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Distribution, quantitative morphological variation and preliminary molecular analysis of different growth forms of wild rooibos (*Aspalathus linearis*) in the northern Cederberg and on the Bokkeveld Plateau

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Abstract

Aspalathus linearis (Fabaceae) is endemic to the Cape Floristic Region in the Western Cape and Northern Cape Provinces of South Africa. The reddish leaves and stems, primarily of one cultivar, are used to make a commercially important tea which is marketed locally and internationally as 'rooibos' or 'redbush' tea. In historical times rooibos was collected in the wild. In the twentieth century cultivation of a single cultivar increasingly replaced wild harvest to meet growing demand. Recently, tea from wild forms of the species, which vary significantly in growth form and reproductive strategy, has been marketed by small-scale farmers in Wupperthal and on the Suid Bokkeveld plateau in the northern part of the species' distribution. Little information on the wild forms of this species has been published, although a rich body of knowledge exists amongst local harvesters and other land-users. In this study, we focus on the northern part of the species' distribution area where wild rooibos is harvested for commercial sale to niche organic and fair-trade markets. We adopt a transdisciplinary approach to (1) document the different growth forms, (2) develop a bioclimatic model of the potential distribution of the species, (3) quantify the morphological variation that exists between growth forms relative to the established cultivar and (4) use molecular techniques to provide a preliminary insight into the infraspecific diversity of different wild *A. linearis* growth forms. Our results show that local land users in the region identify four main growth forms of wild *A. linearis*. These are an 'erect form' and a 'prostrate form' in the Wuppertal area, a 'shrub form' in the Suid Bokkeveld, and a 'tree form' that has been observed at specific sites at Wupperthal, Biedouw and the Suid Bokkeveld. The PCA analysis of seven morphological traits identified three growth forms, which support the land user descriptions except in the case of the 'tree' and 'erect' forms which co-occurred in coordinate space. Both shrub and prostrate forms are wider than they are taller and possess more stems closer to the ground than erect forms. While the stems of both shrub and prostrate forms lie relatively flat on the ground, stem thickness is significantly greater in shrub forms. The tree type, the erect form and the cultivar studied possess the highest harvestable biomass. Prostrate forms and shrub forms resprout after fire while erect and tree forms regenerate from seed only. Haplotypic variation was assessed using DNA sequences from a single chloroplast region and revealed strong genetic differences between the different growth forms. Although preliminary, there is some evidence that sprouting and nonsprouting forms of the species are genetically isolated. This has important taxonomic implications for the species. Additional chloroplast regions and a nuclear region were also identified as variable and potentially useful markers for a multi-locus molecular approach to studying taxonomic and ecological questions within the species.

Keywords: *Aspalathus linearis*; Bioclimatic modelling; Local knowledge; Morphological variability; *trnL-F* spacer

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1. Introduction

Aspalathus (Fabaceae, Tribe Crotalarieae) consists of 279 species (Dahlgren, 1988; Cupido, 2007) and is the second largest genus of vascular plants in the Cape Floristic Region

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(Goldblatt and Manning, 2002). The genus is endemic to South Africa and most of the species are concentrated in the Cape Floristic Region, with only six species extending to southern KwaZulu-Natal. *Aspalathus linearis* (Burm.f.) Dahlg. (known locally as 'rooibos' or 'red bush') occurs over a wide geographic range in the western and south eastern parts of the Western Cape Province and in limited areas in the south-western part of the Northern Cape Province (Dahlgren, 1968, 1988; Fig. 1). Prior to the twentieth century rooibos was exclusively collected in the wild. Increasing demand stimulated cultivation of rooibos, and the proportion of wild harvested rooibos steadily diminished. Currently a single cultivated form of the species forms the basis for a commercially important tea industry. In recent years wild rooibos has been harvested and processed as a separate product by small-scale rooibos producers in the Suid Bokkeveld and Wupperthal areas (Arendse and Oettlé, 2001; Malgas and Oettlé, 2007).

Wild populations of *A. linearis* vary considerably from one area to another. The species exists as a series of partially allopatric populations (Van der Bank et al., 1999) which differ in a number of respects, specifically growth form, fire-survival strategy, vegetative and reproductive morphology, isozyme patterns and flavonoid composition (Dahlgren, 1968; Van der Bank et al., 1995, 1999). Substantial variation is observed within the species and distinct geographical forms can be recognised at a population level. Dahlgren (1968, 1988) and Van Heerden et al. (2003), for example, distinguish five and seven main rooibos tea 'types' and describe their distribution and main characteristics, respectively. Local harvesters in the northern Cederberg and on the Suid Bokkeveld plateau also differentiate between wild rooibos tea 'types' and use descriptive Afrikaans names that reflect morphological traits and growth forms which are usually easily observed in the field. The northern Cederberg refers to the area from Clanwilliam north towards the Doring River. The Suid Bokkeveld lies further north between the Doring River (where it forms the border between the Western and Northern Cape Provinces) and the town of Nieuwoudtville (Fig. 1).

Wild populations of *A. linearis* have also been noted to contain both sprouting and nonsprouting individuals (Schutte et al., 1995; Van der Bank et al., 1999). Fynbos is a fire driven system in which plant reproduction is largely centered around recurrent fires (Cowling, 1987). In general, sprouting and reseeding are mutually exclusive strategies (Vesk et al., 2004) used by plants in response to periodic natural disturbances that result in loss of above ground biomass, such as fire. Sprouters are able to regenerate from subterranean lignotubers after a fire, whereas nonsprouters are killed by fire and must re-establish through seeds.

Van der Bank et al. (1999) suggest that the different forms of *A. linearis* may be genetically fixed at the population level, while Van Heerden et al. (2003) mention an area near Citrusdal where four distinct forms co-occur, their distinctness apparently being maintained over multiple generations. A study of leaf chemistry (Van Heerden et al., 2003) provides further support for the

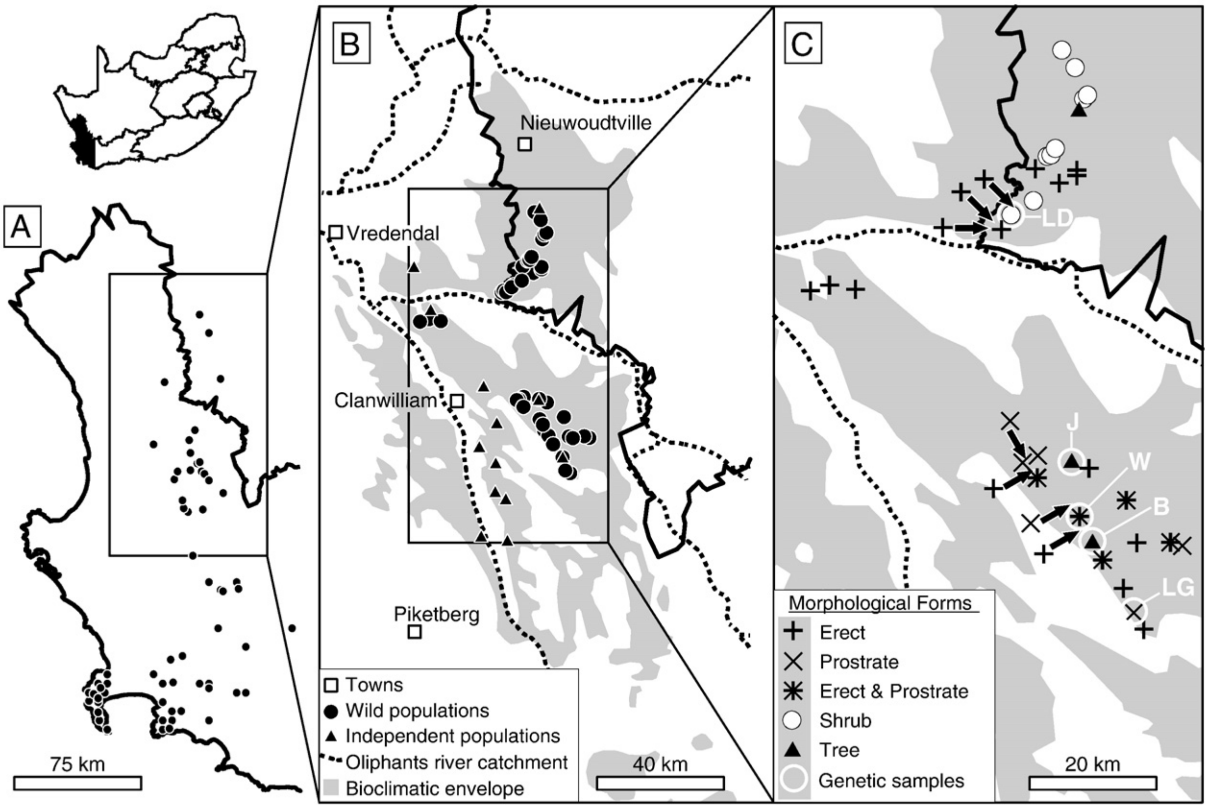

Fig. 1. (A) The distribution of *Aspalathus linearis* based on Dahlgren (1968, 1988). (B) The bioclimatic envelope for the northern part of the species range based on the distribution of 44 wild populations which were surveyed in this study. The distribution of several independent population locations obtained from the literature is also shown. (C) The distribution of different morphological forms of the species observed in wild populations with the populations selected for genetic analyses (LD – Landskloof; J – Jeugkamp; W – Witbank; B – Bo-Valletjie; LG – Langkloof).

uniqueness of these forms. Although the chemical compounds found in leaves are highly variable across the species, some of the forms and several populations have distinctive flavonoid "fingerprints", which means that they could be uniquely identified using their chemical profiles.

Globally there are only two small-scale rooibos producer organisations that supply wild harvested rooibos tea to niche markets. These organisations have their seats in the Cederberg and the Suid Bokkeveld, respectively, where the harvesters and tea producers live. The land-users of those regions are key stake-holders in the conservation and sustainable management of wild rooibos populations there. For this reason this study focuses on the northern range of *A. linearis* (Fig. 1A) in order to: (1) document the presence of different growth forms of *A. linearis* as perceived and classified by local harvesters of those regions; (2) locate several wild *A. linearis* populations identified by local harvesters and develop a bioclimatic model for the species in the northern range; (3) quantify the morphological variation that exists within wild *A. linearis* growth forms as identified by local harvesters; and (4) use molecular techniques to evaluate the genotypic diversity of different wild *A. linearis* growth forms.

2. Methods

2.1. Local land user perceptions of the morphological variation in A. linearis

A rich body of knowledge about *A. linearis* exists amongst local land users and harvesters in the study area. Members of these communities have managed this wild resource for generations maintaining an oral history of management practices. Local knowledge was integrated with scientific knowledge through a transdisciplinary Participatory Action Research (PAR) approach (Kemmis and McTaggart, 1988). Key research partners included scientists, land-owners, land-users, decision-makers and development practitioners in a context that ensured an exchange of information, insights and knowledge about wild rooibos management. With limited time and resources to cover large areas where isolated populations of wild rooibos occur and in the absence of written records or confirmed information, meaningful participation of local harvesters was crucial to the study.

Information was obtained from individual interviews and group workshops carried out between December 2004 and July 2006. Interviews were conducted with five land owners and four harvesters who live and work either in the northern Cederberg or in the Suid Bokkeveld. The nine informants were identified as 'experts in wild rooibos' by members of their respective communities and were between the ages of 55 and 75. All but one interviewee were male. Interviews were based on a semi-structured, open-ended questionnaire focused on the identification, distribution, local taxonomy and management practices for wild rooibos. Interviews were conducted at the homes of the respective interviewees at pre-arranged times. Informal conversations also took place in the field during scouting and surveying trips. Formal interviews were tape recorded and transcribed, while information gained from informal conversations was recorded as field notes. Eight workshops, with a total of 102 participants, were held in the settlements situated in the mountainous areas surrounding the Wupperthal Valley between March and April 2006. The highest proportion of Wupperthal's small-scale rooibos producers and wild rooibos harvesters are residents of the outlying settlements. These remote locations high up in the catchment areas are also the sites where wild rooibos populations are most prolific. Interviewees were thus selected from these communities. In addition, one workshop was held at a communal farm in the Suid Bokkeveld.

While the workshops also focused on farmer-to-farmer knowledge exchange and a discussion of current wild rooibos harvesting and veld management practices, only the results of the participatory mapping exercises and growth form analyses are included here (see Malgas and Oettlé, 2007 for all of the collected information). During the workshops participants were asked to describe the main forms of rooibos tea that occurred in the areas from which they harvested. More specifically, land-users were able to point out areas where certain growth forms occurred relative to locally familiar landmarks and settlements. This information provided the basis for the field surveys and thus the development of a bioclimatic envelope for the species.

2.2. Mapping the distribution of A. linearis and the development of a climatic envelope

Based on information obtained from the individual interviews and group workshops, field surveys were conducted in the northern Cederberg between October 2005 and June 2006 (see Malgas and Oettlé, 2007). Field surveys in Wupperthal were usually conducted with one or two land-users familiar with the area, who had participated at the community mapping workshops and who volunteered their time. Land-users identified areas where they either harvested wild rooibos themselves, or knew of existing populations. These areas were marked on a set of self-drawn maps produced during a series of workshops. Field surveys were conducted with local harvesters to locate and verify many of the identified populations in the study area.

The potential distribution of wild rooibos in the northern Cederberg and on the Suid Bokkeveld Plateau further north was assessed using an approach similar to that used by Midgley et al. (2003) for *Protea* species in the Cape Fynbos. With this approach, generalised additive modelling (GAM) of climate constraints, based on known distribution records was used to construct a climate envelope for *A. linearis*. Climatic parameters that are considered crucial for plant physiological functioning and thus survival were used (Midgley et al., 2003). The resulting climate envelope represents the area that has a similar combination of climate variables as those sites where the species is known to occur and provides a graphical representation of the likely potential distribution of the species. This potential distribution will always be larger than the actual distribution, as apart from the fact that the species may not have dispersed to all suitable sites, other factors which are not considered in the model such as competition and soil factors will reduce the actual distribution. In the current case soil factors may be important at a fine scale, however the available soils information is coarse and was not included as it did not contribute to a refinement of the model. The climate variables that were included in this study were: daily maximum temperatures of the hottest month (°C), daily minimum temperatures of the coldest month (°C), heat units during winter and summer (degree days), potential evaporation (mm), water stress (% days under stress) and elevation (m). These variables were obtained from the climate database compiled for South Africa by Schulze (1997) who also explains the derivation and biological significance of these variables. The locations of sixteen additional sites where wild *A. linearis* has been collected were sourced from the literature and used as an independent data set to test the validity and fit of our model.

2.3. Quantification of morphological variation between A. linearis growth forms

Based on the information about wild *A. linearis* growth forms derived from the individual interviews, workshops and field surveys, seven populations were sampled in June 2006 from five localities in the northern range in order to capture the greatest morphological variation in the region. For each growth form within the separate localities, eight to 11 individual plants were selected for morphological analyses, and leaf material was collected from the same individuals for genetic analyses.

Measurements for seven morphological traits were undertaken on each individual (Table 4). The variables are self-explanatory except for the following. The 'height to diameter ratio' was measured as the estimated pre-harvested height divided by the average diameter of the plant. The 'Harvestable Biomass Index' (HBI) ranged from 1 to 10 with 1 reflecting a shrub with no harvestable biomass and 10 representing a shrub with the maximum amount of harvestable biomass which could be obtained from a fully-productive, cultivar species in its prime and given a sustainable harvest height of between 50% and 70% (Louw, 2006). The angle of branching was measured with a protractor relative to the horizontal plane of the soil surface.

Differences between morphological variables in the different growth forms were assessed using an analysis of variance (ANOVA) and a post-hoc Tukey HSD test at a 5% level of significance in Unistat Statistical Package Version 5.6 (Unistat Limited, 2005). Correlations between the variables were also determined using a Pearson product moment correlation coefficient. The morphological variables were then subjected to a Principal Components Analysis (PCA) using Community Analysis Package 2.13 (Henderson and Seaby, 2002).

2.4. Molecular analyses

Genomic DNA was extracted from dried leaf material stored in silica using a modification of the extraction method of Gawel and Jarret (1991). Polyvinylpyrrolidone-40 (PVP) was added when grinding the leaf material in liquid nitrogen using a mortar and pestle. For screening purposes, four of the fastest evolving regions of the chloroplast genome (Shaw et al., 2005) and one region from the nuclear genome were amplified using PCR for seven samples representing all populations and morphological forms (Table 5). These samples were then examined for variability. Sequence lengths, number of distinct haplotypes, number of base pair differences and indels for each marker analysed in the screening process for seven individuals are reported in Table 1. Based on this survey, the primers trnL^{UAA}F and trnF^{GAA} (Taberlet et al., 1991) were selected to assess the genetic identity of the morphological groupings within the seven *A. linearis* populations sampled in the Northern Cederberg. Three to five samples from each of the populations were used for the assessment. PCRs were performed in volumes of 30 µl containing 2 µl of template DNA, 3 µl of 10X *Taq* polymerase reaction buffer (Bioline, London, UK), 3 µl $MgCl_2$ (50 mM), 1 µl of each primer (10 µM), 1.2 µl of dNTPs (10 mM), 0.2 µl *Taq* polymerase and sterile H_2O up to 30 µl. PCR for all primers was performed on a GeneAmp 2700 PCR System (Applied Biosystems, USA) with an initial 2 min denaturing step at 94 °C; 28 cycles, each comprising 94 °C for 1 min, 50 °C for 30 s, 72 °C for 1 min; and a final 6-min extension step at 72 °C. PCR products were sequenced using BigDye technology and run on an ABI 3300 sequence analyser by Macrogen, Korea (http://dna.macrogen.com).

All sequences for each region were assembled and edited using CODON CODE ALIGNER 1.6 (www.codoncode.com/), then aligned in CLUSTAL W (www2.ebi.ac.uk/clustalw/) set to default parameters. All sequences were trimmed to exclude ambiguous sections at each end. We used the program TCS 1.2.1 (Clement et al., 2000) with default options to estimated a haplotype network under statistical parsimony (SP) (Templeton et al., 1992) for the trnL^{UAA}F–trnF^{GAA} chloroplast gene region. As the option in TCS 1.2.1 which "treats gaps as a 5th state" treats a single multiple base indel as multiple indels, all indels were shortened to a single base prior to analysis. The haplotype network was generated using a 95% confidence interval to calculate the maximum number of mutational steps between two sequences that constituted a parsimonious connection. The

Table 1
The sequence length, number of distinct haplotypes (H), number of base pair differences (BPD), number of insertion/deletion events (Indels), primer source and GenBank Accession numbers of four chloroplast (cDNA) and one nuclear markers from seven individuals of *Aspalathus linearis* sampled from five localities in the Cederberg Fynbos region.

Locus	Length	H	BPD	Indels	Source	Accession #
trnL^{UAA}F^{a}–trnF$^{GAA\,a}$	893	4	4	1	Taberlet et al. (1991)	FJ620662–FJ620665
trnT^{GGU}–trnD^{GUC}F^{a}	193	2	1	0	Demesure et al. (1995)	FJ620675–FJ620676
trnS$^{GCU\,a}$–trnG^{UCC}	312	2	0	1	Shaw et al. (2005)	FJ620673–FJ620674
trnT^{UGU}F^{a}–5′trnL^{UAA}R	779	7	7	3	Taberlet et al. (1991)	FJ620666–FJ620672
PIII-PIV^{a}	342	5	7	0	Cox et al. (1992)	FJ620677–FJ620682

[a] Indicates the primer used to sequence the gene region.

network was rooted on an accession of *Aspalathus cordata* obtained from GenBank (AF518124; Crisp and Cook, 2003). The inter-simple sequence repeat (ISSR) technique was also used but did not identify any grouping corresponding to the populations or growth form types and the results are not reported here but are described in detail by Potts (2007).

3. Results

3.1. Local land user perceptions of the morphological variation and ecology of A. linearis

The growth forms and ecology of *A. linearis* in the northern species distribution range based on land user perceptions are summarised in Table 2. Names of growth forms with similar descriptions as described by Van Heerden et al. (2003) are also included. Land users and harvesters in the Wupperthal region differentiate between four primary growth forms of wild *A. linearis* and use local descriptive Afrikaans names that reflect morphological traits that can be easily observed in the field. Workshop participants and interviewees primarily use "langbeentee" (long-legged tea) or "regoptee" (upright tea) when referring to plants with an erect growth form. These plants would have been described by Van Heerden et al. (2003) as the "Wupperthal type". "Ranktee" or "rankiestee" (creeper tea) refers to plants that possess stems which grow along the ground before sending vertical shoots upward (prostrate growth form). These populations bear similar traits to those described by Van Heerden et al. (2003) as the "grey resprouter" In the Kleinvlei area, a single stemmed, taller nonsprouting tree-type form was also identified by local participants and called "boomtee" (tree tea). Local harvesters in Wupperthal make a definite distinction between "regoptee"/"langbeentee" and "boomtee" (tree tea), the latter similarly described by Van Heerden et al. (2003) as "tree type". Further north in the Suid Bokkeveld, local harvesters are more familiar with "bossietee" (shrub tea), a bushier, multi-stemmed, robust form. There are thus four locally identified growth forms in the northern distribution range of the species. However, a principal components analysis (PCA; described later; Fig. 2) showed no separation of the tree form from the other erect forms, resulting in the expression of only three growth forms for the selected morphological variables (Table 4) within the study area (Fig. 3).

Plants with an erect growth form ("langbeentee" and "boomtee") grow in sandier, cooler habitats, are usually single stemmed and are generally considered to be nonsprouters. The tree form in particular has so far only been observed at moist sites, either on the banks of streams, or near waterways. Individuals with a prostrate habit occur more frequently in drier, rockier habitats and resprout after fire. The prostrate form ("rankiestee") grows in the transition zones between Fynbos and Succulent Karoo vegetation types to the east and south of Wupperthal on exposed, rocky slopes, and in exposed and rocky locations in the exclusively Fynbos areas of Wupperthal. The shrub form ("bossietee") found in the Suid Bokkeveld grows abundantly on the sandier bottomland soils in the region.

According to local land-users at Wupperthal, the two erect growth forms are not able to resprout after fire. The parent plants easily die during fires, but at the same time, fires stimulate the germination of seeds produced by these plants. Prostrate plants ("rankiestee") are able to withstand severe disturbance, surviving up to three consecutive years of drought conditions. These plants are locally known to resprout after disturbances such as fire and harvesting. Land-users in the Suid Bokkeveld had the same to say of the shrub form ("bossietee") in that region. In both instances, resource users warned that, despite their ability to survive harsh conditions, sprouting wild *A. linearis* plants are slower to regenerate than their nonsprouting counterparts, and should be managed accordingly.

Table 2
Growth forms identified during this study based on local knowledge of land-users and harvesters.

Growth form	Local names	Distribution	Fire response	Main characteristics
Erect (Wupperthal type)	Langbeen/ regop tee	Heuningvlei, Langkloof, Vaalheuning (Wupperthal) and the Agter Pakhuis area. Grows in deep acidic soils.	Dies after fire	Plants grow upright and are 0.7–1.8 m tall. Many leaves grow on each individual branch. Shares some characteristics with cultivated rooibos, including prolific leaf and seed production.
Tree-type (tree type)	Boom tee	Observed at one site in the Suid Bokkeveld, in the Biedouw, and near Wupperthal Favours cool, moist habitat, e.g. near waterways and on the banks of streams.	Dies after fire	Tall and upright. A single stem branches 10–40 cm above the ground. Young bushes grow to chest height. Leaves are long and thick. Production is relatively high.
Prostrate (grey resprouter)	Rank/ rankies tee	Grows in mountainous areas to the east and south around the Wupperthal Valley. Grows on highly acidic soils; grows at transition between "sour" (acidic) and "sweet" (basic) soils. Favours rocky/stony areas	Resprouts after fire	Grows low on the ground (0.2–0.6 m high), and spreads out. At Wupperthal it occurs in the same area as the langbeen type at three sites. Production is relatively low. Leaves are long and sparsely arranged along prostrate branches.
Shrub (northern resprouter)	Bossie tee	Occurs in sandstone regions of the Suid Bokkeveld, north of the Cedarberg Mountains.	Resprouts after fire.	Bossie tea of the Suid Bokkeveld is a re-sprouter. Intermediate growth form; usually between 0.5 and 1.1 m tall. Shrubs grow wide and round, and produce many branches and shoots in its lower portions. The plants have a spreading growth habit, but grow much larger and more thickly than rankies tea bushes.

Includes local names, distribution, fire responses and main characteristics of wild *Aspalathus linearis* plants growing the Northern Cedarberg and Suid Bokkeveld regions. Based on Malgas and Oettlé, 2007. Names of growth forms with similar descriptions by Van Heerden et al., 2003 are indicated in brackets in the first column.

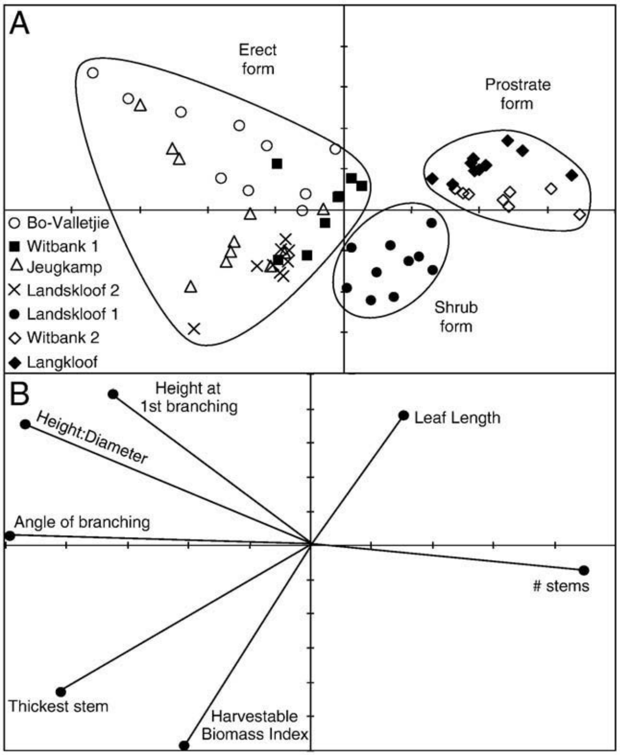

Fig. 2. (A) Plot of the first two principle components (PC1 and PC2) obtained from a principle component analysis based on seven morphological variables listed in Table 2 from 70 individuals of *Aspalathus linearis* sampled from five localities in the Cederberg Fynbos region, namely Langkloof (X), Bo-Valletjie (○), Witbank (◊ and □), Jeugkamp (+), and Landslides (cultivated - , Δ-wild). Stippled lines are used to group individuals with similar morphological traits. (B) illustrates the correlation of the original variables with PC1 and PC2.

3.2. The surveyed populations and bioclimatic envelope of A. linearis in its northern distribution

During participatory mapping exercises, participants pointed out generic regions where rooibos could be found. Of the more than 60 areas identified during participatory mapping exercises at the land-user workshops, 44 populations were visited and recorded during the subsequent field surveys (Fig. 1B). There was little emphasis at that time on the distribution of the various rooibos growth forms and the fact that different growth forms exist and are managed slightly differently emerged as the workshops evolved.

It became clear that the prostrate form (“rankiestee”) grew to the east and north in the more arid parts of the Wupperthal mountains (Fig. 1C). The erect form (“regop”/“langbeentee”) was said to occur in the vicinity of the western and southern villages of Heuningvlei and Eselbank, in areas receiving relatively higher annual rainfall. The tree-like erect form (“boomtee”) occurs in isolated patches in the Biedouw and at Kleinvlei. There were also areas where the different growth forms were observed to co-occur, and others where growth forms were difficult to tease apart based on observation of their morphology alone. The maps and the descriptions of resident harvesters of where different growth forms occur provided key information on where other populations of wild rooibos might be found (see Malgas and Oettlé, 2007).

The bioclimatic envelope for *A. linearis* in the northern distribution is shown in Fig. 1B. The bioclimatic model indicates that, within the Cederberg Mountains, *A. linearis* is likely to occur at intermediate altitudes. The valley bottoms, particularly those along the Olifants and Doring River valleys, as well as the higher mountain peaks are not likely to be suitable habitats for *A. linearis*. Major potential distribution centres include the northern Cederberg and a large area of the Suid Bokkeveld. Despite the relatively restricted set of *A. linearis* population localities used in the model, the localities obtained from the independent data set all fell within the boundaries of our model, providing some corroboration. It could be argued that this results from the coarse nature of the model and the large resultant potential distribution, making its inclusivity inevitable. However, this is clearly not the case and the map demonstrates that the model is quite refined, picking up suitable and unsuitable areas within a relatively small area. Given the land user descriptions of the preferred habitat for the different forms and the DNA results of this study, it should also be borne in mind that the results are representative of the wild populations of *A. linearis* regardless of growth form and more specific studies will be required to elucidate exactly how the bioclimatic envelope may be refined for the different forms of *A. linearis* within the region.

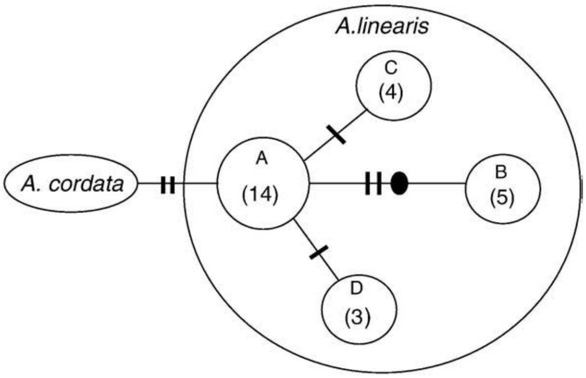

Fig. 3. Statistical parsimony haplotype network of 26 trnL´UAAF – trnFGAA sequences of *Aspalathus linearis* sampled across five localities in the Cederberg Fynbos region. An accession of *Aspalathus cordata* is used as an outgroup to root the network. A dash represents a base pair change and a dot represents a deletion event. The number of individuals found with each haplotype is also displayed in brackets below the haplotype designation.

3.3. Quantification of morphological variation between A. linearis growth forms

The allometric relationship between different morphological traits measured in the 66 plants is shown in Table 3. Leaf length was negatively related to stem thickness, the HBI and the angle of branching. Plants with more stems had a lower height to diameter ratio, branched closer to the ground and lay flatter on the surface of the ground. Plants with thicker stems were taller, possessed a greater HBI, and were more upright. The height to diameter ratio was positively correlated with the angle of branching (i.e. as found in more erect growth forms) and with the height at first branching. Plants which grew more erect branched higher up the stem than those which lay flat on the ground.

Table 3
Pearson product moment correlation coefficient between seven variables measured on 66 individuals from seven locations in the northern Cederberg.

	Leaf length	No. stems	Thickest stem	Height: diamete*r*	Harvestable Biomass Index (HBI)	Angle of branching
No. stems	0.05959					
Thickest stem	−0.2945**	−0.4475***				
Height:Diameter	−0.02878	−0.5659***	0.2874**			
Harvestable Biomass Index (HBI)	−0.2134*	−0.1402	0.7492***	−0.06773		
Angle of branching	−0.3118**	−0.627***	0.4708***	0.6889***	0.1596	
Height at 1st branching	0.1586	−0.3171**	0.1481	0.7075***	0.1177	0.3243**

*$p<0.05$; **$p<0.01$; ***$p<0.001$.

The selected morphological traits displayed significant variability amongst the sampled populations (Table 4). The regeneration strategy is based on the descriptions by local land users who suggest that prostrate and shrub forms resprout after fire while erect and cultivar forms regenerate from seed only. The full data set was also subjected to a PCA to determine the degree of similarity between individuals and populations (Fig. 2). The PCA analysis of seven morphological traits identified three growth forms, which matched the land user descriptions except in the case of the 'tree' ("boomtee") and 'erect' ("langbeentee") forms were largely overlapping in coordinate space. The first two axes of the PCA accounted for 68.3% of the variance in the data. The first axis separates the prostrate from the erect and shrub forms, while the second axis separates the shrub form from the erect and prostrate forms. The first axis is most strongly related to the angle of branching ($r=-0.49$), the height to diameter ratio ($r=-0.47$), the number of stems at 5 cm ($r=0.45$) and the thickest stem at 5 cm ($r=-0.41$). The second axis was best related to the HBI ($r=-0.59$) and the height to first branching ($r=0.44$).

Our results indicate clear morphological differentiation between the three main growth forms studied. While individuals of the tree form from Bo-Valletjie clustered within the erect growth form (Fig. 2), they differed in having relatively longer leaves, fewer but moderately thicker stems at 5 cm, the largest height to diameter ratio of all populations, a moderate HBI, a relatively upright branching angle and a very variable height at first branching. Individuals representing the two erect forms at Witbank and Jeugkamp appeared very similar to the cultivar at Landskloof. The wild *A. linearis* individuals measured at Landskloof are distinct from all individuals in this study and are best described as large shrubs with many branches at the base which lie relatively flat on the ground at first but then curve upwards providing the highest HBI value of all forms. The stems are similar in thickness to the erect forms and support relatively long leaves. Their height to diameter ratio, however, is similar to that of the prostrate forms. The prostrate forms are very distinctive in the PCA ordination even though the two populations visited differed significantly in leaf length. Relatively similar mean values for the two populations were obtained for all the other variables measured. The HBI values are the lowest for all the growth forms.

3.4. Molecular analyses: haplotype variation

Using the trnL^{UAA}F–trnF^{GAA} gene region, four unique haplotypes were identified by the statistical parsimony network analysis, with three haplotypes directly connected to an ancestral haplotype (Fig. 3). All of the prostrate forms and the erect form from Bo-Valletjie possess the ancestral haplotype (haplotype A; Table 5). Both haplotypes C and D are separated from haplotype A by single transversion events, while haplotype B is separated by one transition, one transversion and one deletion event. The three shrub form individuals from Landskloof possess a unique derived haplotype (haplotype D). The erect individuals from Witbank,

Table 4
Mean (±std.dev.) values for seven morphological traits recorded in seven populations from the northern range of the *Aspalathus linearis*.

Region	Wupperthal (Kleinvlei)	Wupperthal (near Brugkraal)	Biedouw Valley	Suid Bokkeveld	Suid Bokkeveld	Wupperthal	Wupperthal
Location	Bo-Valletjie	Witbank 1	Jeugkamp	Landskloof 2	Landskloof 1	Witbank 2	Langkloof
Latitude (S)	32.29399	32.25928	32.17942	31.83085	31.82659	32.25928	32.40197
Longitude (E)	19.18808	19.17043	19.15858	19.07286	19.06901	19.17043	19.24067
Growth form	Erect^{1}	Erect	Erect^{1}	Cultivar	Shrub	Prostrate	Prostrate
Regeneration strategy	Nonsprouter	Nonsprouter	Nonsprouter	Nonsprouter	Sprouter	Sprouter	Sprouter
N	10	8	11	9	10	8	10
Leaf length (mm)	46.9±6.9^{a}	22.4±2.4^{c}	25.5±2.5^{c}	24.1±3.1^{c}	33.4±5.3^{b}	25.9±1.6^{c}	53.2±8.6^{a}
No. stems at 5 cm	2.7±2.2^{c}	4.8±1.2^{bc}	3.8±2.6^{bc}	7.7±2.0^{bc}	14.2±7.0^{b}	26.6±15.2^{a}	14.5±8.2^{b}
Thickest stem at 5 cm (mm)	18.8±6.1^{a}	15.5±8.1^{a}	22.5±7.5^{a}	23.9±10.3^{a}	21.5±5.1^{a}	4.0±0.0^{b}	3.8±1.2^{b}
Height to diameter ratio	2.3±0.7^{a}	1.2±0.3^{b}	1.8±0.7^{ab}	1.1±0.2^{b}	0.7±0.1^{b}	0.6±0.1^{b}	0.6±0.1^{b}
Harvestable Biomass Index (1–10)	5.3±1.3^{b}	3.9±2.2^{bc}	6.5±2.3^{b}	8.6±0.7^{a}	8.8±1.3^{a}	3.1±0.6^{c}	3.6±0.5^{bc}
Angle of branching (degrees)	56.5±11.1^{ab}	47.5±12.2^{b}	69.1±7.7^{a}	65.0±4.3^{ab}	0.2±0.6^{c}	3.0±3.7^{c}	8.0±5.4^{c}
Height at 1st branching (cm)	127.0±157.2^{a}	5.0±0.0^{b}	54.2±89.4^{ab}	0.8±1.3^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}

[1] Identified as a 'boomtee' (tree tea) by local land users.

Dissimilar superscripts denote significant differences between populations at $p<0.05$. All quantitative data were analysed statistically using one-way ANOVA and post-hoc Tukey HSD in Unistat 5.6 (2005).

Table 5
Populations analysed in this study, the growth forms, regeneration strategy, numbers of individuals (N) and haplotype frequencies.

Population	Growth form	Regeneration strategy	N	Haplotype frequencies			
				A[a]	B[b]	C[c]	D[d]
Witbank 2	Prostrate	Sprouter	4	4			
Langskloof	Prostrate	Sprouter	5	5			
Bo-Valletjie	Tree-type	Nonsprouter	5	5			
Jeugkamp	Tree-type	Nonsprouter	3		1	2	
Witbank 1	Erect	Nonsprouter	4		3	1	
Landskloof 2	Erect[e]	Nonsprouter	2		1	1	
Landskloof 1	Shrub	Sprouter	3				3

[a] FJ620665.
[b] FJ620663.
[c] FJ620662.
[d] FJ620666.
[e] Cultivated ‘rocklands’ variety.

Jeugkamp and from the cultivated Landskloof population possess two other haplotypes derived from the ancestral one (haplotypes B and C). The haplotype frequency for each form from each locality is summarised in Table 5. There is a striking correspondence between haplotypic and morphological variation.

4. Discussion

Focussing on the northern distribution of the species we (1) identify four different growth forms as perceived by local harvesters and quantified this morphological variation, (2) develop a bioclimatic envelope that reflects the potential distribution of the species, and (3) provide preliminary molecular data that shows a high level of genetic diversity that displays a striking correspondence between the morphological and haplotypic variation within *A. linearis*. This study is an example of the immense value of informal local land user knowledge with regard to important species. Not only were populations easily located that would otherwise have required extensive and time-consuming field work, but local form descriptions also provided the basis for quantifying the morphological variation within the species — a novel undertaking that adds value to existing descriptions of morphological variation (e.g. Dahlgren, 1968; Van Heerden et al., 2003).

4.1. Growth form distribution and morphological variation between growth forms

Land users in the northern Cederberg and on the Bokkeveld Plateau utilize a local classification system which differentiates between four main growth forms within wild *A. linearis* populations. An erect form is differentiated from a prostrate form of *A. linearis* in the settlements and outstations of the Wuppertal region. Another erect form takes on the appearance of a tree. In a few localities erect and prostrate growth forms occur together. Further north, a shrub form dominates the sandy and stony plateaus of the Suid Bokkeveld. The quantitative morphological trait analysis provided support for three of the four forms described by the land users. However, a critical trait may have not been recorded that would separate the erect and tree forms in coordinate space, and thus the distinction between the two should not be unnecessarily discarded.

Morphological traits associated with these growth forms often differ from the commercial cultivar in a number of important respects. The morphological, chemical and genetic variability (Dahlgren, 1968; Van der Bank et al., 1999; Van Heerden et al., 2003) prevalent in *Aspalathus linearis* appears to have hindered the resolution of the taxonomy of the species (Van Heerden et al., 2003). The results of this study suggest that without taking direct account of this variation and including samples from all the different known biotypes, little further progress is likely. Descriptions of wild growth forms offered by local land-users from the study area correspond in several ways with those offered by Van Heerden et al. (2003). The different names for the various growth forms may seem redundant in the context of taxonomic differentiation. However, resource users use these traits to assess the risks and advantages of fires and the effects of over-harvesting and grazing on the different growth forms (Malgas and Oettlé, 2007).

While the climate envelope for *Aspalathus linearis* suggests that it has the potential to exist across a wide area in the northern Cederberg, the wild populations are fragmented as indicated by local user descriptions and observed in field surveys. Local users were also very specific and accurate about where different growth forms occur. This suggests that *A. linearis* is fairly habitat specific, and the key variable is likely to be soil type. Extensive transformation of relatively deep sandy soils for the commercial production of *A. linearis* has probably negatively impacted on particular biotypes that favour these habitats (Pretorius, 2008). Competition for habitat between cultivated and wild *A. linearis* potentially threatens the persistence of the wild biotypes. Furthermore, such habitat-specific transformation has been identified as a major threat to the biodiversity of the area, and as many as 50 of the 90 Bokkeveld Sandstone Fynbos endemic species are now Red Data Book-listed (CAPE, 2008; Pretorius, 2008).

The land-users who harvest wild rooibos in the study area are key stakeholders in the conservation and sustainable management of wild *A. linearis* populations in the northern species distribution range. Their knowledge contributions and active participation in this kind of research are thus of great conservation value. However, to sustain this relationship it must be mutually beneficial and scientific results must be reported back to the land users in an accessible form. Feedback has to be in the local language if at all possible, should be simplified and free of jargon, yet accurate and true to the findings. In this instance, one of the project outputs was a harvest manual collectively produced by members of the research team (see Malgas and Oettlé, 2007).

4.2. Are morphological forms genetically distinct?

We find a high level of genetic diversity that displays a striking correspondence between the morphological and haplotypic variation within *A. linearis*. This suggests that there may be a genetic basis for the different morphologies and reproductive strategies. However, the results from this study should only be considered an introductory glimpse into the sequence DNA variation within the species because a) of the low sample size, and b) different parts of an organism's genome may have different evolutionary histories and analysing the data from the chloroplast genome alone can be misleading (Takahata, 1989; Doyle, 1997). The level of genetic diversity is surprising given that low levels of DNA sequence diversity have been observed in the genus *Aspalathus* (Edwards et al., 2008) and chloroplast gene regions are often found to be inadequate for plant population level studies (Schaal et al., 1998).

The trnL^{UAA}F–trnF^{GAA} region revealed strong genetic differences between the sprouters and non-sprouters, confirming the findings of Van der Bank et al. (1999). In addition, there are genetic differences between morphological forms. The prostrate populations possessed only haplotype A. This haplotype was, however, not unique to the prostrate form as it was also present in the erect population at Bo-Valletjie. The erect nonsprouters, however, all possessed haplotypes B and C, and the shrub form only possessed haplotype D. Two to five samples of the chloroplast trnL^{UAA}F–trnF^{GAA} region is too small a sample size to allow population level dynamics within a species to be inferred accurately. However, if the apparent fixation of haplotypes in the prostrate, erect (excluding the Bo-Valletjie population as an anomaly that requires further investigation) and shrub populations is a true reflection of the haplotype distribution and diversity between these forms then this would suggest that there may be genetic isolation between the different forms. Thus, our results confirm that there is a genetic component underlying the different morphological forms.

4.3. Genetically isolated erect and prostrate forms at Witbank

The maintenance of different morphologies with corresponding genetic divergence at Witbank suggests the existence of more than one reproductive isolated species within what is currently defined as *A. linearis*. Both prostrate and erect forms were found at Witbank, growing in sympatry and randomly distributed over a very small area (< 1000 m2). The fact that they do not share any haplotypes (Table 5) and that no hybrids (i.e. morphological intermediates) have been observed suggests that these two forms are reproductively isolated from one another. This divergence is not likely due to differences in pollinator exploitation. Seventeen species of Hymenoptera have been observed visiting the flowers of *A. linearis*, most of which were found to be generalist pollinators within the genus *Aspalathus* (Gess and Gess, 1994). The evidence that sprouters and nonsprouters are genetically isolated supports the findings of Van der Bank et al. (1999) who found that sprouting populations found over 300 km apart, in Franschoek and Gifberg, were genetically more similar to each other than they were to any of the five nonsprouting populations found within 50 km of the Gifberg population.

Taken together, the data indicate a need for a comprehensive re-evaluation of the species limits within *A. linearis*. Based on the evidence of this study and that of Van der Bank et al. (1999), the splitting of *A. linearis* would be supported by both the biological species concept (BSC; Mayr, 1942) and the genealogical species concept (Baum and Shaw, 1995).

4.4. Future research directions

Wild *A. linearis* is an economically important species for small-scale producers in the northern range of the species' distribution range. However, more scientific research is needed to complement the body of existing local knowledge amongst land-users in those regions. Four morphologically distinct growth forms are locally identified in the northern Cederberg and on the Bokkeveld Plateau. Harvesters use functional traits to distinguish between sprouting and nonsprouting growth forms, and manage the wild resource accordingly. The forms appear to have a genetic basis, maintaining their integrity even in sympatry. This suggests the existence of multiple species currently resorting under *A. linearis*. In view of the very limited sampling applied here, there is a clear need for more work, both in the directions of morphological and molecular research. This should be geared towards a re-evaluation of species limits in the complex, and a comprehensive revision of the group. The necessity of these research directions stem from a growing need for proper management and conservation of a highly valued natural resource.

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