek	-34.192	19.110	18	18	18	18	17	17	209	163+173+185+200
R. bergii × *rigidus*	RSA41/17	SA, WC, between Sandbaai and Caledon, Creation	-34.333	19.331	19	19	19	19	18	18	209+221	163+167+173+185+204
R. bergii × *rigidus*	RSA42/17	SA, WC, SW margin of Genadendal	-34.049	19.553	20	20	20	20	19	19	209+221	163+173+185+200
R. bergii × *rigidus*	RSA44/17	SA, WC, between Genadendal and Helderstroom	-34.062	19.438	21	21	21	21	20	20	209+221	163+167+171+193+200
R. bergii × *rigidus*	RSA45/17	SA, WC, Helderstroom	-34.066	19.370	22	22	22	22	21	21	209+221	163+173+193+200
R. bergii × *rigidus*	Beek2018.15	SA, WC, Stellenbosch	-33.912	18.942	23	23	23	23	22	22	209	163+167+173+185+198
R. bergii	RSA29/17	SA, WC, ca 8.7 km NE of Kleinmond, Elgin Valley	-34.280	19.086	24	24	24	24	23	23	209+221	167+185+193
R. bergii	RSA35/17	SA, WC, Table Mt., Disa Gorge	-33.978	18.399	24	24	24	24	23	23	209+221	167+185+193
R. bergii	RSA38/17	SA, WC, Table Mt., Woodhead Dam	-33.975	18.407	25	25	24	24	23	23	209+221	167+185+193
R. bergii	RSA43/17	SA, WC, Genadendal	-34.036	19.556	26	26	24	24	23	23	209+221	167+185+193
R. bergii	RSA08/17	SA, WC, Rondebosch, 750 m W of the Rhodes Memorial	-33.953	18.451	24	24	24	24	23	23	209+221	167+185+193
R. bergii	RSA16/17	SA, WC, Stellenbosch, Devonvallei	-33.948	18.819	24	24	24	24	23	23	209+221	167+185+193
R. bergii	RSA17/17	SA, WC, Stellenbosch, base of Pappegaaiberg	-33.940	18.845	24	24	24	24	23	23	209+221	167+185+193
R. bergii	RSA19/17	SA, WC, Kylemore, at the road Stellenbosch - Pniel	-33.912	18.944	24	24	24	24	23	23	209+221	167+185+193
R. bergii	RSA22/17	SA, WC, between Simondium and Cillie	-33.816	18.951	24	24	24	24	23	23	209+221	167+185+193
R. bergii	RSA23/17	SA, WC, Paarlsberg	-33.735	18.947	24	24	24	24	23	23	209+221	167+185+193
R. bergii	RSA24/17	SA, WC, Paarlsberg, under Victoria Dam	-33.759	18.948	24	24	24	24	23	23	209+221	167+185+193
R. bergii	R151/11	Germany, Lower Saxony, Voltlage	52.430	7.753	24	24	24	24	23	23	209+221	167+185+193
R. bergii	VŽ-Vig1	Germany, Hessen, Rotgau	50.035	8.903	24	24	24	24	23	23	209+221	167+185+193
R. bergii	VŽ-Vig2	Germany, Hessen, Urberach	49.956	8.773	24	24	24	24	23	23	209+221	167+185+193

Detected alleles						
SSR5 = Rubus275a	**SSR17 = Rub236b**	**SSR27 = Rubus26a**	**SSR18 = Rub238h**	**SSR14 = RubPa-ra_SQ005_K23**	**Rub4 = Rubusr47a**	**SSR13 = ERubLR_SQ01_G16**
134+160+178+248	167+186	158	145+156+163	215+242+244	231+233+244	231
134+162+178	167+184	158	145+163	215+242+244	233+237+244	231
134+162+248	167+188	158	145+156+163	215+242+244	231+233+235+237+244	231
134+160+178	167+174	158	145+156+163	215+242+244	233+237+244	231
148+160	167+174+180	162	135+145+156+163	215+242+244	223+231++233+244	225
152+160	167+174+180	138	135+145+156+163	215+242+244	231+235+237+244	231
158+162+178	165+176	158	145+156+163	215+242+244	233+235+237+244	231
160+178+248	180	null	156+163	244	223+233+237	null
160+178+248	180	null	156+163	244	223+233+237	null
160+178+248	182	null	156+163	244	223+233+237	null
162+178+248	180	null	156+163	244	223+233+237	null
160+178+248	180	null	156+163	244	223+233+237	null
160+178+248	180	null	156+163	244	223+233+237	null
160+178+248	180	null	156+163	244	223+233+237	null
160+178+248	180	null	156+163	244	223+233+237	null
160+178+248	180	null	156+163	244	223+233+237	null
160+178+248	180	null	156+163	244	223+233+237	null
160+178+248	180	null	156+163	244	223+233+237	null
160+178+248	0	null	156+163	244	223+233+237	null
160+178+248	180	null	156+163	244	223+233+237	null
160+178+248	180	null	156+163	244	223+233+237	null

Supplementary Table S3. Samples used for SSR analyses, allelic data and genotype assignment as determined by GENOTYPE (continued)

Taxon/hybrid	Collection code	Locality	Latitude	Longitude	Genotype assignment at different thresholds						Detected alleles	
					Th=0	Th=1	Th=2	Th=3	Th=4	Th=5	Rub5 = Rubusr76b	Rub6 = Rubus105b
R. bergii	VŽ-Vig4	Germany, Hessen, Babenhausen	49.980	8.929	24	24	24	24	23	23	209+221	167+185+193
R. bergii	VŽ-Vig5	Germany, Lower Saxony, Voltlage	52.430	7.753	27	27	24	24	23	23	209+221	167+185+193
R. aff. bergii	MS24/18	SA, KZN, Ingelabantwana Forest, N of Bulwer	-29.725	29.744	28	28	25	25	24	24	209	165+169+193

Detected alleles						
SSR5 = Rubus275a	**SSR17 = Rub236b**	**SSR27 = Rubus26a**	**SSR18 = Rub238h**	**SSR14 = RubPa-ra_SQ005_K23**	**Rub4 = Rubusr47a**	**SSR13 = ERubLR_SQ01_G16**
160+178+248	180	null	156+163	244	223+233+237	null
160+178+248	176	null	156+163	244	223+233+237	null
138+148+160	165+172	null	156+163+165	244	215+233+244	null

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First record of *Amaranthus crassipes* subsp. *warnockii* (I.M.Johnst.) N.Bayón (Amaranthaceae) outside of the Americas, with nomenclatural notes

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Background: The genus *Amaranthus* is taxonomically complex because of its high morphological variability, which led to nomenclatural confusion, misapplication of names and misidentifications. Unfortunately, floristic and taxonomic studies on this genus are still incomplete. A population of *Amaranthus crassipes* subsp. *warnockii* was discovered in Monastir Governorate, Tunisia, representing the first record for both the Tunisian and the African floras, as well as the first one outside of its American native distribution area.

Objectives: The main aim of the present study was to record *Amaranthus crassipes* subsp. *warnockii* (I.M.Johnst.) N.Bayón in Tunisia and Africa for the first time. Morphological characters and ecological data were provided. Clarification about the typification of the names *Amaranthus crassipes*, *A. warnockii* and *Scleropus amaranthoides* was also presented.

Methods: The work was based on field surveys, analysis of relevant literature and examination of specimens preserved in the herbaria GH, HAL, P, RO, NY, US and the Herbarium of the Faculty of Pharmacy of Monastir (Monastir University).

Results: Nomenclatural notes were provided for Schlechtendal's *A. crassipes* (lectotype at HAL, designated by Henrickson in 1999 but here corrected according to Art. 9.10 of the ICN); Schrader's *Scleropus amaranthoides* [a superfluous and illegitimate name (Arts. 52.1 and 52.2 of the ICN) and regarded as a homotypic synonym of *A. crassipes* s.str.]; and Moquin-Tandon's *Scleropus amaranthoides* [an invalid name (Art. 36.1a of the ICN)].

Conclusion: *Amaranthus crassipes* subsp. *warnockii* is an alien species in Tunisia, growing in human-made habitat on clayey and sandy substrates within ruderal vegetation where it can be considered a casual. We hope that in the future continuous monitoring of the population will take place, to verify the possible naturalisation and spread of this taxon in Tunisia. If the latter happens, actions for eradication of the plants are needed.

Keywords: African flora; alien species; *Amaranthus*; Monastir city; Tunisia.

Introduction

The genus *Amaranthus* L. (Amaranthaceae Juss.) comprises 65–70 species of which approximately half are native to the Americas (see e.g., Mosyakin & Robertson 1996; Iamonico 2015a). Some American species are used as ornamentals, food or medicines and are able to escape from cultivation, negatively impacting the agricultural systems through a reduction in both productivity and crop quality (see Iamonico 2010, 2015a; Das 2016).

Amaranthus is a taxonomically complex genus due to its high phenotypic variability, which has resulted in the current nomenclatural confusion and

misapplication of several names (see e.g., Costea et al. 2001; Bayón 2015; Iamonico 2014a, 2014b, 2016a, 2016b, 2016c, 2020a, 2020b, 2020c; Iamonico & Palmer 2020).

According to Le Floc'h et al. (2010) and Iamonico (2015b) nine *Amaranthus* species occurred in Tunisia. More recently two further species were found, i.e. *A. palmeri* S.Watson (Iamonico & El Mokni 2017) and *A. spinosus* L. (Iamonico & El Mokni 2019) and a new species – *A. tunetanus* Iamonico & El Mokni – was described from Monastir Governorate, central Tunisia (Iamonico & El Mokni 2018). The total number of Tunisian species belonging to the genus *Amaranthus* is now 12.

As part of an ongoing investigation on the Tunisian *Amaranthaceae* sensu lato (Sukhorukov et al. 2016; Iamonico & El Mokni 2016, 2017, 2018, 2019), we found a population identifiable as *Amaranthus crassipes* Schltdl. subsp. *warnockii* (I.M.Johnst.) N.Bayón, which represents the first record for both the Tunisian and the African floras, as well as the first indication of the species out of the Americas. Morphological notes and data on the habitat are presented here and the typification of the linked names is clarified.

Material and Methods

The work is based on field surveys, analysis of relevant literature and examination of specimens preserved at GH, HAL, P, RO, NY and US (acronyms according to Thiers 2022) and in the personal collection of one of the authors (R. El Mokni) deposited in the herbarium of the Faculty of Pharmacy of Monastir, Monastir University (not listed in *Index Herbariorum*). The articles cited throughout the text follow the Shenzen Code (Turland et al. 2018).

Results and Discussion

Notes on the typification of *Amaranthus crassipes* sensu stricto

Amaranthus crassipes was validly described by Schlechtendal (1831: 757–758) through a detailed description and a morphological comparison with *A. polygonoides* L., which was considered by Schlechtendal (1831) as the most similar species. The provenance ('*In locis paludosis ad rivulos insulae Sti. Thomae*') was also provided.

Henrickson (1999: 787) indicated the holotype for *Amaranthus crassipes* sensu stricto in a specimen preserved at HAL collected by C. Ehrenberg. Bayón (2015: 318) specified the barcode of this specimen (HAL076208), as well as the locality and date of collection ('Isla Vírgenes: St. Thomas Island, 1826–1828'). However, Schlechtendal (1831: 757–758) did not cite any holotype and, according to Arts. 9.1, 9.3 and 9.4 of the ICN, a lectotypification would be necessary (see also the considerations given by McNeill 2014). On the basis of Art. 9.10 of the ICN, Henrickson's use of the term 'holotype' (which precedes Bayón's publication, see Art. 10.5 of the ICN) is an error to be corrected to lectotype.

Notes on the typification of *Amaranthus warnockii*

Johnston (1944: 153–154) validly proposed *Amaranthus warnockii* by a detailed description (in Latin), a morphological comparison with the related *A. crassipes*, and a list of examined specimens. In particular, the author reported 'COAHUILA: 1 mi. [1.6 km] southeast of Ocampo, silty plain near mogote, *Johnston 8886* (TYPE, Gray Herb.)'. According to HUH Index of Botanists (2013 onwards), Grey Herbarium and types are preserved at GH, whereas further material is kept in many other American and European herbaria. Johnston's statement 'Gray Herb.' would therefore refer to a GH specimen. We traced just one specimen at GH (barcode GH00037034) bearing three plants and the following original label: 'MEXICO: western Coahuila | *I.M. Johnston no. 8886* | Sept. 8, 1941 | *Amaranthus warnockii* n sp | a mile S.E. of Ocampo low place near mogote on plain'. Since no further sheets were found at GH, GH00037034 is the holotype of the name *Amaranthus warnockii*, as correctly reported by both Henrickson (1999: 788) and Bayón (2015: 319).

Notes on the name *Scleropus amaranthoides*

Schrader (1835: 5) proposed *Scleropus amaranthoides* as a replacement name of *Amaranthus crassipes*, which was listed as synonym. A short diagnosis was given in a note ('*Character essentialis. Flores monoici. Mas. Cal. 5phylli foliola ovato-oblonga, inaequalia, exteriora carinata. Cor. 0 ...*'). According to Arts. 52.1 and 52.2 of the ICN, *Scleropus amaranthoides* is a superfluous and illegitimate name since the valid *Amaranthus crassipes* was cited in synonymy. Therefore, the type of *Scleropus amaranthoides* is that of *Amaranthus crassipes* (homotypic synonyms). Note that Henrickson (1999: 787) listed the name *Scleropus amaranthoides* as synonym of *Amaranthus crassipes* var. *crassipes* stating 'Type: unknown'; so, he probably did not examine Schrader's protologue and wrongly considered *Scleropus amaranthoides* as a heterotypic synomyn.

Moquin-Tandon (1849: 271–272) listed *Scleropus amaranthoides* as a synonym of the new proposed combination *Scleropus crassipes* (Schrad.) Moq. reporting '*Scleropus amaranthoides* Schrad. l.c.' where 'l.c.' (=

loco citato) refers to the previous listed name, i.e. '*Amaranthus crassipes* Schlecht. in Linnaea 6: 757, n. 278 (1831)'. However, Schlechtendal (1831: 757–758) did not cite the name *Scleropus amaranthoides*. Moquin-Tandon (1849) intended to synonymyse *S. amaranthoides* with *S. crassipes*, but he inadvertently published a new name ('*Scleropus amaranthoides* Schrad. ex Moq.'). According to Art. 36.1a, this name is invalid from a nomenclatural point of view.

Taxonomic Treatment

Amaranthus crassipes

Amaranthus crassipes Schltdl. in Linnaea 6: 757–758 (1831) subsp. ***crassipes*** = *Euxolus crassipes* (Schltdl.) Hieron. in Bol Acad. Nac. Sci. 4: 13 (1881) = *Scleropus amaranthoides* Schrad. in Index Sem. Hort. Acad. Gottingen.: 5 (1835), *nom. superfl. et illeg.* (Arts. 52.1 and 52.2 of the ICN) = *Scleropus crassipes* (Schltdl.) Moq. in Prodr. [DC.] 13(2): 271 (1849). Type: U.S.A., Virgin Islands: 'in locis paludosis ad rivolus ins. [insulae] St. Thomas, 1826–1828', *Ehrenberg s.n.* (HAL076208, lecto.!, designated by Henrickson 1999: 787 as 'holotype', here corrected according to Art. 9.10 of the ICN). Image of the lectotype available at http://141.48.4.202/djatoka/jacq-viewer/viewer.html?rft_id=hal_0076208&identifiers=hal_0076208.

– *Scleropus amaranthoides* Schrad. ex Moq. in Prodr. [DC.] 13(2): 271 (1849), nom. inval. pro syn. of *Scleropus crassipes* (Art. 36.1a of the ICN).

Description

Herbs 100–400(–500) mm tall, monoecious, annual (therophyte). *Stems* erect, ascending or decumbent, glabrous, green or reddish, branched. *Leaves* green, orbicular or ovate, 10–50 × 10–15 mm, glabrous, margins usually entire, apex obtuse to slightly emarginate with apical mucro, base cuneate, petiolate, with veins more or less prominent on the abaxial surface. *Synflorescences* arranged in axillary glomerules 4–10-flowered, 4–10 mm in diameter, with axes much thickened becoming indurate at fruiting stage. *Floral bracts* 1, ovate-deltoid, 0.5–1.5 × 0.5−1.0 mm, about half the length of the perianth, membranaceous with median vein light green, apex acute-mucronate, margin entire, glabrous. *Staminate flowers* with 5 tepals, equal to each other, lanceolate, 1.0–1.5 × 0.5–1.0 mm, with median vein visible, apex acute; stamens 3(5). *Pistillate flowers* with usually 5 tepals, lanceolate (1.2–2.0 × 0.2–0.4 mm), connate in the proximal $^1/_5$, spatulate with the distal part expanded, hyaline distally; stigmas 2(3), 0.7–0.8 mm long. *Fruit* indehiscent, subglobose to ellipsoidal, 1.4–2.0 × 1.2–1.7 mm, shorter than the perianth, verrucose at maturity in the distal part. *Seeds* ovoid to lenticular, 0.9–1.4 mm in diameter, dark brownish to reddish (often reddish at the margins), shiny.

Iconography

Bayón (2015: 320, Fig. 28).

Vernacular names

Clubfoot amaranth, spreading amaranth, tropical spreading amaranth (Mosyakin & Robertson 2003).

Distribution

Native to Colombia, the Caribbean (Aruba, Bahamas, Cayman Islands, Cuba, Dominican Republic, Haiti, Jamaica, Leeward Island, Netherland Antilles, Puerto Rico, Turks and Caicos Islands, Winward Island), North México, southern U.S.A. (Alabama, Arizona, Florida, Louisiana, New Mexico, South Carolina, Texas), and Venezuela; alien to South México, Perù and Trinidad & Tobago (see POWO 2022 and literature therein). Outside of the Americas, the taxon is recorded in Europe in Great Britain (Clement & Foster 1994), and in Asia in Japan (Randall 2017) and Pakistan (Jamshed et al. 2018); there is also a doubtful record for southern Italy, but it was never confirmed (see Iamonico 2015a). Concerning Pakistan, Jamshed et al. (2018) did not specify the subspecies but, on the basis of the picture given (Jamshed et al. 2018: 3, Fig. 2), the leaves are clearly ovate and green, and the plant is identifiable as the subsp. *crassipes*.

Taxonomic notes

On the basis of the classification proposed by Mosyakin and Robertson (1996), *Amaranthus crassipes* sensu lato is a species belonging to the subgen. *Albersia* (Kunth) Gren. & Godr. sect. *Pentamorion* (G.Beck) Mosyakin & K.R.Robertson, which would include taxa with indehiscent fruits and five tepals. *A. crassipes* sensu lato can be easily distinguished from all the other members of the sect. *Pentamorion* by its peculiar axes of the synflorescences, which appear much thickened and becoming indurate at fruiting stage. The more similar *Amaranthus* species is *A. scleropoides* Schrad., which was included by Mosyakin and Robertson (1996) in the sect. *Pyxidium* Moq. (this section comprises taxa of subgen. *Albersia* with dehiscent fruit). In fact, the main difference between *A. crassipes* sensu lato and *A. scleropoides* refers to the fruit, which is, respectively, indehiscent with surface verrucose in the distal part, and dehiscent with surface smooth or verrucose in the proximal part (see Bayón 2015: 319, 357).

Representative specimens examined

ANTIGUA. **North Sound:** weed in fallow, 6 Apr. 1937, *Box 573* (NY1373779). BAHAMAS. **Little Exuma:** Mr Bowe's farmland on south edge of William town, 23 Apr. 1975, *Correll & Correll 44848* (NY01373732). BARBADOS. **Christ Church:** Chaucery Lane, 30 Jul. 1906, *Dash 360* (US01884380). COLOMBIA. **La Guajira:** Uribia, en los bordes y cauce del arroyo en la salida hacia Maicao, 29 Mar. 1962, *Saravia et Johson 324* (US03541795). CUBA. **La Habana:** Cojimar, 24 Aug. 1910, *Britton 154* (NY1036594). DOMINICAN REPUBLIC. **Valverde:** El Maguenal, Jaibon, Mao, alt. 100 m, 2 Feb. 1974, *Liogier 21195* (NY1373790). GUADALUPE. **Anse-Bertrand:** alt. 10 m, 7 Nov. 1937, *Stehlé 2515* (P05002553). HAITI. **Dep. du Sud:** Trémé, 30 Jun. 1980, *Peeters 80/60* (P04944386); Plaine d'Aquin, début route 44 vers Flamand, en zone paturée, 6 Jul. 1980, *Sastre et Polynice 7341* (P04944385). **Massif de la Selle:** 2 km al este de Petionville, alt. 200–240 m, 14 Jun. 1985, *Zanoni et al. 35236* (NY1373768). JAMAICA. Grounds of St. Benedict's School east of Harbour View, open waste ground, alt. 25–50 ft. [7.6–15.3 m], 13 Aug. 1963, *Proctor 23937* (NY1373776). PUERTO RICO. **Montalva:** roadside, 2–4 Mar. 1915, *Britton et al. 4876* (US00707046); **Island of Celebra:** waste places, 3–12 Mar. 1906, *Britton 154* (NY1036594). TURKS AND CAICOS ISLANDS. **South Caicos:** 14–16 Dec. 1907, *Wilson 7643* (NY1373730). VENEZUELA. **Lara:** Savanas around Barquisimeto, May 1925, *Saer 206* (US 0354180). U.S.A. **Arizona:** Pima County, Cabeza Prieta National Wildlife Refuge, José Juan Tank (Represo), and artificial dirt charco on San Cristobal Wash, 1.2 km W of the western boundary of Organ Pipe Cactus National Monument, 14 Sept. 1992, *Felger 92-713* (US03540276); **Florida:** 1842–1849, *Rugel 31* (US03540289). VIRGIN ISLANDS. **St. Thomas Island**: shore of harbour, 8–9 Feb. 1913, *Britton et al. 475* (US00707047). SAINT CROIX. West Indies Lab Compound, open bare rocky soil, alt. 5 m, 11 Jan. 1972, *Fosberg 53930* (NY1373756).

Amaranthus crassipes

Amaranthus crassipes Schltdl. subsp. ***warnockii*** (I.M. Johnst.) N.Bayón in Ann. Missouri Bot. Gard. 101: 319 (2015). Type: México, Coahuila, W. Coahuila, 1 mi. [1.6 km] SE of Ocampo, silty plain near mogote, 8 Sept. 1941, *Johnston 8886* (GH-00037034, holo.!). Image of the holotype at https://plants.jstor.org/stable/viewer/10.5555/al.ap.specimen.gh00037034?loggedin=true.

Diagnostic features

Subsp. *warnockii* differs from subsp. *crassipes* by the leaves, which are obovate to oblanceolate, 6–10 mm wide (vs 10–15 mm), and green-glaucous on the abaxial surface (vs green, never glaucous) (Figure 1).

Figure 1. *Amaranthus crassipes* subsp. *warnockii* from Monastir Governorate; A, plant; B, details of two floral glomerules. Photographs: R. El Mokni.

Figure 2. Distribution of *Amaranthus crassipes* subsp. *warnockii* in Tunisia.

Vernacular name

Warnock's amaranth.

Phenology in Tunisia

Flowering time October–November; fruiting time November–December.

Distribution

U.S.A. (SE Texas) and Mexico (E Chihuahua, E Coahulia). Not recorded outside of the Americas up to the present paper (see e.g., Bojian et al. 2003; Mosyakin & Robertson 2003; Palmer 2009; POWO 2022 and literature therein; African Plant Database version 3.4.0).

Habitat and distribution in Tunisia

Human-made habitat on clayey and sandy substrates within ruderal vegetation (almost similar to native habitat and vegetation, which consists of open areas and matorral, see Bayón 2015: 319). *Amaranthus crassipes* subsp. *warnockii* is restricted in Tunisia to one locality of Monastir city at alt. 8 m (Figure 2). The population found covers an area of about 100 m^2. The taxon is an alien for Tunisia and Africa and can be considered as casual.

Representative specimens examined

MÉXICO. **Coahuila:** W. Coahuila, 1 mi. [1.6 km] SE of Ocampo, silty plain near mogote, 8 Sept. 1941, *Johnston 8886* (GH00037034). TUNISIA. **Monastir:** Monastir city, 35°45'39"N, 10°49'52"E, on clayey and sandy substrates in ruderal vegetation, alt. about 8 m, 8 Oct. 2019, *El Mokni s.n.* (HFLA!, *Herb. R. El Mokni*). U.S.A. **Texas:** Hudspeth Co. Gypsum Flat: 10 Oct. 1944, *Waterfall 5842* (NY3363738); near Rio Grande, common and abundant, low places, 8 Aug. 1919, *Hanson 822* (US03540282).

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

DI conceived the research, searched pertinent literature and specimens for the typification purposes, and prepared the first draft of the manuscript; REM carried out field surveys, checked the manuscript and gave further considerations.

Ethical considerations

This article followed all ethical standards for research without direct contact with human or animal subjects.

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Data availability statement

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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A new species of *Thilachium* (Capparaceae) from the Analanjirofo Region, Madagascar

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Madagascar is a centre of speciation for the genus *Thilachium* Lour., which includes several species of small trees and shrubs occurring in a wide range of habitats. A new species of *Thilachium*, *T. latifolium* Fici, from the Analanjirofo Region of northeastern Madagascar is here described and illustrated. It is characterised by 1-foliolate leaves, leaf blades widely ovate or elliptic with shortly acuminate or acute apices, flowers in terminal, dense subumbels or corymbs, short pedicels and ellipsoid, ribbed fruit. The new species is related to *T. madagascariense* Fici, a species recently described from eastern Madagascar, differing in the wider, coriaceous leaves with shorter, mucronulate tip, flowers in terminal, 7–16-flowered subumbels or corymbs, shorter pedicels and longer anthers. The distribution, autecology and affinities of the new species are discussed, its conservation status is assessed, and an updated key is provided for the species of the genus *Thilachium* known from Madagascar.

Keywords: Capparoideae, conservation status, endemism, historical collections, Mananara, Masoala National Park, *Thilachium latifolium*.

Introduction

The classification of the tropical and subtropical family Capparaceae Juss., traditionally split into the three subfamilies Cleomoideae Pax, Dipterygioideae Pax and Capparoideae Pax, has undergone several changes due to new evidence from phylogenetic analyses (Hall et al. 2002; Hall 2008; Cardinal-McTeague et al. 2016). In addition, various genera in the neotropical area have been described or reinstated (Cornejo 2017; Cornejo & Iltis 2008a, 2008b, 2008c, 2008d, 2009; Iltis & Cornejo 2007, 2010, 2011). Based on these considerations, the Cleomoideae and Dipterygioideae are now referred to family Cleomaceae Horan., with 34 genera currently recognised within the Capparaceae (Fici 2020).

In Madagascar and the Comoro Islands the Capparaceae are represented by six genera, i.e. *Crateva* L., *Capparis* L., *Cadaba* Forssk., *Boscia* Lam., *Maerua* Forssk. and *Thilachium* Lour. After the treatment in *Flore de Madagascar et des Comores* (Hadj Moustafa Haddade 1965), little attention has been paid to these genera in the area, apart from the description of two new species of *Capparis* and *Thilachium* (Fici 2011, 2021a), while several studies were carried out during the last decades in southern Asia (Viswanathan 2000; Srisanga & Chayamarit 2004; Sy et al. 2013, 2015, 2016, 2017, 2020; Fici et al. 2018, 2020; Souvannakhoummane et al. 2018, 2020; Fici & Souvannakhoummane 2020; Murugan et al. 2020; Julius 2022), Indonesia (Fici 2012, 2021b) and New Caledonia (Fici 2017). The genus *Thilachium* includes 15 species of trees or shrubs (with a single herbaceous species), with 1- or 3-foliolate leaves, occurring in eastern Africa, Madagascar and Mauritius along a wide range of habitats (Elffers et al.1964; Thulin 1993; Harvey et al. 1995; POWO 2019; Fici

2021a). The genus is characterised by the absence of petals and by the connate sepals rupturing transversally with a conical calyptra often remaining attached at one side. De Wolf (1962) regarded *Thilachium* as 'somewhat anomalous' within the African Capparaceae and hypothesised an affinity with *Ritchiea* R.Br. and *Maerua,* a relationship which has been confirmed by molecular phylogenetic data (Hall et al. 2002; Su et al. 2012; Cardinal-McTeague et al. 2016; Tamboli et al. 2018).

While studying the collections of *Thilachium* at the Muséum National d'Histoire Naturelle (P), flowering material collected in 1912 by Perrier de La Bâthie in the coastal forest of Mananara, in the Analanjirofo Region of northeastern Madagascar, was examined. This collection was formerly studied by Hadj Moustafa Haddade (1965), who regarded it as an undescribed species 'insuffisamment connu', characterised by large, coriaceous, 1-foliolate leaves and white flowers with short pedicels, arranged in compact inflorescences. The occurrence in the same herbarium (P) of a more recent fruiting specimen, collected in the same region, allowed for the completion of the morphological characterisation of this new species, which is here described.

Materials and methods

Herbarium investigations were carried out on historical and recent collections from Madagascar kept at P. To my knowledge the new species has been collected two times, in October 1912 (*Perrier de La Bâthie* 5029) and in April 1996 (*Aridy et al*. 260). The description and illustration are based on this herbarium material. The species concept follows the one adopted by Elffers et al. (1964) and Hadj Moustapha Haddade (1965). The main diagnostic characters among the new species and related taxa are based on the same treatments (Elffers et al. 1964; Hadj Moustapha Haddade 1965) and on Fici (2021a). The herbarium acronyms follow Thiers (continuously updated), while authors and plant names are based on the International Plant Names Index (IPNI) (2020). The examination of the type specimens of other species was carried out through electronic images available at JSTOR Global Plants (n.d.). The available online collections at MO and the Catalogue of the Vascular Plants of Madagascar (Anon 2022) were also consulted. The conservation status was provisionally assessed according to IUCN Red List Categories and Criteria (IUCN 2012).

Taxonomic treatment

Thilachium latifolium Fici, sp. nov.

TYPE: MADAGASCAR, **Analanjirofo Region**, Mananara, Côte Est, [16°10'S / 49°46'E], Oct. 1912, *Perrier de La Bâthie 5029* (P 05457228!, holo.; P 05457229!, iso.).

Description

Shrub up to ± 3.5 m tall. *Branches* reddish or brownish, beset with sparse lenticels; twigs glabrous. *Leaves* 1-foliolate, alternate; blade coriaceous, persistent, widely ovate or elliptic, (60–)70–140(–160) × (30–)40–93 mm, with entire margins; base attenuate or obtuse; apex acuminate, with tip up to ± 8 mm long, or acute, mucronulate; surfaces glabrous; nerves (4–)5–6(–8) on each side of the midrib; petiole (7–)12–41(50) mm long, glabrous, articulate at the top. *Flowers* in 7–16-flowered terminal, subsessile, dense subumbels or corymbs; pedicels 8–12 mm long, glabrous; bracts ± 0.5 mm long or lacking; flower buds (5.0–)5.5–8.0 × (4–)5–8 mm, with whitish, ovoid or ellipsoid calyx at maturity rupturing transversally, the calyptra often remaining attached at one side; petals 0; androgynophore ± 1.0–1.5 mm long; stamens ± 51 to 72, filaments 22–28 mm long, anthers 2.5 mm long; gynophore ± 25–26 mm long, glabrous; ovary oblong, ± 3–4 mm long, glabrous. *Fruit* ellipsoid, 45–63 × 29–45 mm, 8-ribbed; seeds ovoid, brownish, ± 13–18 × 10–11 mm. Figure 1.

Distribution and habitat

The new species is known from two localities of the Analanjirofo Region (Figure 2), at 16°10'S / 49°46'E and 15°40'S / 49°57'E, where it has been collected in coastal forest and in dense evergreen, humid forest, from sea level up to ± 300 m elevation. Based on the available material, flowering occurs in October, fruiting in April.

Etymology

The specific epithet is composed of the Latin words *latum*, meaning wide, and *folium*, meaning leaf.

Diagnosis and relationships

The new species is related to *Thilachium madagascariense* Fici, from which it differs in the coriaceous, widely ovate or elliptic, (30–)40–93 mm wide leaf blade (leaf blade chartaceous, narrowly obovate or elliptic, (20–)33–50 mm wide in *T. madagascariense*); leaf apex with tip up to ± 8 mm long, mucronulate (tip up to 15 mm long, not mucronulate in *T. madagascariense*); inflorescence a 7–16-flowered terminal, dense subumbel or corymb (2 or 3 flowers conferted at the top of lateral twigs in *T. madagascariense*); pedicels 8–12 mm long (12–18 mm long in *T. madagascariense*); and anthers 2.5 mm long (1.5–2.0 mm long in *T. madagascariense*).

With regard to other species with 1-foliolate leaves from Madagascar, *T. latifolium* shows also affinities with *T. laurifolium* Baker, which differs from the former in the

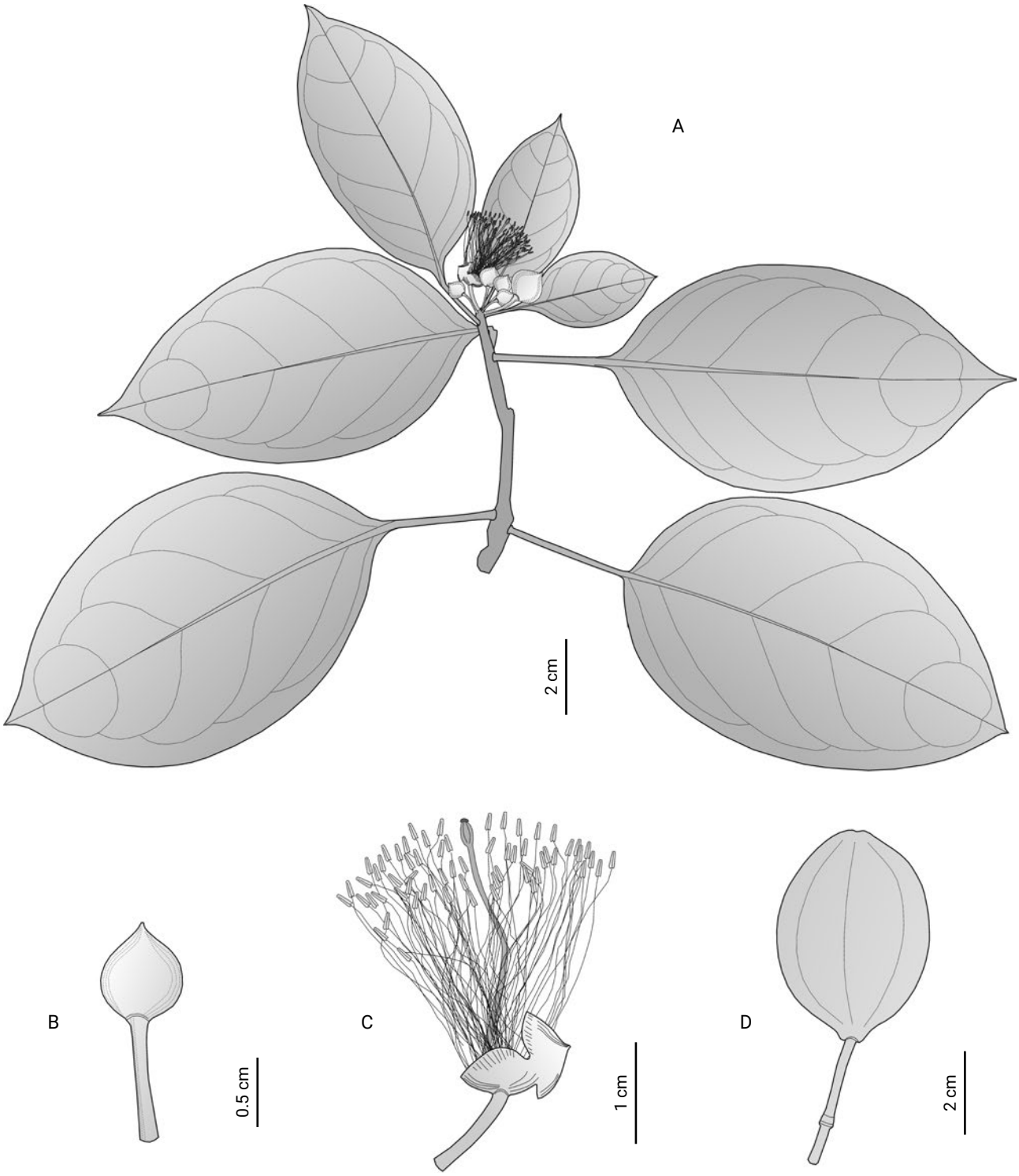

Figure 1. *Thilachium latifolium*. A, flowering branch; B, flower bud; C, flower; D, fruit and stipe. A, B, C from *Perrier de La Bâthie 5029* (holotype), D from *Aridy et al. 260*. Artist: Silvio Fici.

smaller, subemarginate leaf blade, 25–75 × 25–30 mm; inflorescence a 4–6-flowered terminal, loose corymb; pedicels (30–)35–50 mm long, stamens ± 30–46 with filaments 31–35 mm long; and gynophore ± 50–70(–80) mm long. Among the 1-foliolate species of the genus recorded from eastern Africa, *T. latifolium* is closer to *T. thomasii* Gilg, a species widespread in Kenya and southern Somalia (Elffers et al. 1964), which is distinguished by the petiole (4–)5–9(–14) mm long; leaf blade with apex obtuse or rounded, not acuminate; inflorescence a 1–10-flowered terminal, loose corymbose raceme; pedicels up to 20 mm long; and stamens ± 18–25.

Conservation status

Lacking information to assess its risks, *Thilachium latifolium* is assessed here as Data Deficient (DD). However,

it is to be underlined that one of the known localities falls within a conservation area (Masoala National Park).

Other material examined

MADAGASCAR, **Analanjirofo Region:** Park National de Masoala, Andranobe, Fok. Ambanizana, Fir. Anjahana, Fiv. Maroantsetra, 15°40'S / 49°57'E, 200–300 m, 17 Apr. 1996, *Aridy et al. 260* (MO, P 04746459).

Notes

Apart from the widely ovate or elliptic leaves with apex shortly acuminate, *Thilachium latifolium* is mainly differentiated from the other 1-foliolate species of the genus in Madagascar by its terminal, dense subumbels or corymbs. The inflorescence in *Thilachium* is commonly reported as a terminal, axillary or on short lateral branches, corymbose raceme (Elffers et al. 1964; Kers 2002), or more rarely 2 or 3 flowers are conferted at the apex of lateral twigs (Fici 2021a). Among the known species a dense, many-flowered corymb is reported only for *T. densiflorum* Gilg & Gilg-Ben., a 3-foliolate species from Tanzania, with buds up to 5 mm in diameter and receptacle elongating to ± 8 mm at anthesis (Elffers et al. 1964). As mentioned above *T. latifolium* shows some affinities with *T. madagascariense*, a species recently described from eastern Madagascar, and with *T. laurifolium*, known from the central and eastern parts of the island (Hadj Moustapha Haddade 1965; Fici 2021a; Anon 2022). A key to the species of *Thilachium* known from Madagascar is here provided.

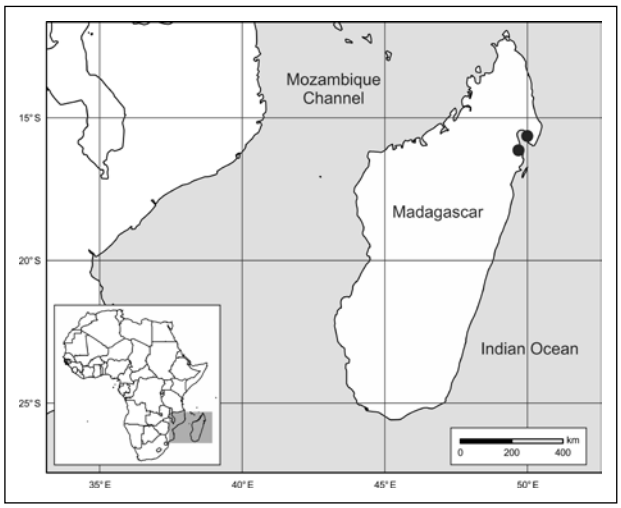

Figure 2. Distribution of *Thilachium latifolium*.

Madagascar constitutes a centre of speciation of the genus *Thilachium*, which is represented here by nine endemic species, besides one species also native to eastern Africa and one to Mauritius; among these, five species have 1-foliolate leaves and six species 3-foliolate leaves. The description of this new species confirms that the historical herbarium collections still represent a remarkable and inspiring source of data for plant taxonomists (Fici 2021a) in areas such as eastern Madagascar.

Key to the species of *Thilachium* in Madagascar

1a. Leaves always simple or 1-foliolate:
 2a. Inflorescence dense subumbel or corymb up to 16-flowered; pedicels 8–12 mm long. . . . . **T. latifolium** Fici, sp. nov.
 2b. Inflorescence loose corymbose raceme up to 10-flowered, or 2 or 3 flowers at the top of lateral twigs or on the branches; pedicels ≥ 12 mm long:
  3a. Petioles ≥ 80 mm long; leaf blade ≤ 10 mm wide . . . . . *T. pouponii* Aubrév. & Pellegr.
  3b. Petioles ≤ 40 mm long; leaf blade ≥ 18 mm wide:
   4a. Petioles 8–12 mm long; inflorescence 8–10-flowered; stamens 25–30; anthers 2.5 mm long . . . . . *T. monophylum* Hadj-Moust.
   4b. Petioles (11–)19–40 mm long; inflorescence 2–6-flowered; stamens ≥ 30; anthers 1.5–2.0 mm long:
    5a. Leaf blade submarginate, 25–75 mm long; 4–6 flowers in terminal corymbs; pedicels (30–)35–50 mm long . . . . . *T. laurifolium* Baker
    5b. Leaf blade acuminate (75–)100–153 mm long; 2 or 3 flowers at the top of lateral twigs; pedicels 12–18 mm long . . . . . *T. madagascariense* Fici
1b. Leaves 3-foliolate, rarely with simple leaves intermixed or on fertile branches:
 6a. Leaflets linear or narrowly oblong, 2–12 mm wide; fruit 8–10 mm wide . . . . . *T. angustifolium* Bojer
 6b. Leaflets elliptic, lanceolate, oblanceolate, ovate, obovate or panduriform, (6–)10–53 mm wide; fruit 12–40 mm wide:
  7a. Leaflets heteromorphic, lanceolate and panduriform. . . . . *T. panduriforme* Juss.
  7b. Leaflets not heteromorphic:
   8a. Pedicels 6–8 mm long. . . . . *T. sumangui* Bojer
   8b. Pedicels 10–21 mm long:
    9a. Gynophore 23–32 mm long; filaments 27–36 mm long . . . . . *T. africanum* Lour.

9b. Gynophore 7–20 mm long; filaments ± 10 m long:
10a. Pedicels ± 10–13 mm long; stamens ± 15; fruit 40–50 mm long . . . . . *T. humbertii* Hadj-Moust.
10b. Pedicels 15–20 mm long; stamens 30–40; fruit 7–8 mm long . . . . . *T. seyrigii* Hadj-Moust.

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Competing interests

The author declares that he has no financial or personal relationships that may have inappropriately influenced him in writing this article.

Ethical considerations

This article followed all ethical standards for research without direct contact with human or animal subjects.

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An online survey on user perceptions of natural science collections in South Africa

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Background: In South Africa, and globally, the value of natural science collections for scientific research is not widely recognised and has led to its marginalisation, which in turn has resulted in low funding, staffing and use of the collections.

Aim & objectives: To this end, as part of the effort to increase understanding and appreciation of the collections, a cross-sectional web-based survey was administered to users of natural science collections (NSCs) in South Africa. The objectives of the study were to identify the perceived value of NSCs to the research community; perceived or experienced barriers in accessing NSCs and associated data for use in research; perceptions of NSCs' current performance in serving the needs of stakeholders; and how performance is judged and what the expectations are to improve future performance of NSCs to better serve the needs of stakeholders.

Methods: The survey consisted of 26 questions, distributed by email to relevant researcher community mailing lists, and posted on relevant social media groups. The survey was completed by 131 respondents.

Results & conclusion: The study indicated the overall perception of the importance of NSCs and their accessibility to the student and researcher community in South Africa and internationally to be extremely important to their research. Lack of funding for operations and staff impedes the ability of researchers and other users alike in using NSCs to optimise their research and contribute to issues of societal concern. A sustained commitment is required from NSC institutions to work together to solve various challenges, including improvement in serving stakeholder needs, which will in turn assist with demonstrating the value of NSCs to policymakers, in order to lobby for support and funding. Improved recognition of the importance of NSCs for research by the scientific community will assist NSCs in demonstrating their impact. Political priority should also be given to the long-term upkeep and ongoing assistance of institutional infrastructures.

Keywords: natural history collections, natural science collections, natural history museums, collections management.

Introduction

South Africa has an estimated 100 natural science collections at approximately 40 institutions (NSCF 2019). Together they provide over 18 million objects or specimens representing about 100 000 different species of plants, animals and fungi, which have been accumulated over the last 200 years and represent life on earth since its origins (NSCF 2019).

The documentation and study of natural science specimens underpin our understanding and further research into the diversity of life, its origins and evolution, and its distribution in space and time. This contributes to biodiversity conservation, pest and disease control, solving crime, public health, food security; and allows for future predictions, including for climate change impacts, that can inform

decision-making by policymakers (Suarez & Tsutsui 2004; Figueira & Lages 2019; Jacobs 2020). The collections also constitute an invaluable record of the natural heritage of the subcontinent (Davison 1994). Museum exhibitions, events and lectures based on biological collections contribute to a greater public understanding and appreciation of nature, both local and worldwide, and why it needs to be conserved. NSCs directly contribute to the success of a museum by providing knowledge to local communities but could also indirectly contribute to the growth of tourism and the local economy (NatSCA 2005; Powers et al. 2014; Proa & Donini 2019).

The impacts or outcomes of research and data emanating from collections, however, are generally indirect or downstream, which means that their significance is often not well understood or recognised, resulting in a lack of appreciation of this infrastructure. This in turn has resulted in low funding, staffing and use of the collections in South Africa and globally (Drew 2011; Hamer 2012). Several initiatives have been directed towards addressing dwindling capacity and resources for NSCs and associated research in South Africa. The establishment of the Southern African Society for Systematic Biology (SASSB) in 1999 and the South African Biosystematics Initiative (SABI) in 2002, both aimed to address the country's declining capacity in biological systematics and taxonomy, and to increase public appreciation of the value of systematics and natural science collections (SASSB 2023). Despite these efforts, capacity and resource challenges persist for NSCs.

An assessment of South African zoological research collections (Hamer 2012) recommended two actions required to improve engagement with collections: 1) multilateral discussions between relevant government departments under which the collections' institutions are governed; and 2) making use of the collections to address questions of societal relevance. These recommendations are currently being addressed by the Natural Science Collections Facility (NSCF) project, funded by the Department of Science and Innovation through the establishment of a virtual network of South African institutions housing NSCs. This virtual network is directed towards collaboratively dealing with challenges faced by the South African NSC community.

One of the objectives of the NSCF is to research and demonstrate the importance and use of the collections and data by the global research community in solving issues of societal relevance and protecting the systems that sustain life. This is critical to ensure the long-term sustainability of the collections.

A survey by Astrin and Schubert (2017) captured a snapshot of the values and opinions regarding natural history collections from 525 poll participants from predominantly North America and Europe, mostly based in academia (41%) and at natural history institutions (32%), or students (10%). It was found that natural history collections are intriguing or interesting places for almost all respondents. Fundamental research, collection care and educating the public were the three most often selected natural history collections' core roles. The general importance of vouchering and the treatment of type specimens were considered to be satisfactory. Molecular vouchers, data accessibility, sample documentation and taxonomic expertise at the natural history collections were considered to deserve more attention, with less satisfaction expressed. Insufficient funding was the strongest concern of most survey participants. Such a study has, to date, not been carried out on South African NSCs.

To this end, as part of the effort to increase understanding and appreciation of the collections, a survey of the stakeholder community's perceptions of the value and current performance of South African NSCs was conducted. The study aimed to identify the perceived value of NSCs to the stakeholder community; perceived or experienced barriers in accessing NSCs and associated data for use in research; perceptions of NSCs' current performance in serving the needs of stakeholders; how NSC performance is judged and what the expectations are to improve future performance of NSCs to better serve the needs of stakeholders.

Research method and design

Target group

The target group of the survey consisted of the user community who access and use specimens, images of specimens and specimen data from South African NSCs to conduct research or related work. This included students, taxonomists, Environmental Impact Assessment experts, citizen scientists, and scientists in the fields of climate change, ecology, ethnobotany, evolution, nature conservation, pest and disease control, and agriculture.

Study design

A cross-sectional web survey design was employed for the study. The survey collected responses for a period of two months.

The survey link was distributed by email to relevant researcher community mailing lists, and to collections curators and managers at the NSCF partner institutions, to share with users of their respective collections. A link to complete the survey was also posted on natural science and researcher community Facebook pages and groups.

Methods

The survey, adapted from Astrin and Schubert (2017), consisted of 26 questions (Annexure A), and was set up and administered through the Survey Monkey website. The first seven questions constituted of background questions and only two of these were compulsory. The compulsory questions prompted participants to state in which discipline/s they conduct their research, and whether they were a South African resident. This enabled analysis of data based on the type of researcher, and analyses of perceptions from both the local and international communities.

The remainder of the survey questions were not compulsory and were divided into the specific objectives of the research. Eight questions related to **Objective 1**: perceived value of NSCs to the research community; two questions related to **Objective 2**: perceived or experienced barriers in relation to access to natural science collections and associated data for use in applied research; six questions related to **Objective 3**: perceptions of NSCs' current performance in serving the needs of applied research, and three questions related to **Objective 4**: how performance is judged and what future expectations for performance are.

Data analysis

The results from the survey were analysed using the Survey Monkey (www.surverymonkey.com) outputs summary tool and Microsoft Excel. The filter tool was applied to determine: a) types of respondents based on residency status, employment sector, relevant research discipline, and b) the different types of services that collections offer. The two open-ended questions, relating to barriers to access experienced and the area that NSCs can improve on most, were analysed by grouping answers according to themes.

Ethical considerations

Ethical clearance

The survey study was approved by the South African National Biodiversity Institute Animal Research Ethics and Scientific Committee, with reference number SANBI/RES/P2021/21.

Risks or negative impacts associated with research and mitigation

To ensure no harm came to participants, the respondents were able to complete the survey anonymously. In the case where respondents had chosen to provide their names, the risk to participants was reduced by not naming any individual or their affiliation in the survey results.

Recruitment and informed consent

An informed consent form (Annexure B) including the aim of the study and details regarding the protection of participants' personal information was prepared for this study and was distributed to participants to complete and sign. Participation was voluntary and participants were free to withdraw from the study at any time without consequence.

Data protection

The raw data from the study is stored in a password-protected file, and the password is only available to the first author. The raw data, which includes participant details, will be deleted upon completion of the research.

Results

Profile of respondents

Of the 131 responses received, 74% of respondents were South African residents and 26% were international. Respondents mostly indicated that they worked in more than one discipline, with most national and international respondents working in the disciplines of taxonomy and ecology (as depicted in Figure 1).

The disciplines listed on the 'other' option by respondents included geomorphology, biodiversity informatics, soil science, plant virology, biogeography and genetics.

Of those respondents who indicated their place of work, 47 indicated they were employed at universities, 25 at science and research councils, 13 at museums, 10 as consultants at private companies, three at conservation trusts/councils, and two were employed in government departments. Eleven respondents indicated

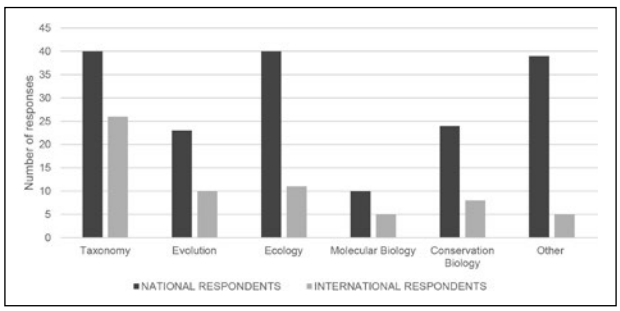

Figure 1. Respondents' field of research.

that they were students. The majority of international respondents were employed at universities. Responses from those employed at government departments were underrepresented. This suggests that either government departments were not sampled adequately, or only a small number of government departments use basic taxonomic outputs.

The value of NSCs to the researcher community

Access to specimens and data

The results indicate that respondents access natural science specimens or data for their research daily (23%), weekly (15%), monthly (13%), biannually (16%), annually (11%), and less frequently (22%). These respondents indicated that they worked with one or more of the following types of specimens and data: animal specimens and data (52%), followed by plant specimens and data (23%), fossil specimens and data (10%), and fungi specimens and data (1%). Fourteen per cent of respondents indicated that they worked with other specimens, which included soil, shells and bacteria. The majority (97%) of international respondents worked with animal and plant specimens and data.

Contribution of access to specimens and data to research

For Question 14 respondents were asked, 'How important is access to NSCs (specimens, associated data and collaboration with NSC staff) to your research?' Responses were indicated on a Likert scale (1 – not at all, 2 – slightly, 3 – moderately, 4 – very, 5 - extremely). Seventy-one per cent (71%) and 17% of respondents indicated access to NSCs as 'extremely' and 'very important', respectively.

Eighty-nine per cent (89%) of respondents indicated that access to NSC specimens directly contributed to their research, with access to NSC specimens referring to loans of physical specimens and tissue/DNA samples; images of specimens; laboratory space and equipment; specimen data; expertise and advice from curators/researchers; identification services; depositing collected specimens; and/or collaborations with associate researchers.

Respondents indicated that their research using NSCs contributed to one or more of the fields listed in Table 1.

Seventy per cent (70%) of respondents indicated that access to NSC specimens and data contributed to the curation process of the collection and/or led to the formation of collaborations with NSC staff. Sixty-four per cent (64%) of respondents indicated that they inform the NSC once their research has been published and/or send the NSC a copy of the published paper.

Table 1. Use of natural science collection's contribution to fields of research

Field of research	Response count
Documentation and classification of biodiversity	86
Nature conservation	57
Evolution	40
Agriculture	24
Pest and disease control	24
Environmental impacts of climate change	23
Other	16
Food security	5
Solving crime	2
Public health	2

Perceived or experienced barriers to access

For Question 17 'Do you find the access request procedure overly onerous?', 77 respondents answered no, 11 indicated yes and 27 were uncertain. Twenty-one respondents (18%) indicated that they have been denied access to specimens or data. Responses to the open-ended question on how access was denied, organised according to themes, included:

- Staff shortages – seven responses.
- Institutional policy (on destructive sampling, loaning of physical type specimens) – three responses.
- Collection closed (due to renovations or Covid-19) – three responses.
- Institution access committee decision – two responses.
- Perceived bias by collection curator – two responses.
- Embargo on specimens due to pending research – one response.

A comparison of national and international respondents indicated that 14% of national respondents and 21% of international respondents experienced barriers to access.

Perceptions of NSCs' current performance in serving the needs of research

Responses to the six questions dealing with the perceptions of NSCs' current performance in serving the needs of applied research are summarised below:

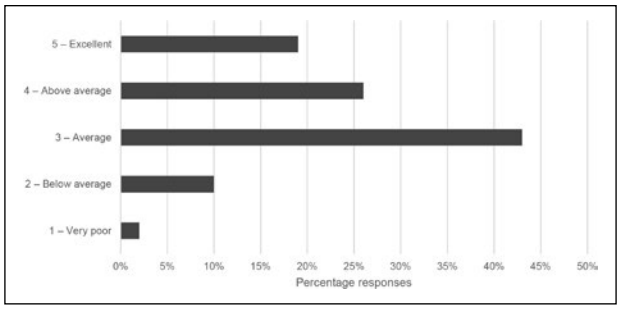

Figure 2. South African natural science collections' performance in providing services compared to other countries.

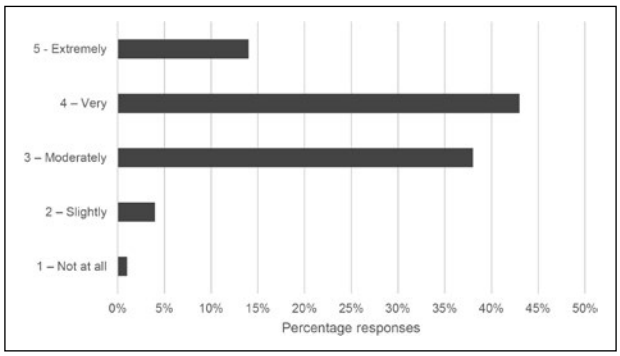

Figure 3. Satisfaction with services offered by natural science collections.

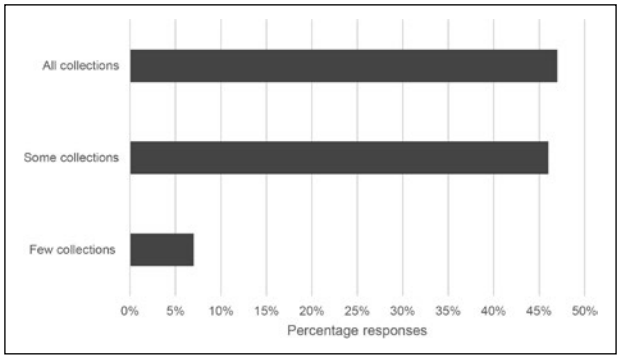

Figure 4. The physical curation of specimens is to an acceptable standard.

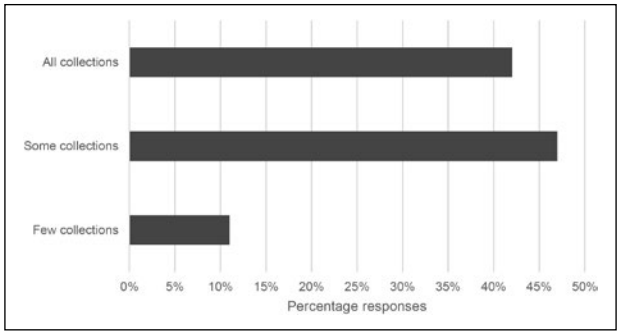

Figure 5. The data associated with specimens are accurate, up to date and usable.

The majority of respondents (43%) indicated that South African NSCs perform 'average' in providing services compared to NSCs in other countries. Twenty-six per cent (26%) of respondents indicated that the NSCs performed 'above average', and 19% of respondents indicated that NSCs performed 'excellent' in providing services compared to NSCs in other countries (Figure 2).

The majority of respondents (43%) indicated that they were 'very happy' with services offered by South African NSCs, 14% of respondents indicated that they were 'extremely happy' with services offered and 39% of respondents indicated that they were 'moderately happy' (Figure 3).

Forty-seven per cent (47%) of respondents indicated that 'all' collections' physical curation was to an acceptable standard, and 46% indicated that 'some' collections' physical curation was to an acceptable standard (Figure 4).

The majority of respondents (47%) indicated that the data associated with the specimens were up-to-date, accurate and usable for 'some' collections. Forty-two per cent (42%) of respondents indicated that the data associated with the specimens were up-to-date, accurate and usable for 'all' collections (Figure 5).

Perceived performance according to collection type is summarised in Table 2. The majority of respondents were 'moderately happy' with the services offered for animal collections, 'very happy' with services offered for plant collections and 'extremely happy' with services offered for fossil collections. The majority of

Table 2. Perceived performance according to the type of collection

Highest response count per type of collection	Animal collections	Plant collections	Fossil collections	Fungi collections	Other
Overall, how happy are you with the services offered by NSCs? (*not at all – slightly – moderately – very – extremely)*	Moderately	Very	Extremely	Too few responses (<3)	Moderately
Physical curation to an acceptable standard? *(few – some – all collections)*	Some	Some	All	Too few responses (<3)	Some
Data associated with specimens up-to-date, accurate and usable? *(few – some – all collections)*	Some	Some	Some	Too few responses (<3)	Some

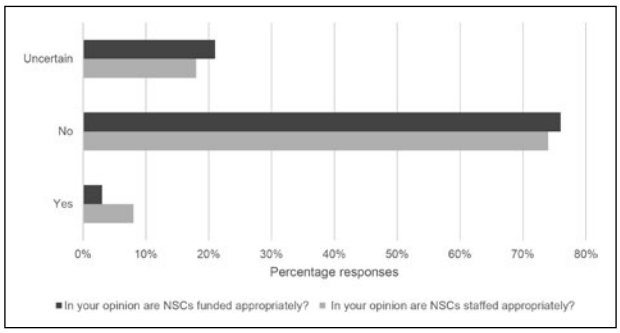

Figure 6. Natural science collection resources.

respondents indicated that the physical curation of some animal collections, some plant collections and all fossil collections were to an acceptable standard. Most respondents indicated that the data associated with specimens were up-to-date, accurate and usable for some plant, some animal, and some fossil collections.

The majority of respondents (76%) indicated that NSCs were not funded appropriately in their opinion and most respondents (74%) indicated that NSCs were not staffed appropriately in their opinion (Figure 6).

Performance: how is it judged, and what are the future expectations

The five most important roles of NSCs, as identified by 114 respondents, were: collection care and conservation; documenting biodiversity; availability of taxonomic expertise; collecting new specimens; and making data openly accessible.

National and international respondents rated NSC performance in the below-mentioned service areas as average, except for collection care and conservation performance, which was rated as above average (Table 3).

Table 3. Performance of natural science collections in service areas

Service area/role	Rating (indicated by highest response count)
Collection care and conservation	Above average
Documenting biodiversity	Average
Availability of taxonomic expertise	Average
Collecting new specimens	Average
Making data openly accessible	Average
Providing accurate datasets	Average
Making specimens digitally accessible	Average
Conducting basic research	Average
Supporting biological surveys	Average
Educating the public	Average

The majority of respondents believed that the areas in which NSCs should improve on most were an increased staff complement (25), followed by collection care and conservation (19) and availability of taxonomic expertise (19) (Figure 7).

Discussion

Outline of the results

Perceived value of NSCs to the research community

A mutually beneficial relationship exists between the users and the collections (including staff expertise and access to specimens and data), with a large percentage of respondents indicating that access to NSC specimens directly contributed to their research and/or the curation process of the collection, which often leads to the formation of collaborations with staff. This is supported by the wide use of the collections as reported by the 16 NSCF partner institutions reporting an average of 1 157 national visitors using the collections per year, 204 international visitors using the collections per year and an average of 479 454 data records provided to external users per year over a five-year period from 2017 to 2021 (NSCF 2022).

To encourage and support increased funding for NSCs, there is an argument that the scientific community must improve recognition of the role of NSCs in research so that NSCs can more effectively demonstrate their impact (Miller et al. 2020). While a large percentage of the respondents (64%) indicated that they inform the NSC once their research has been published and/or send the NSC a copy of the published paper, there is a lack of a standardised method of citation for collections and institutions for tracking publications. This has been one of the challenges associated with attribution for NSCs and a possible solution is to acknowledge NSCs along with their specimens through citation of the institutions (or their individual departments) by Digital Object Identifier (DOI, found in GBIF) in concert with complete voucher lists containing sample accession numbers (Miller et al. 2020).

Perceived or experienced barriers in relation to access and current performance in serving the needs of research

Many collections are understaffed or not staffed at all, and the loss of even a single staff member frequently results in a collection being neglected and unused (Hamer

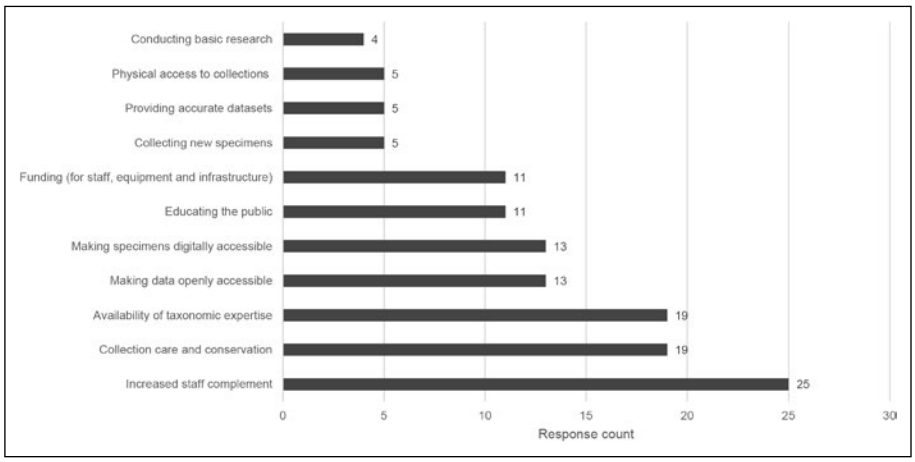

Figure 7. Areas in which natural science collections should improve.

2012). Staff shortages slow the distribution of specimen loans and provide fewer resources for visitors at a time when collections are being used by more researchers in increasingly diverse fields (Schindel & Cook 2018). Although the majority of respondents did not find the access procedure to specimens and data overly onerous and did not experience barriers to access, the majority of respondents indicated that very few collections fared extremely well (Table 1) in serving the needs of research and indicated that NSC current performance was linked to a lack of staffing and funding.

Institutions that hold NSCs have indeed experienced insufficient funds for operations and staffing from governing bodies to care for the collections under their control (Herbert 2001). The 1997 White paper on the conservation and sustainable use of South Africa's biological diversity stated that South Africa's museums and other collection-based institutions were facing serious funding problems, endangering existing collections and the professional staff of these institutions (Herbert 2001). A comprehensive inventory and review of South African NSCs commissioned by the National Research Foundation in 2011 also highlighted several significant challenges with the collections, which meant that their full potential as a national research infrastructure was not being realised and several important collections were at risk (Hamer 2012). The establishment of the NSCF is geared toward collaboratively addressing the challenges that NSCs face, with investment in infrastructure and research equipment upgrades, capacity development and appointment of short-term staff to address collections at risk. However, funding for operations and appointment of permanent staff at NSCs do not fall within the NSCFs ambit, but rather the national, provincial and municipal departments from which the collections receive the core of their funding.

Many (if not all) South African institutions housing NSCs that are accessible to external researchers have suffered further financial losses during the COVID-19 period. The impact of the pandemic on the South African economy has resulted in subsidy cuts from the national, provincial and municipal departments, which has exacerbated the financial constraints (NSCF 2022). Thus, the challenges of sustainability from a funding and staffing point of view have indeed worsened and will have a negative impact on the service delivery of NSCs to the research community. NSCF partner institutions report that these budget cuts inevitably result in the 'freezing' of what the government perceives to be non-critical vacancies, even though they are critical to the performance of NSCs in serving the needs of research. Examples of such vacancies include natural science curators, collection managers and research assistants.

How performance is judged and what the future expectations for performance are

The results indicated that respondents believed that the most important roles of NSCs were also the areas in which they should focus and improve on in future. These included collection care and conservation, documenting biodiversity, availability of taxonomic expertise, collecting new specimens and making data openly accessible. This echoes the views of curators who see 'the collections as serving a scientific and research purpose rather than a cultural or historical purpose, with taxonomic research and reference collection or identification value rated as the most important functions of the collections, and cultural, aesthetic, and tourism value rated as the least important' (Hamer 2012:2).

To improve and address gaps in collection care and conservation, the NSCF partner institutions have collaboratively produced policy guidelines, standards and procedures for collections and data management, published as a freely available Collections Management and Conservation Manual (NSCF 2021). The NSCF also developed a Collections Management and Conservation course linked to the manual, with webinars and tutorials accessible on the NSCF website at https://nscf.org.za/resources/collections-management/, in an effort to improve collection and data management practices across NSC institutions.

One of the main criteria for participation of collection institutions in the NSCF is that the collections and data are openly accessible to external researchers and students. This was agreed upon and accepted by the institutions that are participating in the NSCF (NSCF 2022). For data, the NSCF objective is that the specimen data sets of NSCF partner institutions will be made accessible through a single portal. While the development of this portal is under way, submission of data to the Global Biodiversity Information Facility (GBIF) platform is encouraged and technical support for this is provided for institutions that require this (NSCF 2022).

Although 'educating the public' scored low on overall importance compared to other roles, respondents believed that NSCs should improve in this area. This can be linked to the fact that collections are kept behind the scenes and, while NSCs have an underestimated value to society in terms of providing the foundational information to promote national/global economic, historic and scientific prosperity, communicating their value to society has not been given adequate attention. Although some NSCF partner institutions have made a concerted effort to focus on improving learner education and public understanding of the importance of NSCs, more work is required in this area (NSCF 2022). Specimens and natural history collections typically offer a perfect platform for the public to engage in science and support the collections through volunteer programs and community science activities (Sforzi et al. 2018). Through public education and outreach, training programmes and research collaborations, NSCs have the potential to increase participation of historically underrepresented groups in museum sciences, which can increase public investment, while benefiting participating communities (Miller et al. 2020). Promoting and collaborating with citizen science initiatives also hold, often untapped, opportunities for promoting the collections. Citizen science programmes like iSpot and iNaturalist encourage scientific enquiry and raise public awareness of the value of protecting the environment (Silvertown et al. 2015).

Practical implications

Lack of funding for operations and staff impedes the ability of researchers and other users alike in using NSCs to optimise their research and contribute to issues of societal concern. Political priority should be given to the long-term upkeep and ongoing assistance of institutional infrastructures by national, provincial and municipal government departments.

Limitations of the study

Due to the varied state of NSCs in the country, the study aimed to capture the general perceptions and experiences of users of NSCs, and not at an individual collection or institution level. The NSCF, through funding received from the Department of Science and Innovation, will be conducting comprehensive collection assessments during 2023, with an aim to address issues and challenges, and lobby for support at an individual collections level. Future research linking user perceptions and the outcomes of the assessments holds the potential to provide a three-hundred-and-sixty-degree view of the state of collections and recommendations for specific targeted interventions.

Limitations that could have affected the response rate negatively might be the limited period that the survey was available online (two months), as well as the distribution of the questionnaire by email and through social media only. Given the average number of users of NSCF partner NSCs of 1 192 per year (NSCF 2022), the response rate of the survey was 11%. This was comparable with the finding of an examination of response rates for web-based surveys conducted by Saunders et al. (2016), which revealed that online surveys received rates of response of 10 to 20 per cent.

Recommendations

- The scientific community should improve recognition of the importance of NSCs in research for NSCs to successfully demonstrate their influence. This would promote and support more funding for NSCs.
- A sustained commitment from partner NSCF institutions to work together to solve various challenges is required. This includes improvement in serving stakeholder needs, which will in turn assist with demonstrating the value of NSCs to policymakers in order to lobby for support and funding.
- NSCs should improve their outreach efforts and collaborations with stakeholders, including the public, learners and citizen science initiatives, to encourage appreciation and support of NSCs.

Conclusion

This study indicated the overall perception of the importance of NSCs and their accessibility to the student and researcher community in South Africa and internationally to be extremely important to their research. Access to physical specimens, associated data, staff expertise and formation of collaborations all directly contribute to research in the fields of taxonomy, nature conservation, evolution, agriculture, pest and disease control, environmental impacts of climate change, food security, solving crime and public health. In turn, users contribute to the curation process at NSCs and form research collaborations with collection staff. The scientific community can further support NSCs by improving

recognition of the role of NSCs in research so that NSCs can more effectively demonstrate their impact.

Lack of funding for operations and staff impedes the ability of researchers and other users alike in using NSCs to optimise their research and contribute to issues of societal concern. Political priority should be given to the long-term upkeep and ongoing assistance of institutional infrastructures. The establishment of the NSCF through the Department of Science and Innovation has made considerable strides in forming a network of institutions, which enable sharing of resources and expertise, working towards implementing international curation and data management standards across institutions, and advocating for open access data policies, as well as conducting research that answers questions of societal concern. The NSCF project is still in its infancy and will require a sustained commitment from partner NSC institutions to work together to solve various challenges, including improvement in serving stakeholder needs, which will in turn assist with demonstrating the value of NSCs to policymakers in order to lobby for support and funding. This is especially true within a country with competing priorities for basic service delivery, alleviation of poverty and high unemployment rates.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

S.R. (South African National Biodiversity Institute) conducted the data analysis and prepared the first draft of the paper. T.R. (Iziko Museums of South Africa), B.Z. (University of the Witwatersrand), M.S.M. (South African National Biodiversity Institute) and A.M. (South African National Biodiversity Institute) reviewed and edited the paper for final publication. All authors contributed to the development of the study.

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Supplementary Material

Annexure 1 – Survey Questions

Background information

1. Name: (will not be shared)
2. Email address: (only if you would like feedback on survey results)
3. Job title:
4. Where do you work:
5. South African resident (*yes/no*)
6. Other Affiliations, i.e. higher learning institute:
7. In which discipline do you work/conduct your research? (*taxonomy, evolution, ecology, molecular biology, conservation biology, other – please specify*)

Survey questions relating to Objective 1: perceived value of natural science collections (NSCs) to researcher community.

8. When required in your research, do you have access to museum specimens and associated data (physical or digital access)? (*yes/no*)
9. How often do you have access to or have contact with NSCs? (*daily/weekly/monthly/biannually/annually/less frequent*)
10. What type of specimens do you routinely work with? (*animal, plant, fossil, fungi, other – please specify*)
11. Has access to museum specimens directly contributed to your research? (*yes/no – specify how: loans, using lab space/equipment, students, images of types, associate researchers, other*)
12. If access to the museum specimens has directly contributed to your research, do you inform the museum once the research has been published and/or send them a copy of the manuscript? (*yes/no)*
13. To which of the following fields does/did your research that used natural science collections contribute? (*documentation and classification of biodiversity, evolution, nature conservation, environmental impacts of climate change, pest and disease control, solving crime, public health, food security, agriculture, if other, please specify*)
14. On a scale from 1 to 5 how important is access to NSCs (specimens, associated data and collaboration with NSC staff) to your research? (*rate on scale from 1 to 5: not at all – slightly – moderately – very – extremely*)
15. Has your access to the NSCs specimens contributed to the curation process and/or formed collaboration with NSC staff? (*yes/no/uncertain*)

Survey questions relating to Objective 2: perceived or experienced barriers in relation to access to natural science collections and associated data for use in applied research.

16. Have you ever been denied access to museum specimens? (*yes/no/I don't know, if yes reason: text answer*)
17. Do you find the access request procedure overly onerous? (*yes/no/uncertain*)

Survey questions relating to Objective 3: perceptions of NSCs current performance in serving the needs of applied research:

18. How do South African NSCs perform in providing services compared to other countries? (*Rate performance from 1 to 5: very poor – below average – average – above average – excellent*)
19. Are the collections you use for your research maintained properly? The physical curation of specimens are to an acceptable standard (*few collections, some collections, all collections*)
20. Are the collections you use for your research maintained properly? The data associated with the specimens are accurate, up to date and usable (*Few collections, some collections, all collections*)
21. In your opinion are NSCs funded appropriately? (*yes/no/uncertain*)
22. In your opinion are NCSs staffed appropriately? (*yes/no/uncertain*)
23. Overall, how happy are you with the services offered by NSCs? (*Rate from 1 to 5: not at all – slightly – moderately – very – extremely, comments:*)

Survey questions relating to Objective 4: how performance is judged and what future expectations for performance are:

24. What are the most important roles of NSCs? (*Choose up to 4: collection care and conservation, documenting biodiversity, collecting new specimens, supporting biological surveys, preservation of molecular samples, providing accurate datasets, making data openly accessible, making specimens digitally accessible, conducting applied research, conducting basic research, training students, availability of taxonomic expertise, contributing to science policy, educating the public, other (please specify*)
25. How do NSCs perform in the following areas? (*Rate performance from 1 to 5: very poor – below average – average – above average – excellent*):

- collection care and conservation,
- documenting biodiversity,
- collecting new specimens,
- supporting biological surveys,
- preservation of molecular samples,
- providing accurate datasets,
- making data openly accessible,
- making specimens digitally accessible,
- conducting applied research, conducting basic research,
- training students,
- availability of taxonomic expertise,
- contributing to science policy,
- educating the public.

26. In which areas/services should NSCs improve on most? (*open ended text answer*)

Annexure 2 – Research Consent Form

An online survey on user perceptions of natural science collections in South Africa

Please read and complete this form carefully. If you are willing to participate in this study, tick the appropriate boxes, sign with your full name and date the declaration at the end. If you do not understand anything and would like more information, please contact Shanelle Ribeiro (shanelle@nscf.org.za).

Note:

1. All information about participants will be treated in strict confidence and participants will not be named in any written work arising from this study.
2. Any data collected will be used solely for research purposes and will be erased on completion of the research.

I confirm that:

- I have read and understand the information contained in the Survey Research Proposal Page for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.
- I understand that my participation is voluntary and that I am free to withdraw from this study at any time without giving any reason and without any consequences to me.
- I understand that all information about me and my organisation will be treated in strict confidence and that participants and organisations will not be named in any written work arising from this study.
- I understand that any data collected will be used solely for research purposes and personal data of survey participant will be erased on completion of the research.
- I freely give my consent to participate in this research study and have been given a copy of this form for my own information.

Name and surname: . . . . . .

Date: . . . . . .

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A nomenclatural correction in *Colchicum* L. (Colchicaceae: Colchiceae) in southern Africa: two new combinations for *C. coloratum* J.C.Manning & Vinn., nom. superfl.

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The new combinations *Colchicum burchellii* (Baker) J.C.Manning & Vinn. and *C. burchellii* subsp. *pulchrum* (Schltr. & K.Krause) J.C.Manning & Vinn. are provided for the taxa currently known respectively under the names *C. coloratum* subsp. *burchellii* (Baker) J.C.Manning & Vinn. and *C. coloratum* J.C.Manning & Vinn., nom superfl.

Keywords: Africa; *Androcymbium* Willd.; nomenclature; taxonomy.

Introduction

The circumscription of the genus *Colchicum* L. (Colchicaceae: Colchiceae) was substantially enlarged by Manning et al. (2007) to include the largely sub-Saharan *Androcymbium* Willd. on the basis of their molecular phylogenetic analysis showing that *Colchicum* (including *Bulbocodium* L. and *Merendera* Ramond) constituted a clade that was deeply nested within *Androcymbium*. This relationship and its taxonomic implications have been confirmed by subsequent analyses (Nguyen et al. 2013). At the time, Manning et al. (2007) provided the necessary combinations in *Colchicum* for the taxa previously treated in *Androcymbium*. It has emerged, however, that they erred in their treatment of the species *A. burchellii* Baker and *A. latifolium* Schinz (= *A. pulchrum* Schltr. & K.Krause) and this is corrected here.

Nomenclature

Androcymbium burchellii Baker and *A. latifolium* Schinz (= *A. pulchrum* Schltr. ex K.Krause) were treated as two distinct species in *A.* series *Therocymbia* U.Müll.-Doblies & D.Müll-Doblies (Müller-Doblies & Müller-Doblies 1998, 2002). The two taxa appear to differ, however, solely in the colouring of the foliage leaves and the bracts, and *A. latifolium* was subsequently treated as a subspecies of *A. burchelli* under the name *A. burchellii* subsp. *pulchrum* (Schltr. & K.Krause) Pedrola et al. (2003). This taxonomy was followed by Manning et al. (2007) when they proposed new combinations for these taxa in *Colchicum*.

The two earlier names *Colchicum latifolium* Sibth. & Sm. (Sibthorp 1823) and *C. pulchrum* Herb. ex Baker (Baker 1879) preclude the transfer of both of these epithets to *Colchicum* (Turland et al. 2018: ICN, Art. *53.1*) and Manning et al. (2007) accordingly proposed the replacement name *C. coloratum* J.C.Manning & Vinn. for the taxon, with the two subspecies *coloratum* and subsp. *burchellii* (Baker) J.C.Manning & Vinn. The replacement name *C. coloratum* for the combined taxon is, however, nomenclaturally superfluous if this subspecific taxonomy is followed since the name *A. burchellii* Baker (Baker 1874), as a synonym, is available as an earlier epithet and should have been used (Turland et al. 2018: ICN, Art. *11.4*). The combination *A. burchellii* subsp. *pulchrum* (Pedrola et al. 2003) establishes the priority of this epithet at subspecific rank (Turland et al. 2018: ICN, Art. *11.2*). The correct combinations are provided below.

Should the two taxa be treated as distinct species, however, then the combination *C. coloratum* J.C.Manning & Vinn. is available for the component that includes the type of *A. pulchrum*.

Colchicum burchellii (Baker) J.C.Manning & Vinn., comb. nov. *Androcymbium burchellii* Baker in J. Bot. (British & foreign) 12: 246 (1874). *Colchicum coloratum* subsp. *burchellii* (Baker) J.C.Manning & Vinn. in Taxon 56: 879 (2007).

Colchicum burchellii subsp. **pulchrum** (Schltr. & K.Krause) J.C.Manning & Vinn., comb. nov. *Androcymbium pulchrum* Schltr. & K.Krause in Krause in Notizbl. Bot. Gard. Berlin 7: 522 (1921). *A. burchellii* subsp. *pulchrum* (Schltr. & K.Krause) Pedrola et al. in Bot. Macaron. IV Ci 24: 113 (2003). *Colchicum coloratum* J.C.Manning & Vinn. in Taxon 56: 879 (2007), nom. superfl. [as a new name for *A. pulchrum* Schltr. & K.Krause].

= *Androcymbium latifolium* Schinz in Bull. Herb. Boiss. 4: 415 (1896).

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The new combination *Coleus leemannii* (N.H.Hahn) A.J.Paton for *Rabdosiella leemannii* N.Hahn (Lamiaceae: Nepetoideae: Ocimeae)

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The new combination *Coleus leemannii* (N.Hahn) A.J.Paton is provided for *Rabdosiella leemannii* N.Hahn (Lamiaceae: Ocimeae) from South Africa, a species that was overlooked in the recent synopsis of subtribe Plectranthinae.

Keywords: new combination; nomenclature; South Africa; taxonomy

Introduction

The genus *Rabdosiella* Codd (1984) was established for two species of Lamiaceae: Ocimeae that differed from other genera in the tribe in their densely paniculate synflorescences with ascending branches; erect, shortly cylindrical and distinctly 10-nerved fruiting calyx with suberect or incurved teeth; and declinate, basally saccate corolla. *Rabdosiella* was considered to be allied to both *Plectranthus* L'Hér. and *Isodon* (Schrad. ex Benth.) Spach (Codd 1975, 1985). In addition to the type species *R. calycina* (Benth.) Codd from southern Africa, the genus also included the southeast Asian *R. ternifolia* (D.Don) Codd.

A later morphological and cytological analysis (Ryding 1993) concluded not only that *Rabdosiella* was polyphyletic, but that its continued recognition was not justified; the southern African species being best returned to *Plectranthus* as *P. calycinus* Benth. and the Asian species readily accommodated in the genus *Isodon* under the name *I. ternifolius* (D.Don) Kudô.

These findings were supported by the subsequent molecular phylogenetic analyses of plastid DNA regions in Ocimeae by Paton et al. (2004) and Paton et al. (2018), which retrieved *I. ternifolius* deeply embedded among other species of *Isodon* and *P. calycinus* nested in the genus *Plectranthus sensu lato* in a subclade that included species of *Pycnostachys* Hook. and *Holostylon* Robyns & Lebrun. It was clear from these analyses that substantial changes in generic circumscriptions in Ociminae–Plectranthinae were required to render the genera monophyletic. As a result of these findings, Paton et al. (2018) proposed that *Coleus* be expanded to include all species in the clade containing the type of the genus, *Plectranthus* be restricted to the clade sister to *Thorncroftia* N.E.Br. plus *Tetradenia* Benth., and the new genus *Equilabium* Mwany., A.J.Paton & Culham be described for the clade containing the mainly tropical African species and Indian species formerly included in *Plectranthus*.

This taxonomic proposal was followed by a nomenclatural synopsis of the genera *Coleus*, *Equilabium* and *Plectranthus* (Paton et al. 2019). *Rabdosiella* (type species only) was among several genera that were included in the expanded circumscription of *Coleus*, and the new combination *C. calycinus*

(Benth.) A.J.Paton for *R. calycina* was among the 130 new combinations that were provided in that genus. In its expanded circumscription, *Coleus* is a genus of 295 species of the Old World tropics and subtropics, diagnosed by an oblique-based calyx with the pedicel attached asymmetrically to the base of the tube opposite the posterior lip, and a strongly zygomorphic corolla with the upper lip mostly shorter than the cymbiform lower lip enclosing the declinate stamens (Paton et al. 2019).

Paton et al. (2019), however, overlooked the existence of a third species of *Rabdosiella* that had been described from southern Africa several decades after the revision of the genus by Codd (1985). *Rabdosiella leemannii* N.Hahn (Hahn & Bredenkamp 2007) is a narrow endemic of quartzitic substrates on the Soutpansberg and Blouberg in Limpopo, South Africa. It is morphologically very close to *R. calycina* and the two species are evidently an edaphic-allopatric species-pair. It falls within the currently expanded circumscription of *Coleus*, and we provide the necessary new combination for *R. leemannii* in *Coleus* here.

Coleus leemannii *(N.Hahn) A.J.Paton*, comb. nov. *Rabdosiella leemannii* N.Hahn in Hahn & Bredenkamp in Bothalia 37(1): 37 (2007). Type: South Africa, Limpopo, 2329 (Polokwane): Soutpansberg, Lejuma, (–AB), 13 Apr 2005, *N. Hahn 2086* (ZPB, holo.; PRE!, PRU, iso.).

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First record of the North African *Launaea arborescens* in southern Africa

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The first record of the non-native, naturalised *Launaea arborescens* (Batt.) Murb. in the Namib Desert raised questions of its origin and whether or not it could pose a threat to the indigenous vegetation. The North African plant was introduced in a forestry nursery in the Kuiseb Delta, and some individuals were also planted outside the nursery in the early 1970s. They have maintained a likely viable population for nearly 50 years but have so far not been observed elsewhere and thus appear not to be spreading.

Keywords: exotic plant; forestry; invasive alien; Kuiseb Delta; Namib Desert.

Introduction

Invasive alien plants displace native vegetation and change ecosystems all around the world (Chapin et al. 2000). Because of their harsh environments, deserts have so far been spared the deluge of invading plants typically encountered on islands and other ecosystems that are easy to invade (e.g. Duarte et al. 2005; Davies et al. 2011). However, even deserts are not immune to these invasions, and particularly habitats that are moister and receive water regularly such as ephemeral rivers, seeps and fountains can provide suitable habitats for invaders. All western-flowing rivers of the Namib Desert, for example, are locally invaded by numerous exotic species such as *Datura, Prosopis, Nicotiana* and *Ricinus* species (e.g. Boyer & Boyer 1989; Auala et al. 2014). The level of infestation varies and is currently localised, but the invasives are nevertheless there. The densest infestations are usually in the vicinity and downstream of settlements where human activities such as woodcutting and overgrazing have disturbed the natural balance.

However, exotic, non-native plants also turn up in unexpected places – for example, in the northwest corner of the Kuiseb Delta, far from settlements and in an area hardly ever touched by the infrequent Kuiseb River floods. This was not recognised for decades, though the spiky desert shrub *Launaea arborescens* (Batt.) Murb. was found here recently and this raised numerous questions: How did it get there? As this plant is not known in Namibia, has it been reported elsewhere in southern Africa and in similar habitats? Has it established a viable population, and does it pose a threat as an invader? Whether *Launaea arborescens* has established a viable population in the Kuiseb Delta was investigated by posing two subquestions: What is the extent and size of the population, and what is the size distribution of the plants. These questions are addressed and background information on the plant is presented in this article.

Materials and methods

The study area

The study area is situated in the central Namib Desert, in the Erongo Region of Namibia, just south of Walvis Bay (Figure 1). The central Namib lies between the ephemeral Ugab and Kuiseb rivers, and is bounded by the Atlantic Ocean in the west and the escarpment in the east. It falls into the Desert Biome (Irish 1994; Rutherford & Westfall 1994). The coastal zone at Walvis Bay lies within a 'cool desert' region of Namibia, an environment influenced by the South Atlantic Anticyclone, the cold, northward-flowing Benguela current and the divergence of the southeast trade winds along the coast. According to Mendelsohn et al. (2002), average daily temperatures vary between a minimum of 10°C in the coldest month and a maximum of 24°C in the warmest month in the area, although temperatures as low as 2°C have been recorded (Jürgens et al. 2013). Temperatures are variable both daily and seasonally, with the highest temperatures recorded during 'berg wind' episodes, when cold air from the interior flows towards the coast and is heated by compression (catabatic wind), resulting in temperatures of up to 40°C or more. Southerly, westerly, and southwesterly winds are prevalent, and are usually strongest between late afternoon and early evening (Mendelsohn et al. 2002).

Rainfall in the Namib Desert is highly variable, unpredictable and patchy. It varies from 0 to approximately 100 mm per annum, increasing from west to east. In the west, where precipitation from rain is lowest, fog is carried inland by wind passing over the cold Benguela current of the Atlantic Ocean. It is a vital source of moisture for many desert organisms. Walvis Bay has a mean annual rainfall of 13.5 mm, with most rain falling in summer between January and April, and the wettest month being March when about 50% of annual rainfall is recorded (Atlas of Namibia Team 2022).

Coastal dune hummocks are the prevalent habitat in the study area. They are important components of coastal ecosystems and are formed mainly by the shrub *Salsola nollothensis* Aellen, which traps windblown sand and results in the vertical formation of dune hummocks. These sand-stabilising hummocks are ecologically important, providing shelter, forage and habitats for the local fauna and flora.

The study plant

Launaea arborescens is growing in a dune hummock area, where hummocks are of medium size (1–2 m high). Most of the other plants in this area grow on these hummocks, but also in valleys in between. However, they are absent in valleys where the ground seems to be wet regularly (i.e. closer to the water table and likely saline conditions).

Vegetation cover in the dune hummocks averages approximately 20% and includes, in addition to *L. arborescens*, the shrubs *Salsola nollothensis* and *Lycium tetrandrum*, as well as the herb *Crotalaria colorata*, the grass *Odyssea paucinervis* and the sedge *Scirpus dioicus*. *Phragmites australis* reed beds adjoin the area to the west. A few isolated trees such as *Tamarix usneoides*, the exotic *Acacia cyclops, Myoporum serratum* and *Eucalyptus camaldulensis* appear to have escaped from the forestry nursery at Wortel and are also found in the hummock area nearby.

The plant was originally identified by recognition of its similarity in flower structure to its closest indigenous relatives in Namibia, namely *Launaea intybacea* (Jacq.) Beauverd and *Lactuca inermis* Forssk. They all belong

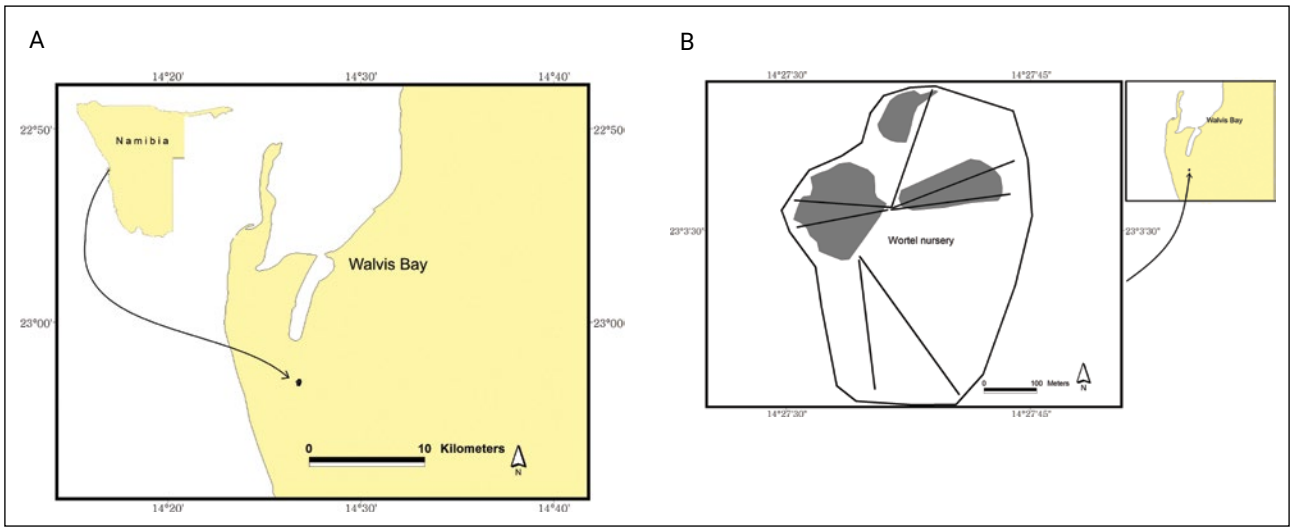

Figure 1. A, Position of the study area near Walvis Bay in Namibia; B, Extent of field survey of *Launaea arborescens* (outline), survey transects (lines), and area of occupancy of population (grey polygons) in the Kuiseb Delta in Namibia.

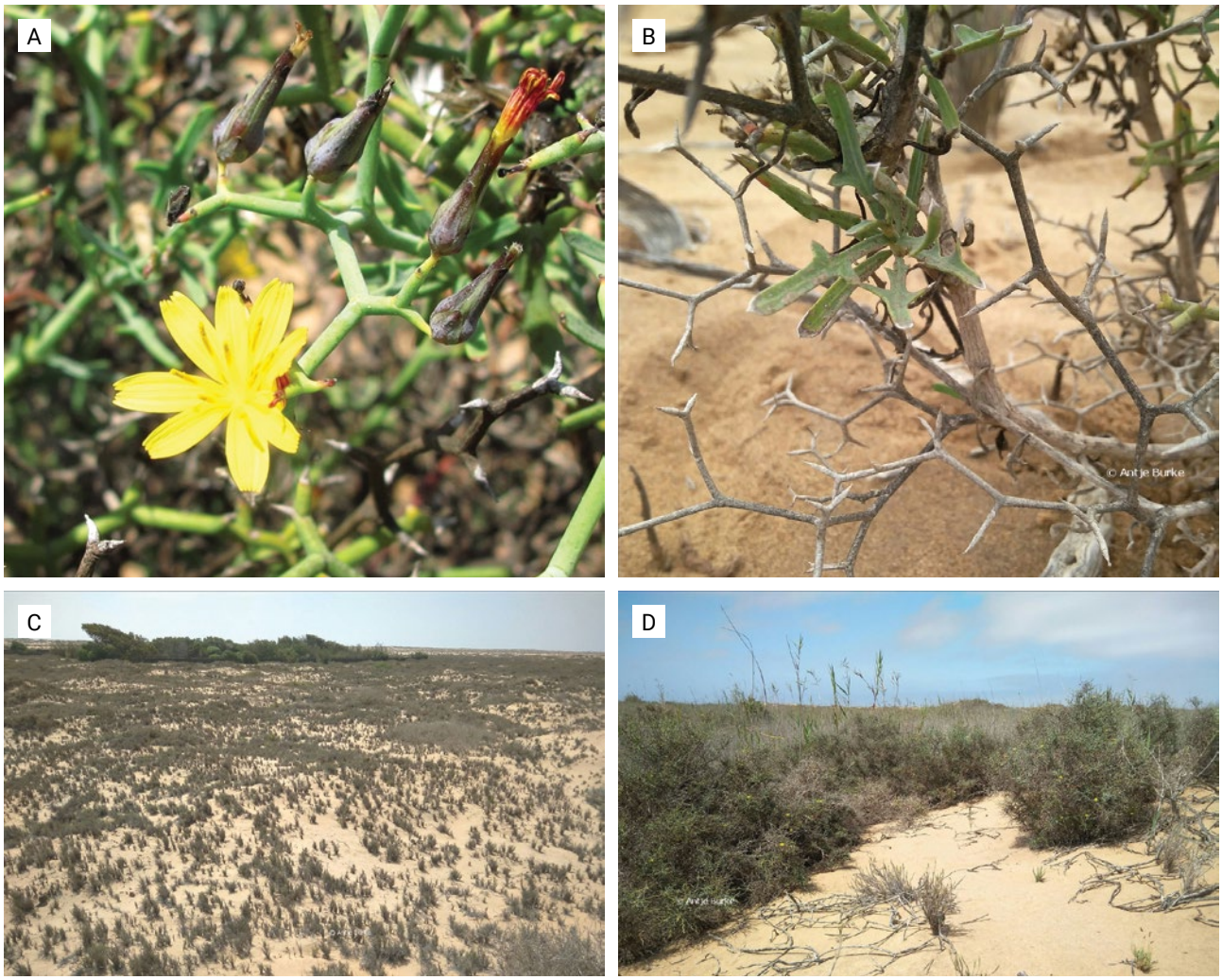

Figure 2. *Launaea arborescens* in the Kuiseb Delta; A, flower; B, leaves; C, general habitat with Wortel nursery in background; D, plant in habitat. Photographs: A, C. Mannheimer; B–D, A. Burke.

to the Tribe Cichorieae within the daisy family (Asteraceae), which is characterised by the presence of milky latex and (in most species) bisexual ligulate florets with five teeth at the tips (i.e., strap-shaped 'petals' with toothed tips. This tribe includes many edible, medicinal and weedy species, in genera such as *Lactuca* (lettuce), *Cichorium* (chicory/endive), *Sonchus* (milk thistle) and *Taraxacum* (dandelion). *L. arborescens* is native to Algeria, Baleares, Canary Islands, Cape Verde, Madeira, Mauritania, Morocco, Saudi Arabia and Spain (GBIF, January 2021; https://www.gbif.org).

Launaea arborescens (Batt.) Murb. (adapted from Kilian 1997)

Dense, intricate, spinescent, irregularly hemispherical shrub, usually up to 1 m high, almost leafless (Figures 2 and 3). Branches terete, divaricately and intricately branched, with distinct joints and spinescent terminal segments; young branches stiff, green, smooth, waxy, becoming greyish brown with age. Latex whitish with unpleasant smell. Leaves clustered at the bases of the lower branches, somewhat succulent, blue-green, mostly narrowly spathulate to linear in outline, soon deciduous, higher up the shoots reduced to inconspicuous, ovate-acute bracts. Capitulae ('flowers') bright yellow, up to 16 mm in diameter, always terminal and single on the peduncles, which persist as spines after shedding of the capitulae. Capitula with 7–19 florets ('petals'), each with five 'teeth' at the tip. Involucre up to 15 mm long, cylindrical to conical. Achenes with 5 main ribs accompanied by 2 secondary ribs, with transverse, roundish, and tuberculate wrinkles, often somewhat powdery-papillose, brown. Pappus 5–8 mm long, comprising numerous white, setaceous rays.

A specimen (*Antje Burke AB20007*) was deposited at the National Botanical Research Institute in Windhoek, which is the first record of the plant in southern Africa.

Review of information

To establish whether this plant is recorded for the first time in southern Africa, global biodiversity databases

Figure 3. *Launaea arborescens* in the Kuiseb Delta near Walvis Bay. Photograph: A. Burke.

(GBIF, GloNAF), citizen science databases (iSpot, iNaturalist), Namibia's National Botanical Research Institute records and the authors' own records of over 30 years of plant survey work across the entire Namibia were consulted.

Extent of the population

The extent of the population was established by mapping the occurrence of the plant in the field. The locality of all plants encountered was recorded with a geographic positioning device (GPS). These were then plotted, and the apparent centre determined, which appeared to be just outside the entrance to the nursery. From this centre transects were walked in all compass directions and the distribution boundary recorded. Seven transects of 150–320 m length were walked. The plants were counted along the transects and in the areas between these transects, which were also searched thoroughly. The nursery area was also surveyed (Figure 1B). The GPS positions of recorded individuals and groups of individuals were plotted and the area of occupancy (i.e., the habitat where the plant grows) mapped (Figure 1B).

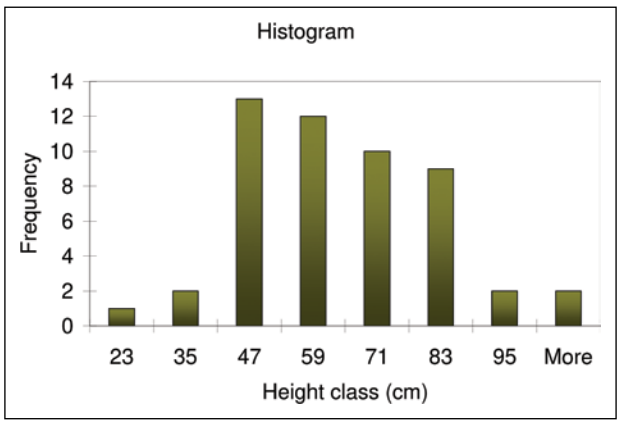

Figure 4. The distribution of mean height measurements of 51 random individuals of *Launaea arborescens* in the Kuiseb Delta.

Size distribution of the population

The dimensions of 51 haphazardly selected plants (by walking backwards and throwing a bottle over the shoulder and then selecting the nearest plant to where the bottle landed) were measured. At each plant, height and diameter of the plant was measured three times, resulting in six measurements for each individual. Repeated measurements were used to accommodate the irregularity of plant shape and the irregular surface. Height measurements were averaged per individual and plotted in a histogram (Figure 4). The data are provided in the supplementary (Appendix 1).

Results

The species' history in Namibia

This species' history in Namibia is directly linked to the forestry nursery at Wortel, which was established in the early 1970s to raise and test plants for dune stabilisation around Walvis Bay (Piet le Roux, pers. comm. January 2021). Considerable research was undertaken by Mr le Roux at the time, and plants from other deserts were tested as suitable candidates to stabilise the dunes around Walvis Bay (Le Roux 1974). As seawater irrigation was planned, only plants with a high salt tolerance were used. Seeds of selected species were obtained from North Africa and Australia, and this included the North African desert shrub *Launaea arborescens* (Le Roux 1974). Initially plants were raised at the forestry nursery in Grootfontein (19°30'S and 18°15'E) and then transplanted into the Walvis Bay dunes. However, a small nursery was eventually established in the Kuiseb Delta at Wortel (23°05'S and 14°45'E), and plants were also raised there and planted out in pilot sites around the nursery, on dunes in the delta and along the road between Wortel and Walvis Bay. Initially the plants were irrigated with treated sewage water or seawater (Le Roux 1974), but no watering has taken place for decades now. The current *Launaea arborescens* population originates from these trials.

Considering that this plant was introduced some 50 years ago and still likely maintains a sizable population, it is clear that the plant is well adapted to the conditions in the Kuiseb Delta (Figure 3). However, the question arises whether these are still the original plants that were planted in trials, or if the species has established a self-sustaining, or perhaps even an invading population. Unfortunately, Mr le Roux could not enlighten us on the exact number of plants per species used in the trials, nor the precise localities where these were planted in the study area. However, this is not surprising after such a long time.

Extent of population

The plants are growing to the west and north of the former forestry nursery at Wortel in three distinct local populations. The three mapped areas amount to approximately 3.49 ha. Some 360 individuals were counted. Although the survey aimed to count all individuals, there are possibly more plants as some may not have been discovered in the uneven terrain and between the other plants present. However, the survey provides a reasonably good estimate.

Size distribution of the population

The extent and size of the population contribute to determining whether a population can be considered self-sustaining and therefore viable. Isolated individuals of a species far from other individuals of the same species can hardly present a viable population, particularly if these are out-crossers and therefore rely on genetic exchange with other individuals (Harper 1977; Drew & Kaufman 2013). The size distribution of the plants provides an indication of the population status (Figure 4). A healthy population contains individuals of all size classes, usually with the majority in the medium range of sizes measured (Harper 1977; Barbour et al. 1987).

To facilitate incorporation of this new record into global databases (e.g., the Global Naturalized Alien Flora database (GloNAF) (Pyšek et al. 2017), we provide the available information on this species in Appendix 2. The parameters proposed by Zengeya and Wilson (2020) were used to structure the data as well as the criteria provided by the IUCN (2020), with adaptations to the terminology suggested by Groom et al. (2019).

Other observations

Launaea arborescens was not the only plant tested for dune stabilisation in Walvis Bay (Le Roux 1974) and other non-native plants in the study area were also noted. Outside the nursery area a few individuals of *Acacia cyclops, Eucalyptus camaldulensis* and *Myoporum serratum* were observed. Whether these had been planted originally at their current position or escaped from the nursery and established new plants, could not be established. There were also more trials along the road to Walvis Bay where various non-native *Atriplex* species had been planted. These have persisted for some 50 years without management intervention.

Discussion

Does *Launaea arborescens* form a viable population?

The current area of occupancy of the plant of over 3 ha and the well over 300 individuals suggest that this may be a viable population. Also, a bell-shaped size-class distribution of plant sizes is considered indicative of a healthy population in long-lived species such as trees and shrubs (Cousins et al. 2014). *L. arborescens* size class distribution in the Kuiseb Delta is close to this bell-shaped slope with a peak in the medium-sized height class (Figure 4).

Furthermore, the fact that this plant has been present in the Kuiseb Delta for several decades without irrigation or any other management, suggests that these now form a viable population. Although the plants were initially introduced and irrigated (Le Roux 1974), no maintenance has taken place for at least 40 years. This supports the notion that they may have established a self-sustaining population.

Is there a threat of invasion?

Because no detail was available regarding the number of plants initially planted on a trial basis, or exactly in which areas, it is impossible to establish whether the population has grown or spread. The question of whether the plant has invaded natural areas and replaced indigenous species, or whether they have only maintained their presence in the area where they were initially planted, cannot be answered. Although the plant has not been found in the broader area, monitoring of the population is required over several years (likely decades) to establish whether the plant does form a threat. This study provides a baseline against which future monitoring could be evaluated. However, the fact that the plant has to date also not been found further afield, suggests that it has not spread. However, there have been disturbances in the area since the field survey in the form of widening the two-spoor track to the nursery and beyond with a dozer to more than double the original width, which opens new areas to be occupied by the plant. Human disturbances such as the creation of roads often provide convenient pathways for invasive species (e.g. Higgins et al. 1999; Kumschick et al. 2020; Zengeya & Wilson 2020) and monitoring the plant now may be opportune timing.

Regarding the aspect of potential displacement of indigenous species, the dominant native plants in the surrounding area, which *Launaea arborescens* could have displaced, are *Salsola nollothensis* and *Lycium tetrandrum*. They occupy the same habitat and possibly the same niche, both being shrubs and in the case of *L. tetrandrum*, with a very similar growth form. The Namib-endemic *Crotalaria colorata* is a multi-seasonal herb and occupies a different niche. It grows in between the *L. arborescens* shrubs and is unlikely to have been displaced by *L. arborescens*.

The effect this exotic plant has on the environment (e.g., soils, water availability) and animals associated with (potentially displaced) indigenous plants in the area, is unknown and may deserve study. It is well known that exotic plants have the potential to alter the environment in many ways (e.g., Chapin et al. 2000) and this aspect should thus not be dismissed. In terms of potential pathways, like many Asteraceae, the diaspores are wind dispersed and this could potentially enable the plants to spread to new habitats.

At present monitoring rather than eradiation is proposed as management. As the risk of the plant spreading is currently considered low, the plant presents an interesting historic record of past forestry management practices and should thus not be eradicated, unless it can be shown that it does spread and presents a threat to the natural vegetation. Also the number of plants and extent of area occupied are small and thus eradication would be easy, if necessary.

Conclusions

It has taken 50 years for some 360 plants to establish and maintain a population in a very localised (3.49 ha) area and *Launaea arborescens* has so far not been found anywhere else in the Kuiseb River or other rivers or habitats in the Namib, or even southern Africa.

At present we consider the occurrence of the North African *Launaea arborescens* in the Kuiseb Delta an interesting record and testimony of the efforts that were made some 50 years ago to find solutions to the problem of sand intrusion into Walvis Bay. However, we encourage people to look out for this plant and report any further distribution records. Should plants appear in places other than near the Wortel nursery or former dune stabilisation trial sites, further study regarding the plants' invasive potential is recommended.

Acknowledgements

We are indebted to Piet le Roux for sharing historical information on activities related to sand stabilisation around Walvis Bay. An anonymous reviewer provided constructive comments, suggestions and references, which helped us to greatly improve the manuscript.

Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

A.B. undertook the field work and analysis and developed the structure of the paper. C.M. identified the plant, provided taxonomic information and wrote part of the paper. Both authors corresponded with P. le Roux to obtain background information.

Ethical considerations

This article followed all ethical standards for research without direct contact with human or animal subjects.

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Data availability statement

Data sharing is not applicable to this article.

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Supplementary Material

Appendix 1

Position and dimension measurements (in cm) of *Launaea arborescens* individuals in the Kuiseb Delta in Namibia (LAT S= latitude south, LON E= longitude east, H1–H3= height measurements in cm, L1–L3= length measurements in cm).

NO.	LAT S	LON E	H1	H2	H3	L1	L2	L3
1	-23.05808	14.45993	26	21	23	34	28	31
2	-23.05806	14.45994	64	53	58	70	67	68
3	-23.05804	14.45993	63	54	42	84	54	69
4	-23.05805	14.45992	67	42	43	86	71	62
5	-23.05803	14.45993	118	110	108	168	124	157
6	-23.05804	14.45989	40	39	32	43	58	45
7A	-23.05802	14.4599	57	47	50	72	79	86
7B	-23.05802	14.4599	56	50	45	58	48	56
8	-23.05801	14.4599	67	52	58	125	107	85
9	-23.05798	14.4599	27	20	18	34	32	30
10	-23.05799	14.45991	53	56	54	56	60	48
11	-23.05799	14.45991	54	53	41	74	95	78
12	-23.05799	14.45992	27	27	29	26	31	24
13	-23.05796	14.45996	98	80	85	109	117	109
14	-23.05798	14.45996	48	50	42	48	36	60
15	-23.05798	14.45998	34	30	27	52	26	42
16A	-23.05795	14.45999	64	56	58	132	80	78
16B	-23.05795	14.45999	42	39	34	32	39	42
17	-23.05795	14.45998	76	92	82	112	112	77
18	-23.05793	14.45998	48	45	42	48	54	52
19	-23.05794	14.45998	78	67	64	98	96	105
20	-23.05793	14.45998	77	80	90	100	107	100
N-01	-23.056073	14.460591	49	45	44	58	35	54
N-02	-23.056095	14.460461	68	56	48	136	95	132
N-03	-23.0561	14.460515	107	104	100	132	170	150
N-04	-23.056119	14.460367	97	88	77	138	77	132
N-05	-23.056119	14.460367	53	37	44	63	39	28
N-06	-23.056119	14.460367	83	92	78	116	121	106
N-07	-23.056271	14.460307	47	45	38	57	58	46
N-08	-23.056277	14.460316	45	32	40	36	23	21

NO.	LAT S	LON E	H1	H2	H3	L1	L2	L3
N-09	-23.05627	14.460281	53	47	42	63	57	62
N-10	-23.056283	14.460289	53	42	40	65	67	74
N-11	-23.056436	14.460311	65	68	62	68	50	69
N-12	-23.056382	14.46035	82	94	99	195	160	165
N-13	-23.056366	14.460312	57	56	58	96	80	74
N-14	-23.056322	14.460399	75	64	73	170	106	110
N-15	-23.056649	14.46024	65	50	50	63	67	54
N-16	-23.056708	14.460389	74	77	63	97	82	60
N19A	-23.057196	14.460645	36	27	36	40	47	39
N-16A	-23.057622	14.462044	53	38	49	49	54	41
N-17	-23.057575	14.461987	106	95	92	164	135	154
N-18	-23.057418	14.461989	60	59	50	73	65	64
N-19	-23.057142	14.461618	49	44	38	33	35	39
N-20	-23.057799	14.460538	108	93	112	186	180	160
N-21	-23.057669	14.460744	92	81	83	154	117	15
N-22	-23.057588	14.461224	142	120	124	202	215	172
N-23	-23.057582	14.46127	52	54	49	53	41	47
N-24	-23.057616	14.461241	36	22	27	35	33	23
N-25	-23.057627	14.46127	34	32	30	41	48	42
N-26	-23.057681	14.461358	15	17	14	16	14	12
N-27	-23.05774	16.461307	70	65	68	115	107	110

Appendix 2

Summary statistics of the first record of *Launaea arborescens* in Namibia.

Scientific name	*Launaea arborescens*
Taxon ID	AB20007
Vernacular name	*mol-albina* (Algerian Arabic); wicked dandy, barbed wire brush (English); *aulaga*, *cardaviejo*, *jadionda*, *rascavieja*, *volavientos* (Spanish)
Regulatory listing	Unlisted[1]
Is native	False
Occurrence status	Present
Degree of establishment	C3[2]
Introduction status	Naturalised
Pathway	Forestry planting
First record	1974
Range Broad Admin	Erongo Region
Range QDSC	1qds: 2314AB
Range Exact	3.49 ha
Organism Quantity	~360
Impact EICAT	Data deficient
Risk assessment	No
Realm	T[3]

1 New record for Namibia – so not listed on national plant species list, which also provides the status of each taxon.
2 Individuals surviving outside of captivity or cultivation in a location, reproduction occurring, and population self-sustaining.
3 T= terrestrial

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Bothalia – African Biodiversity & Conservation.

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Full length articles report on complete, comprehensive pieces of original research, as well as reviews, strategies or innovative case studies in any field of work aligned with the scope of the journal. Full length articles include a maximum of 8 000 words and 60 references.

Short communications are concise reports on narrow investigations. These include new species descriptions. They have a maximum of 2 000 words and 30 references.

In the case of reviews, strategies and short communications, not all of the headings and subheadings specified below may be relevant. In such cases authors will need to use their discretion in selecting appropriate headings.

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Manuscripts must be submitted as an MS Word document. Documents compiled in other software, including Google Documents, cannot be accepted.

Low resolution versions of figures and tables can be inserted into the document. High resolution of figures must, however, also be included separately, with each figure as a separate, appropriately labelled file (see details of requirements for figures below).

Please do not use hidden formatting, including character styles in the manuscript. Also avoid nested tables and text boxes. Many of these cause corruptions in the design software, and can usually be avoided if authors refrain from copying and pasting from various sources, including other MS Word documents.

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- **Font:**
  - **Font type:** Times New Roman
  - **General font size:** 12pt
- **Line spacing:** 1.15
- **Headings:** Ensure that formatting for headings is consistent in the manuscript.
  - First headings: normal, bold and 14pt
  - Second headings: normal, bold and 12pt
  - Third headings: normal, underlined and 12pt
  - Fourth headings: normal, bold, running-in text and separated by a colon, and 12pt.

Scientific names: Names of genera and infrageneric taxa are italicised, with the author citation not italicised. Exceptions include specific cases in taxonomic treatments (see details of such manuscripts below); new taxa in the abstract; and in checklists where the position is reversed – correct names are not italicised and synonyms are italicised. Names above generic level are not italicised. The complete scientific name of a species, as well as the author citation should be given at the first mention in the text. The generic names should be abbreviated to the initial thereafter, except where references to other genera with the same initial could cause confusion.

Authors of botanical names are abbreviated according to Authors of Plant Names (Brummitt & Powell 1992, Royal Botanic Gardens, Kew).

In names covered by the International Code of Zoological Nomenclature, the date of publication should be separated from the authority by a comma (e.g. *Anthomyza bellatrix* Roháçek, 1984). When a species or subspecies is transferred to a genus other than that in which it was first classified, the original authority, including the date, is placed in parentheses.

Adjectives and nouns derived from genus names become Roman with a lower case initial (e.g. *Felis*→feline, *Libellula*→libellulids, *Streptococcus*→streptococcal infection). Those derived from higher taxonomic groups also begin with a lower case letter and are presented in Roman (e.g. Ostracoda→ostracods, Cactaceae→cacti).

A scientific name given at its first mention after a vernacular name should be separated from it by a comma if the two names are exact synonyms (e.g.…the two-spotted cricket, *Gryllus bimaculatus*,…), but not if the vernacular name may apply to more than one species (e.g. the starfish *Asterina pectinifera*, the medaka *Oryzias latipes*).

Abbreviations should be used sparingly but consistently. No full stops are placed after abbreviations ending with the last letter of the full word, after units of measure,

after compass directions, after countries and after well-known institutions.

FIGURES AND TABLES

The word Figure should be written out in full and should begin with a capital F, in both the text and captions.

Figures (original or electronic submissions):

- Figures should be planned to fit, after reduction, into a width of either 80, 118 or 165 mm, with a maximum vertical length of 230 mm. Allow space for the caption in the case of figures that will occupy a whole page.
- Graphics, i.e. drawings, graphs or photographs, should be submitted as separate files. Low resolution copies of the figures should be included in the manuscript for review purposes.
- If extensive changes to image files are proposed by the editor, the author will be contacted and the specific image file will have to be re-submitted after the indicated corrections have been implemented.
- Scale bars or scale lines should be used on figures where relevant.
- Captions should not be added as part of the figure file. Number captions clearly and correctly and include either in the main text close to where the figure should be inserted or as a list of captions at the end of the text; not as a separate document.
- Authors wishing to use illustrations already published elsewhere must obtain written permission before submitting the manuscript and provide this to the editor at the time of submission, along with appropriate acknowledgements.
- Do not resample low resolution images to a higher resolution.
- Mosaics should be submitted as separate photographs as TIF/JPG files at 600 dpi or higher. A mock-up of the layout should also be submitted. Final layout of the mosaic will be done by our graphics department. Do not number the original images, but do include a scale bar. Indicate the lettering on the mockup and not on the original photographs.
- Manuscripts for which the figures, including line drawings, photographs, graphs and histograms, and maps, do not comply will be rejected for design and layout, even though the paper was accepted for publication, until such time that the authors can provide suitable images. This can significantly delay publication.

Line drawings:

- The original artwork should be in jet-black Indian ink, on fine art paper, 200 gsm. Lines should be clear enough to accommodate reduction. Do not use draughtman's film.
- Drawings in pencil will not be accepted.
- Provide original drawings electronically as bitmap TIF files, 1200 dpi.
- At the request of the author, the Graphic Design Section of SANBI will assist with the scanning of original material. Authors wishing to have the originals of figures returned must inform the editor in writing and mark each original 'To be returned to author'.

Photographs:

- Provide photographs electronically as either TIF or JPG files, 600 dpi or higher.

Graphs and histograms:

- The typeface for all graphs and histograms is Arial.
- Provide graphics originated in CorelDraw (version 16 or lower), as a .CDR file.
- Graphs and histograms generated in MS EXCEL or MS Word, should be provided as is. File conversion into the correct format will be accommodated by SANBI Graphics.
- Images generated in other programmes should be submitted as TIF or JPG files at a resolution of 600 dpi or as encapsulated postscript files (.EPS). If graphs and histograms are submitted in colour, please ensure that the shading used is easily discernible once the file is converted to grayscale.

Maps:

- It is strongly recommended that taxonomic articles include dot maps as figures to show the distribution of taxa. If maps will be reduced to column width (80 mm), the symbols and numbers used must be large enough to accommodate the reduction. The maps should show: numbered grid lines of latitude and longitude; the provinces of South Africa; and a scale line. Maps of neighbouring countries should be treated in the same way, with bordering states clearly labelled. For orientation purposes, a small inset map should appear in a corner of the figure.
- Submit maps electronically as either TIF or JPG files, 600 dpi or higher.
- ArcView GIS maps are acceptable. The layout representing all the appropriate themes (including grid lines) should be submitted as an encapsulated postscript file (.EPS).
- If maps are submitted in colour, please ensure that the shading used is easily discernible once the file converted to grayscale.

Tables:

- Tables should be drawn up in MS Word and not copied and pasted from other software such as MS Excel.
- Avoid copying and pasting text into the table as this often results in nested tables that are problematic to format and edit. Type in all text from scratch.

- Do not submit tables as text with separators such as tabs or commas, submit as MS Word standard tables.
- Do not include text boxes in table cells, type text directly in the primary table cell.
- Use Times New Roman 12pt if possible. However, should the width of the columns and the amount of text make this difficult, the size of the font may be reduced to no less than 9pt.
- If possible, present tables in portrait format. However, if tables must be presented in landscape format, use section breaks before and after the tables to separate it from the main text.
- Do not stretch the table to beyond the size of the paper on screen.
- Use the background fill function to shade cells if necessary. Do not use text highlights.

STRUCTURE OF YOUR ARTICLE

Page 1:

The format of the compulsory cover letter forms part of your submission and is on the first page of your manuscript and should always be presented in English. You should provide all of the following elements:

- **Article title:** Provide a short title of 50 characters or less.
- **Full author details:** Provide title(s), full name(s), position(s), affiliation(s) and contact details (postal address, email, telephone and cellular number) of each author.
- **Corresponding author:** Identify to whom all correspondence should be addressed to.
- **Authors' contributions:** Briefly summarise the nature of the contribution made by each of the authors listed.
- **Summary:** Lastly, include a list containing the number of words, pages, tables, figures and/or other supplementary material with the submission.

Page 2 and onwards:

Title: The article's full title should contain a maximum of 95 characters (including spaces).

Abstract: The abstract, written in English, should be no longer than 250 words and must be written in the past tense. The abstract should give a succinct account of the background, objectives, methods, results and significance of the findings/conclusion

Do not cite references in the abstract and do not use abbreviations excessively in the abstract.

The following points serve as a guide for presenting your manuscript in a well-structure format:

Introduction: The introduction contains two subsections, namely the background section and the literature review.

- **Background:** This section should be written from the point of view of the readers, including those without specialist knowledge in that area and must clearly state and illustrate the introduction to the research and its aims in the context of previous work bearing directly on the subject. The Background section to the article normally contains the following five elements:
  - **Key focus:** A thought-provoking introductory statement on the broad theme or topic of the research.
  - **Context:** Provide the context to the study, which can include the conceptual framework or explain the role of other relevant key variables in this study.
  - **Trends:** Cite the most important published studies previously conducted on this topic or that have any relevance to this study (provide a high-level synopsis of the research literature on this topic).
  - **Objectives:** Indicate the most important controversies, gaps and inconsistencies in the literature that will be addressed by this study. In view of the above trends, state the core research problem and specific objectives that will be addressed in this study.
  - **Contribution to field:** Explanation of the study's academic (theoretical and methodological) or practical merit and its importance (provide the value-add or rationale for the study).
- **Literature review:** The literature review is the second subsection under the Introduction and provides a brief and concise overview of the literature under a separate second-level heading, e.g. literature review. A synthesis and critical evaluation of the literature (not a compilation of citations and references) should at least include or address the following elements (ensure these are in the literature review):
  - Definitions of all key concepts.
  - A critical review and summary of previous research findings (theories, models, frameworks, etc.) on the topic.
  - A clear indication of the gap in the literature and for the need to address this void.
  - A clearly established link that exists between formulated objectives and theoretical support from the relevant literature.

Research method and design (first-level heading):

The methods should include:

- **Materials (second-level heading):** Describe the type of organism/s or material/s involved in the study.
- **Study site (second-level heading):** Describe the site and setting where your study was conducted.
- **Design (second-level heading):** Describe your experimental design clearly. Note: Additional details can be placed in the online supplementary location.
- **Procedure or Methods (second-level heading):** Describe the protocol for your study in sufficient detail (with a clear description of all interventions and comparisons) so that other scientists could repeat your work to verify your findings.

- **Analysis (second-level heading):** Describe how the data were summarised and analysed. Additional details can be placed with the online supplementary information. Do not include lists here as they will be published as supplementary material.

Ethical considerations (first level heading):

- **Ethical clearance (second-level heading):** Articles based on the involvement of animals and/or humans must have been conducted in accordance with relevant national and international guidelines. Approval must have been obtained for all protocols from the author's institutional or other relevant ethics committee and the institution's name and any ethics certificate number/s should be provided at submission.
- **Risks or negative impacts associated with research and mitigation (second-level heading):** This section should consider any risks or negative impacts to the subjects caused by the project (the subject may be a human individual or a population of plants or animals). What precautions were taken to minimise any negative impacts of the research on the subject/s?
- **Permitting (second-level heading):** Projects that required permits for collection, transport or provision of material must provide all relevant permit details.
- **Recruitment and informed consent (second-level heading):** In the case where human subjects were involved, how were subjects recruited? Was there any sense in subjects being obliged to participate or were volunteers recruited. Authors must include how informed consent was handled in the study.
- **Data protection (second-level heading):** Authors must include, in detail, the way in which data protection was handled.

Results (first-level heading):

Results should be presented as follows:

- Present the results of your experiment(s) or research data in a sequence that will logically support (or provide evidence against) the hypothesis, or answer the questions / address the objectives, as stated in the introduction.
- Present the body of the results section in text with the key findings that include references to each of the tables and figures. Report statistical test summaries (test name, p-value) parenthetically (that is, inserted as a parenthesis in brackets) together with the biological results they support. Use the SI unit.
- All units should conform to the SI convention and be abbreviated accordingly. Metric units and their international symbols are used throughout, as is the decimal point (not the decimal comma).

Discussion (first-level heading):

This section normally contains the following four elements. It is suggested that subheadings are used in this section:

- **Outline of the results (second-level heading):** Restate the main objective of the study and reaffirm the importance of the study by restating its main contributions; summarise the results in relation to each stated research objective or research hypothesis; link the findings back to the literature and to the results reported by other researchers; provide explanations for unexpected results.
- **Practical implications (second-level heading):** Reaffirm the importance of the study by restating its main contributions and provide the implications for the practical implementation your research.
- **Limitations of the study (second-level heading):** Point out the possible limitations of the study and provide suggestions for future research.
- **Recommendations (second-level heading):** Provide the recommendations emerging out of the current research.

Conclusion (first-level heading):

This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance, with a recommendation for future research (implications for practice). Provide a brief conclusion that restates the objectives, the research design and the results with their meaning.

Acknowledgements (first-level heading):

If, through your study, you received any significant help in conceiving, designing or carrying out the work, or received materials from someone who did you a favour by supplying them, you must acknowledge their assistance and the service or material provided. *Authors should always acknowledge outside reviewers of their drafts and any sources of funding that supported the research*.

- **Competing interests (second-level heading):** A competing interest exists when your interpretation of data or presentation of information may be influenced by your personal or financial relationship with other people or organisations that can potentially prevent you from executing and publishing unbiased research. Authors should disclose any financial competing interests, but also any non-financial competing interests that may cause them embarrassment were they to become public after the publication of the manuscript.

  Where an author gives no competing interests, the listing will read:

  'The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.'

- **Authors' contributions (second-level heading):** This section is necessary to give appropriate credit to each author, and to the authors' applicable

institution/s. The individual contributions of authors should be specified with their affiliation at the time of the study and completion of the work. An 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study. Contributions made by each of the authors listed, can follow the example below (please note the use of author initials):

> J.K. (University of Pretoria) was the project leader, L.M.N. (University of KwaZulu-Natal) and A.B. (Stellenbosch University) were responsible for experimental and project design. L.M.N. performed most of the experiments. P.R. (Cape Peninsula University of Technology) made conceptual contributions and S.T. (University of Cape Town), U.V. (University of Cape Town) and C.D. (University of Cape Town) performed some of the experiments. S.M. (Cape Peninsula University of Technology) and V.C. (Cape Peninsula University of Technology) prepared the samples and calculations were performed by C.S. (Cape Peninsula University of Technology).

References (first-level heading):

Begin the reference list on a separate page with no more than 60 references for full length articles and 30 references for short notes. The *Bothalia – African Biodiversity & Conservation* Journal uses the **Harvard referencing style**. Note: no other style will be permitted.

If you use any reference editor to add citations in the text, remove all data fields and replace with normal text before submission.

For journal articles, provide DOIs for as many as possible (usually all papers published in or after 2000). The DOI reference can be provided after a comma at the end of each reference.

TAXONOMIC PUBLICATIONS

Bothalia – African Biodiversity & Conservation publishes taxonomic findings where these align with the scope and focus of the journal (see Scope and Focus of *Bothalia – African Biodiversity & Conservation*). For such works the following headings should be used:

The Abstract and Introduction must follow the guidelines for full length articles, as described above.

Research method and materials (first-level heading):

- **Materials (second-level heading):** Briefly explain from which institutions material was studied, and whether any fresh material was collected as part of the study. If field collecting did take place explain where this was carried out, over what time period and how samples were collected.
- **Procedure (second-level heading):** Explain how observations, measurements and illustrations were done, and what equipment was used.

Taxonomic treatments (first-level heading):

This section serves as a guide to understand and standardise the presentation of taxonomy in research articles and short communications.

More details of rules that must be adhered to can be obtained from:

- The International Plant Names Index at http://www.ipni.org/
- International Association for Plant Taxonomy at http://www.iapt-taxon.org/
- The International Commission for Zoological Nomenclature (http://www.iczn.org)

The following sequence and format must be followed for taxonomic treatments in *Bothalia – African Biodiversity & Conservation*:

Species treatments:

- Basionym (the first name validly published, which has priority over other names later given to the same species): **Name** (bold, not italicised), *author citation* (italicised), author/s of paper in which basionym stated (if different from original author, not italicised).
- Name of the journal/publication written out in full (not italicised), volume: page number/range (date of publication), fig/s.
- Type locality: COUNTRY (upper case), as provided in the original description. Type specimen/s: date of collection, *collector (italicised), collector number* (italicised) (where available), institution code (using global acronym), catalogue number (where available), status (holotype, isotype/syntype, lectotype). If specimen was examined, this is indicated by a '!' after the specimen status.
- Additional references, in chronological order, with author: page (year of publication), figure number/s reflected (e.g. Boris et al.: 14 (1966); Boris: 89 (1967), fig. 9.).
- List of synonyms in chronological order, arranged in groups of nomenclatural synonyms (i.e. homotypic synonyms (based on the same type), followed by heterotypic synonyms (based on a different type), arranged chronologically), with references cited as author, page (year of publication), and figure number/s listed in chronological order.
- Identification of illegitimate names in the nomenclatural component must be accompanied by an appropriate indication of the reason for their illegitimacy. The type details for each heterotypic synonym should be included (institution code followed by catalogue number where available and type status), and those specimens examined by the author/s

must be indicated by an exclamation mark. The full reference for citations must be included in the Reference List.

Examples:

1. **Eremiolirion amboense** (*Schinz*) *J.C.Manning & C.A.Mannheimer* in Bothalia 35: 117 (2005), fig. 4. Type: South West Africa [NAMIBIA], Amboland [**Ovamboland**], Ongangua [Ondongwa], without date, *Ruatanen 344* (Z.holo!).

2. **Walleria gracilis** (*Salisb.*) *S.Carter* in Kew Bulletin 16: 189 (1962). *Androsyne gracilis* Salsb.: 61 (1866). Type: SOUTH AFRICA, **Western Cape**, *William Marsden* [BM, holo!; drawing in Salisbury mss.8: 818 (BM)].

   W. armata Scltr. & K.Krause in Krause: 235 (1921). Type: SOUTH AFRICA, [**Western Cape**, near Klawer], [Farm] Windhoek, 8 July 1896, *R. Schlechter 8074* (B, holo [not seen]; BM!, BR!, COI!, GRA!, K, MO!, PRE!, S!. iso).

3. **Plagiotaphrus improvisus** *(Attems 1934) Hoffman* in Revue de Zoologie et de Botanique Africaines, 83 (3–4): 209 (1971), fig. 2. *Megaskamma improvisa:* Attems: 16: 13 (1934), figs 14–17. Type: **ANGOLA**, near Cuanza River, Biéi District, Jan. 1932, *F. Haas* (SMF 1694, holo. [not seen] 1 male).

- Lectotypes or neotypes should be chosen for correct names without a holotype. It is not necessary to lectotypify synonyms. When a lectotype or neotype is newly chosen, this should be indicated by using the phrase "here designated". If reference is made to a previously selected lectotype or neotype, the name of the designating author and the literature reference should be given. In cases where no type was cited, and none has subsequently been nominated, this may be stated as "not designated".

Description of new taxa:

- All newly described taxa and newly proposed synonyms and new combinations should be explicitly designated as such, e.g. fam. nov., trib. nov., gen. nov., sp. nov., nom. nud., syn. nov., comb. nov.

  Name (bold, not italicised) sp. nov. authority (if different to the authors of the manuscript)

  TYPE/S: (holotype followed by paratype/s) (COUNTRY (upper case), **province** (bold), locality as given by original collector (if in foreign language or using archaic or outdated place names then these must be placed in inverted commas, with modern equivalent of collecting locality in square brackets (if relevant)), geographic co-ordinates (if the geographic co-ordinates were not provided on the specimen label or provided by the collector, and were identified by the author using a gazetteer or Google Earth, this must be indicated by including the co-ordinates in square brackets, altitude, habitat or other available, relevant collecting details, date of collection, *collector's name (italicised), collector's number (italicised*) (if available), (institution where specimen is housed (using global acronyms for these), catalogue number (if available), number of specimens by male and female (where relevant)).

  Examples

1. **Lasiosiphon rigidus** J.C.Manning & Boatwr., sp. nov.

   TYPES: SOUTH AFRICA, **Northern Cape**, Tankwa [Tangua] Karoo National Park, SW foot of Leeuberg, along drainage lines, [32°18,2'S / 20°0.3'E, 414 masl], 20 Jun. 2012, *Manning 3363* (NBG, holo., MO, PRE, iso).

2. **Doratogonus microsetus** sp. nov.

   TYPES: SOUTH AFRICA, **Mpumalanga**: Wakkerstroom, 27.36670°S / 30.01670° E, 20 Dec, 2000, *D. Forbes (*NMSA 21786, 1 male holo.; NMSA 21787, 2 males, 1 females, para.).

Second-level headings for taxonomic treatments:

- Description (with third-level headings if required, and according to diagnostic characters for the particular taxon)
- Distribution and habitat
- Ecology
- Etymology
- Local name/s
- Uses / economic value
- Diagnosis and relationships
- Conservation status – comment on whether included in existing Red Lists, or whether the species would potentially qualify as threatened and describe current and potential threats.
- Other material examined (country (upper case), province (bold): locality as given by original collector, modern equivalent of collecting locality in square

brackets (if relevant), co-ordinates (degrees, minutes decimal) (in square brackets if gazetteer or Google Earth used by author), approximate altitude, date of collection, *collector's name (italics), collector's number* (italics) (if available) (institution where specimen is housed (using international acronym or code for these), catalogue number (if available), number of specimens by male and female (where relevant)).

- List of specimens must be arranged alphabetically by country, and within countries, by province in alphabetical order, and within provinces, alphabetically by locality name, and as far as possible keeping those specimens from the same locality together, then in chronological order by collection date.
- Herbarium acronyms follow Index Herbariorum [Thiers, B. [continuously updated]. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/]. The accepted acronyms for other institutions can be obtained from the Global Registry of Biorepositories (GRBio) (http://grbio.org).
- Original locality information in a foreign language or using archaic/outdated place names should be indicated using inverted comas, with any relevant corrections for modern usage, including conversions to metric units, added in square brackets.

The date of collection is to be presented as day, month of the year (abbreviated as Jan., Feb., Mar., Apr., May, Jun., Jul., Aug., Sept., Oct., Nov., Dec.), and year in full.

Geographic co-ordinates must be presented as taken from a GPS, or from an online gazetteer or georeferencer in degrees, decimal minutes (DDM). Records must also indicate the hemisphere (E or W and N or S, and the estimated/approximate altitude. If the geographic co-ordinates and approximate altitude were not provided on the specimen label or provided by the collector, and were identified by the author, this must be indicated by including the co-ordinates in square brackets.

For species that may be threatened by over-collecting, the co-ordinates can be degraded to reflect only the degrees and minutes. In the case of old specimens where the exact locality is unknown the degree and minutes or equivalent, or the degree or quarter degree grid square can be provided.

Examples:

1. SOUTH AFRICA. **Western Cape**: Near Eendekuil, western foot of Piekenierskloof Pass, [32°37.136'S / 18°57.525'E 476masl], 28 Aug. 2009, *Magee, Boatwright, Manning and Goldblatt 161* (NBG, PRE, K, BOL); roadside near Gouda, [33°37.136'S / 19°2.044'E, 85masl], 09 Sept. 1951, *Esterhuysen 18840* (BOL [3 sheets], K, PRE).Tulbagh, 33°17.126'S / 19°8.257"E, 162masl, Sept. 1919, *Bolus 16734* (BOL);
2. SOUTH AFRICA: **KwaZulu-Natal:** Nkandhla Forest, in forest along dirt road, 28°43'38.592"S / 31°07'58.281"E, 1121 masl, 19 Nov, 2001, *A. Armstrong & H. Murray* (NMSA 21970 [1 male, 1 female]).

Language for these sections must be as concise as possible, using principles instead of verbs.

The remaining first-level headings (Discussion, Conclusions, Acknowledgements, Competing interests, Authors' contributions and References) must follow the same format as for full length articles, as detailed above.

Images – low resolution version in the text file; high resolution files – correctly labelled – as separate JPG, TIF or EPS files.

Identification keys: Dichotomous keys must use sequential numbering, with the two parts of the couplet numbered 1a, b; 2a, b etc. New species included in keys must be bolded and not italicised, and sp. nov. must be stated, while other species names must not be bolded, must be italicised, and must include the species authority in the correct format.

Illustrations for taxonomic works: Descriptions of new plant species should include a photograph of the holotype specimen, unless there is a good reason for not providing this. For all taxa, descriptions of new species and taxonomic revisions should include annotated illustrations that clearly show and indicate diagnostic characters.

Nomenclatural changes

Bothalia – African Biodiversity & Conservation will accept notes on nomenclatural changes. Authors are encouraged to include all name changes into a single manuscript and not to split these into separate manuscripts. Note that where a nomenclatural changes are a formality, and not based on research findings presented, the manuscript may not be subjected to a full review process. In such cases the publication will clearly state that the paper has not been peer reviewed.

Range extensions / new distribution records

Bothalia – African Biodiversity & Conservation will accept new distribution records where these have an impact on the conservation status of a species, or they represent a new country record. Single new distribution records will only be considered for publication where these are of major significance, and authors are encouraged to compile all new distribution records into a single manuscript and not to split these into several papers.

SANBI

Biodiversity for Life

South African National Biodiversity Institute

BIODIVERSITY KNOWLEDGE **INTO POLICY AND ACTION**

The **South African National Biodiversity Institute** (**SANBI**) is a state entity under the Department of Forestry, Fisheries and the Environment, whose mission is to champion the exploration, conservation, sustainable use, appreciation and enjoyment of South Africa's exceptionally rich biodiversity for all people. SANBI uses basic information on biodiversity and builds on this foundation through assessments, experiments, models and tools to provide evidence-based advice. In this way we influence policymakers and citizens and contribute to government's higher objectives of resilient biodiversity for poverty alleviation, job creation and improving human wellbeing. SANBI provides knowledge for ecosystem restoration and rehabilitation, leads the human capital development strategy of the sector, and manages eleven national botanical gardens and two zoological gardens as windows to South Africa's biodiversity for enjoyment and education.

FOUNDATIONS OF BIODIVERSITY

Our work starts with a strong foundation through surveying, classifying and mapping South Africa's ecosystems and species.

STATE OF BIODIVERSITY

We build on this foundation through assessments and monitoring, to answer questions about the status of our biodiversity and the best ways to protect it and its benefits to society.

SCIENCE INTO POLICY AND ACTION

Using this knowledge we translate science into policy and action by creating tools and information resources, and giving policy advice that assists those who make decisions about land use and natural resources, while aiming for objectives such as poverty alleviation, job creation and improved human wellbeing. Biodiversity sustains us through providing water and energy; nourishes us through providing food; inspires us through education and art; protects us through providing shelter; connects us through our heritage; and empowers us in terms of development and economic growth. Through all of this biodiversity and natural resources provide opportunities for job creation, improved service delivery and a better life.

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