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Hanging in there: *Aloe lettyae* populations in Critically Endangered grassland fragments

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ABSTRACT

This is the first study documenting the distribution and population biology of the Endangered *Aloe lettyae*, an endemic to the highly threatened Woodbush Granite Grassland (WGG) in Limpopo Province, South Africa. We documented 19 *A. lettyae* populations and calculated the total area of occupied habitat at 17.5 ha within its extent of occurrence of 123 km^2. Most populations were clustered on the south western side of the WGG, with all known localities less than 40 km apart in this severely fragmented vegetation type. Population size varied from 10 to 6547 plants, with ~10,800 individuals estimated in total. Plant size and life history stage demographics were determined in seven of the 19 currently known *A. lettyae* populations, including two large populations, as well as a high- and a low-lying population, and constituted a representative sample of the entire geographical range of the species. For plant size, four metrics (number of leaves, number of leaf layers, height and diameter of the leaf rosette) were recorded for each sampled *A. lettyae* plant, and the presence of an inflorescence indicated its reproductive status. By means of the classification tree technique CHAID (Chi-square Automatic Interaction Detection), we determined that of the four plant size metrics, the 'number of leaves' predicts *A. lettyae*'s life history stages and fecundity most accurately, and revealed significant differences in the life history stage structure of the seven surveyed populations. Five populations occurring in relatively undisturbed WGG fragments, including the two large populations, comprised high proportions of adult individuals (77–89%), while only few *A. lettyae* juveniles were found (3–14%). By contrast, the relatively high percentage of juveniles (25–58%) and low percentage of adults (32–63%) observed in two small populations found in a degraded WGG fragment and in non-natural habitat respectively, appeared atypical. The percentage of subadults varied little across the surveyed populations (8–13%). In slow-growing, long-lived species such as *A. lettyae*, the dominance of adult individuals with a high probability of survival may be considered as indicative of stable populations. This study provides baseline *A. lettyae* population data for long-term demographic monitoring which will aid management and conservation of the range restricted, Endangered *A. lettyae*.

1. Introduction

The Endangered *Aloe lettyae* Reynolds (IUCN: B1ab(iii,v)+2ab(iii,v)) is endemic to the highly threatened Woodbush Granite Grassland (WGG) in South Africa's Limpopo Province (Von Staden and Kremer-Köhne, 2015). Due to large-scale habitat loss through afforestation with exotic trees (Niemandt and Greve, 2016), *A. lettyae*'s distribution has been severely reduced over several decades (Von Staden and Kremer-Köhne, 2015; Kremer-Köhne, 2018). While the destruction of habitat over time, habitat loss, is considered one of the most significant causes of biodiversity decline (Fahrig, 2017), the fragmentation pattern of the remaining habitat affects plant populations by impacting on a species' population size and density, abiotic disturbances such as fire, and the abundance of interacting species (Hobbs and Yates, 2003).

Apart from size and density, age and genetic aspects play a role in the functioning of a population (Hara, 1988). However, the age of the individuals in a plant population is often unknown (Peters, 1996; Condit et al., 1998) and plants of the same age often vary in terms of growth, survival, and fecundity due to genetic variation in the population, variability of environmental conditions, or a combination of the two (Hubbell and Werner, 1979). Plant size is therefore related to life history stages using recognisable criteria to gain an understanding of population dynamics (Hubbell and Werner, 1979; Condit et al., 1998). Furthermore, the size class distribution (SCD) of a population

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reflects recruitment, growth, and mortality over a long period (Wiegand et al., 2000), and the analysis of a population's size structure is useful for predicting population trends and informing management practices (Peters, 1996; Condit et al., 1998).

The size distributions of a great variety of tree species have been particularly well researched (e.g. Condit et al., 1998; Gaugris and Van Rooyen, 2007; Venter and Witkowski, 2010; Helm and Witkowski, 2012). A number of studies have also been undertaken on the population size structures of long-lived, slow-growing species in the family Asphodelaceae, including tree aloes (Midgley et al., 1997; Bolus et al., 2004; Duncan et al., 2006; Cousins et al., 2014), single-stemmed (Stokes and Yeaton, 1995) and stemless aloes (Weisser and Deall, 1989; Pfab and Scholes, 2004; Phama et al., 2014; Arena et al., 2015; Kunonga et al., 2019), *Haworthiopsis koelmaniorum* (=*Haworthia koelmaniorum*) (Witkowski and Liston, 1997) and *Kniphofia umbrina* (Witkowski et al., 2001). However, no published population studies have been conducted on the stemless *A. lettyae*, and the demographics of this WGG endemic *Aloe* species are poorly understood. The aim of this study was therefore (a) to estimate the number of individuals and plant densities for all known *A. lettyae* populations, and (b) to determine plant size and life history stage demographics of selected *A. lettyae* populations to provide baseline *A. lettyae* population data for long-term demographic monitoring. We addressed the following questions: (1) Do population size and density vary across *A. lettyae*'s restricted distribution range?, (2) How are *A. lettyae* plants dispersed within a population?, (3) Which metric of plant size predicts *A. lettyae*'s life history stages most accurately?, and (4) Which are the major SCD and life history stage patterns in a sample of seven *A. lettyae* populations and what inference can be made regarding population trends?

2. Methods

2.1. Study area

The study area encompassed the entire known distribution of *A. lettyae,* which is restricted to the areas immediately around Haenertsburg, Magoebaskloof and Modjadjiskloof (altitude 860–1800 m a.s.l.) on the Woodbush Plateau to the north of the Wolkberg mountain, Limpopo Province, South Africa (23°57′S 29°57′E). Climatically the area is characterised by summer rainfall with a mean annual precipitation of 1166 mm, peaking in January. The mean annual temperature is 16.6 °C, and frost is infrequent with an average of only seven frost days per year (Mucina et al., 2006a). The original vegetation is classified as WGG (Gm 25), a mesic highveld grassland with a high level of plant species diversity (Mucina et al., 2006a; Dzerefos and Witkowski, 2016; Dzerefos et al., 2017) and consequently a high conservation value (RSA, 2011). Due to extensive afforestation during the last century, only approximately 6% of the WGG estimated at 340 km^2 historically (Mucina et al., 2006b) remained in 2008 (Niemandt and Greve, 2016), rendering the WGG Critically Endangered (Mucina et al., 2006a; RSA, 2011; Desmet et al., 2013; SANBI, 2013). The landscape is highly fragmented (Niemandt and Greve, 2016), and two sizeable WGG fragments are situated near the small town of Haenertsburg. The largest remaining WGG fragment is 192 ha in size, 66% of which is formally conserved in the recently established Haenertsburg Nature Reserve (Limpopo Provincial Government, 2016), followed by a peninsula-shaped patch of 60 ha. Several additional and generally smaller WGG remnants, ranging in size from <1 to 86 ha, occur in the Haenertsburg, Magoebaskloof and Woodbush Forest areas (Dzerefos and Witkowski, 2016; Dzerefos et al., 2017).

2.2. Study species

Aloe lettyae plants are succulent, short-stemmed or stemless, and adult individuals have a dense rosette of erectly spreading bluish green leaves with dull cream spots on both surfaces (Reynolds, 1969). *Aloe lettyae* juveniles have a fan-shaped arrangement of leaves (Fig. 1a), which changes to a distinctly rosette-shaped arrangement as the plants mature to subadults (Fig. 1b). The leaves of subadults and adults are arranged in a trifarious spiral, i.e. three leaves form each layer within the rosette. Subadults and young adults (reproductively mature individuals) bear the leaf rosette close to the ground (Fig. 1b and c). As the plants grow older, they develop a short stem (<20 cm long) covered with a persistent skirt of withered leaves, and fall over when they become top-heavy (Fig. 1d). Thereby the stem and roots are sometimes partially exposed and may be damaged by fire, ultimately resulting in the death of the plant (Fig. 1e and f). In the cold, dry winter *A. lettyae* leaves often change colour from bluish green to brownish red and die back from the tips. When plant growth resumes in spring, the youngest leaves in adult *A. lettyae* plants are at first often borne fairly upright before growing more horizontally (Kremer-Köhne, *pers. obs.*). A conspicuous character is the inflorescence with brightly coloured orange-red flowers, which can be up to 2 m tall, branched from about the middle, and bearing distinctly widely upwards curved side branches (Smith et al., 2012). Across its restricted range, *A. lettyae* flowers in summer from early December to mid-April (Kremer-Köhne, 2018). The average adult life span of an *A. lettyae* individual in the wild is not known.

2.3. Locating populations

Eight *A. lettyae* populations were known at the start of the study in January 2016 (referred to as HB, DR, EB, RE, FG, BL, BU, FH; Table 2). Another four populations (O1, O2, W1, W2) were located after consulting the collections of the National Herbarium (PRE), Pretoria. Further searches, enhanced by networking with the local community, were undertaken in the Modjadjiskloof (previously Duiwelskloof) area, and in WGG fragments around Haenertsburg, in the Woodbush Forest and in the Groot Letaba Valley. Searches were conducted during the *A. lettyae* flowering season (early January to late March 2016), as the inflorescences are conspicuous and diagnostic. These efforts yielded another seven small *A. lettyae* populations (SK, VE, LE, RR, G, PA, PR), and hence a total of 19 populations (Table 2). For each *A. lettyae* population, the initially estimated total number of flowering plants was recorded. GPS coordinates were taken at the approximate centre of all known localities using a hand-held Garmin unit (Garmin, GPSmap 62sc, USA). The coordinates were mapped and Google Earth Pro was used to draw polygons around the WGG fragments, measure their size and altitudinal range, and determine distances to nearest neighbouring *A. lettyae* populations.

2.4. Data collection

In addition to the above, GPS coordinates were taken on the peripheries of fairly continuous *A. lettyae* aggregations (clumps) within all known populations, and the number of flowering plants in each clump was estimated during January to March of 2016 and 2017 to obtain an estimate more accurate than the initial one. As flowering *A. lettyae* plants were difficult to detect in a few areas with atypically tall, dense vegetation in populations HB and BL, coordinates on the periphery of *A. lettyae* aggregations were recorded after fire in September 2016; the number of fire damaged rosettes >10 cm in diameter was estimated as proxy for the number of flowering plants in these areas. The coordinates were mapped and the *A. lettyae* polygons were measured using Google Earth Pro to determine the area of occupied habitat (ha). The GPS coordinates taken on the peripheries of *A. lettyae* clumps will be useful for locating these clumps during follow up *A. lettyae* surveys.

Aloe lettyae plant density, population size, plant size and life history stage demographics were determined in seven (HB, EB, RE, FG, LE, BL, W1) of the 19 currently known populations, which included the two large populations (HB and EB), as well as a high- (FG) and a low-lying population (W1) (Table 2). Populations HB, EB, RE, FG and

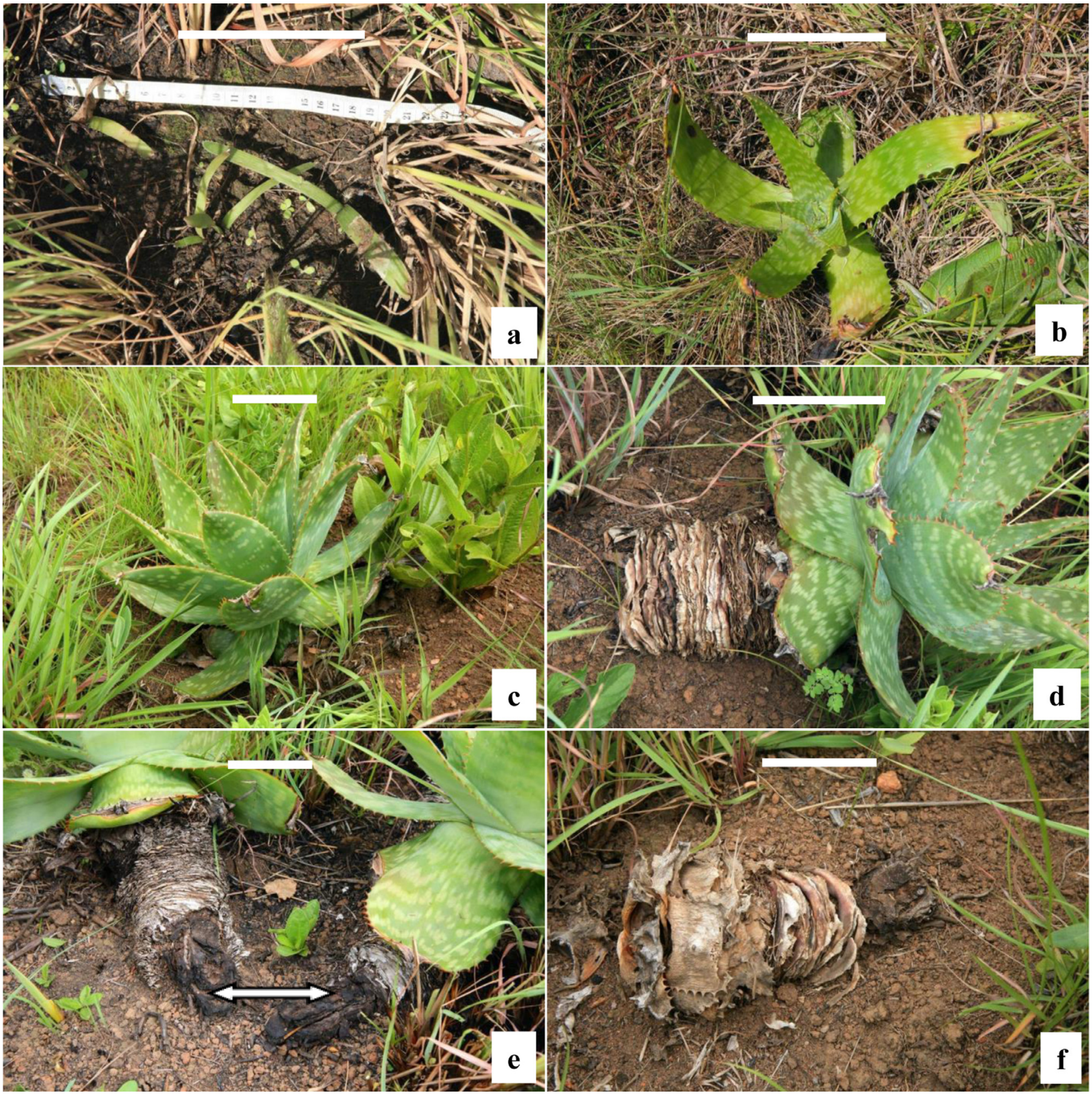

Fig. 1. Life history stages of *Aloe lettyae* (white scale bars=10 cm). (a) Juveniles. (b) Subadult. (c) Young, stemless adult. (d) Old adult that has fallen over due to a top-heavy leaf rosette; persistent skirt of leaf bases is evident on the stem. (e) Top-heavy old adults fallen over, partially exposing stem and roots; fire damage to stems is evident. (f) Dead adult, likely due to fire. Photographs: S. Kremer-Köhne.

BL occur in relatively undisturbed and mostly larger WGG fragments, population LE is found in a degraded WGG fragment and population W1 occurs in non-natural habitat (Kremer-Köhne, 2018). This constituted a representative sample of the entire geographical range of *A. lettyae*. Three sampling methods were used:

a) In summer 2016 (January–April), four smaller *A. lettyae* populations comprising approximately 150 flowering plants (initial estimate) each were selected to obtain preliminary data on plant size and life history stage classes. At this early stage of field work, the collection of plant size metrics was limited to the number of leaf layers and the leaf rosette diameter. Eighty, 40, 60 and 95 plants were randomly selected and measured in populations LE, BL, RE and W1, respectively.

b) From March to May 2017, transects were established across the larger *A. lettyae* clumps (area of occupied habitat 0.1–4.4 ha) in populations HB (clumps A1, A2, B, D, G, L, O and R), EB (clumps 1, 3, 7, 9, 10, 14 and 16) and FG (clump 1), to estimate plant density and the total number of individuals. Sampling points were spaced at 20 m intervals to ensure that individuals were not sampled twice. Two methods based on plant spacing distances, the point-centred quarter method (PCQ) and the Nearest-neighbour method (NN), were used to determine plant densities (Cottam and Curtis, 1956; Marom, 2006; Cousins et al., 2014). In each PCQ, the size of the *A. lettyae* plant closest to the sampling point and that of its nearest neighbour was measured. Placing transects across *A. lettyae* clumps with large variations in plant density, in tall vegetation, and sometimes undulating terrain, proved very challenging. As the dense surrounding vegetation necessitated long, thorough searches for *A. lettyae* plants at each sampling point, a search distance limit of 10 m perpendicular to the transect point was set to increase sampling efficiency.

c) In April–May 2017, all *A. lettyae* plants were counted and their sizes measured in five small clumps (area of occupied habitat <0.1 ha and estimated number of *A. lettyae* <30) to compare the estimates with actual total plant numbers per clump. This was done in two rocky areas (population HB clumps N and Ro1 with visually estimated aerial covers of 13% and 28% rock, respectively in a 1 × 1 m quadrat within 1 m from the aloes; Kremer-Köhne, 2018), and in three non-rocky areas (population HB clumps F and Jo2 and population EB clump 20 with 0% rock cover).

To determine the population size structure, size-related data were recorded for each sampled *A. lettyae* plant. The number of leaves (L) and the number of leaf layers (LL) per rosette were counted, and the height (RH) and diameter (RD) of the leaf rosette were determined with a tape measure. In total, LL and RD were determined for 1084 plants. In addition to LL and RD, L and RH were determined for 809 plants as the latter two metrics were added during the course of the field work. The presence of an inflorescence was noted to determine the percentage of reproductive adults in the study season. During field work, each sampled *A. lettyae* plant was assigned to one of three life history stages:

1) Juveniles (non-flowering plants with a fan-shaped arrangement of leaves; Fig. 1a).
2) Subadults (non-flowering plants with a rosette-shaped leaf arrangement, distinctly smaller than non-flowering adults; Fig. 1b).
3) Adults (plants larger than subadults, with a well-developed leaf rosette, either non-flowering (non-reproductive adults) or flowering (reproductive adults) in the study season).

To assess the health status of the *A. lettyae* populations, the incidence and severity of the fungal rust disease and aloe cancer caused by mites (Smith and Van Wyk, 2008; Van Wyk and Smith, 2014) and the incidence of pests (white scale, aphids) were recorded for each sampled *A. lettyae* plant. The incidences of fungal rust, aloe cancer and pests were calculated as the respective percentage of affected individuals for all populations combined. The severity of fungal rust and aloe cancer was determined by visually estimating the percentage leaf area with the respective symptom; the results are presented as mean ± SE for all populations combined.

2.5. Data and statistical analyses

2.5.1. Estimates of plant density and population size

For the *A. lettyae* clumps sampled along transects, plant densities (plants/m^2) were calculated using the following equations:

For PCQ,

$$N_p = (4(4n-1))/\left(\pi \sum (r_{ij}{}^2)\right)$$

where 'N_p' = Point-quarter estimate of population density; 'n' = number of random points; π = 3.14159; 'r_{ij}' = distance from random point i to the nearest *A. lettyae* individual in quadrant j (j = 1,2,3,4; i = 1, ...n). This is considered to be the appropriate estimate of population density for the point-quarter method, which is the least biased by typical plant clumping (Pollard, 1971; Krebs, 2014).

For NN,

$$\text{Density} = 0.3556 / (\text{sum of r} / \text{n})^2,$$

where 'r' is the distance from each sampled *A. lettyae* individual per quadrat to its nearest neighbour and 'n' is the total number of random points sampled. For both PCQ and NN, the number of *A. lettyae* individuals in the clump or population = density (plants/m^2) × area of occupied habitat (m^2). Subsequently, plant densities were expressed as plants/ha for each clump or population.

Aggregation characteristics of the *A. lettyae* clumps were determined by calculating a non-randomness index according to the equation:

$$a = \pi \text{D w},$$

where 'D' is the population density (PCQ method) and 'w' is the mean of the squares of the point-to-plant distances (Pielou, 1959). If 'a' is equal to, less than or greater than '(n − 1) / n' (where 'n' is the number of point-to-plant distances sampled) the distribution is random, regular or aggregated, respectively (Pielou, 1959).

2.5.2. Plant size and life history stage demographics

The plant size-related data (L, LL, RH, RD) were categorized into the following classes to visualise the SCD curves: ≤5, >5–10, >10–15, >15–20, >20–25, >25–30, >30–35 and >35 leaves/rosette; ≤2, >2–4, >4–6, >6–8, >8–10, >10–12 and >12–14 leaf layers/rosette; ≤10, >10–20, >20–30, >30–40 and >40 cm leaf rosette height, and ≤10, >10–20, >20–30, >30–40, >40–50, >50–60, >60–70 and >70 cm leaf rosette diameter. For all sampled populations combined, and for each population separately, frequencies were calculated for SCD graphs showing the four metrics.

In order to determine which of the plant size metrics (L, LL, RH, RD) predicts *A. lettyae*'s life history stages and fecundity most accurately, and to investigate patterns in the structure of the seven *A. lettyae* populations surveyed, the classification tree technique CHAID (Chi-square Automatic Interaction Detection) was used. CHAID is a nonparametric method that does not require distributional assumptions. It employs a tree growing algorithm to reveal relationships between the dependent variable and the independent variables in a stepwise fashion, i.e. it splits a data set (root node) into groups that differ with respect to the dependent variable (Kass, 1980). Not all variables are evaluated simultaneously, but rather consecutively, using this forward stepwise approach, and the possibility exists that a better separation solution could be found using another variable at an earlier stage in the analyses (Moreno-Fernández et al., 2015). CHAID was run in SPSS (IBM® SPSS® Statistics Version 25).

The independent variable L (number of leaves) was identified as the most accurate predictor of life history stage and fecundity, with 95% and 84% correct predictions, respectively (Table 1), and L was therefore used for further interpretation of the data.

During the CHAID analyses, the algorithm split the *A. lettyae* life history stage class data (juvenile, subadult, adult) into four nodes with significantly different leaf numbers (L) while the fecundity data (reproductive no/yes) were split into five nodes with significantly different leaf numbers (L) (Supplementary Fig. 1 LH a and F a). These results were used to relate L SCD frequencies to *A. lettyae* life history

Table 1
Percentage correct predictions of life history stage (juvenile, subadult, adult) and fecundity (reproduction no/yes) when using the independent variables number of leaves (L), number of leaf layers (LL), leaf rosette height (RH, cm) and leaf rosette diameter (RD, cm), as determined by the classification tree technique CHAID (Chi-square Automatic Interaction Detection) (Supplementary Fig. 1).

Independent variable	Correct prediction (%)	
	Life history stage	Reproduction
Number of leaves (L)	95% (χ^2 = 692.839; df=3; p<0.0001)	84% (χ^2 = 382.965; df=4; p < 0.0001)
Number of leaf layers (LL)	92% (χ^2 = 1072.520; df=6; p<0.0001)	83% (χ^2 = 500.259; df=5; p < 0.0001)
Leaf rosette height (RH, cm)	91% (χ^2 = 571.834; df=4; p<0.0001)	82% (χ^2 = 345.761; df=4; p < 0.0001)
Leaf rosette diameter (RD, cm)	87% (χ^2 = 739.858; df=4; p<0.0001)	81% (χ^2 = 477.812; df=4; p<0.0001)

stages for all surveyed *A. lettyae* plants combined to establish population demographics (Witkowski et al., 2001; Cousins et al., 2014). Following Cousins et al. (2014), plant size in terms of leaf numbers (L) was related to appropriate life history stage classes by defining the approximate size at which the transitions from juvenile to subadult, and from subadult to adult occurred. Juvenile classification was refined as small, non-reproductive individuals with $L \leq 7$ (Supplementary Fig. 1 LH a, Node 1, proportion of juveniles >85%). Subadult plant classification was refined as non-reproductive individuals that occupied the narrow band of the intermediate sizes $7<L\leq 14$ (Supplementary Fig. 1 LH a, Node 2, proportion of subadult plants >65%; Supplementary Fig. 1 F a, Node 2, proportion of non-reproductive individuals outweighed (≥90%) the proportion of reproductive plants). Adults were re-defined as individuals occupying bigger size classes $L>14$ (Supplementary Fig. 1 LH a, Nodes 3 and 4, proportion of adults ≥98%; Supplementary Fig. 1 F a, Nodes 3–5, proportion of reproductive plants ≥64%). Then the L SCD was constructed for non-reproductive versus reproductive individuals (Fig. 2).

3. Results

3.1. Population size and plant density

We located and documented 19 *A. lettyae* populations, most of which were clustered on the south western side of the WGG vegetation type (Mucina et al., 2006b), with the furthest populations occurring less than 40 km apart in this severely fragmented vegetation type (Kremer-Köhne, 2018). The two large populations, HB and EB, occur near Haenertsburg town and are only two km apart while distances between nearest neighbouring *A. lettyae* populations range from 0.9 to 6 km (Table 2). Initial estimates of flowering *A. lettyae* plants, which varied greatly from 1240 in population HB to just 10 in population PR (Table 2), were useful for guiding the sampling strategy. However, these preliminary numbers of *A. lettyae* plants underestimated the size of the populations, as both non-flowering and flowering *A. lettyae* individuals were initially not detected in the surrounding tall, dense vegetation. Counting all plants in small clumps within populations HB and EB revealed an average underestimation of 53%, ranging from 24% to 86% (non-rocky HB F–24%, HB Jo2–58%, EB 20–50%; rocky HB N–46% and HB Ro1–86%).

Populations with a seemingly fairly continuous distribution of *A. lettyae* plants displayed a large variation in plant density, and all *A. lettyae* populations sampled via transects were aggregated (Table 3). Plant densities varied from 473 to 3965 plants/ha between *A. lettyae* clumps sampled along transects in the large populations HB and EB, and the smaller population FG (Table 3). For each clump, plant density estimates differed between the PCQ method and the NN method, sometimes by as much as 185% (clump EB 14) (Table 3). Given these inconsistencies, the plant numbers calculated by the PCQ method, which is considered least biased by plant clumping (Krebs, 2014), were used to estimate the total number of plants in the national *A. lettyae* population at ~10,800 individuals (Table 4). For the large populations HB and EB, the total area of occupied habitat amounted to 10.7 and 2.5 ha, of which 69% and 83% was sampled, respectively (Table 4). For all *A. lettyae* populations combined, the total area of occupied habitat amounted to 17.5 ha (Table 4). Based on our occurrence records and the reference scale of 4 km^2 cells (IUCN Standards and Petitions Subcommittee, 2017), *A. lettyae*'s area of occupancy (AOO) was calculated at 56 km^2 (Von Staden, *pers. comm.*). This AOO falls within an extent of occurrence (EOO) polygon of 123 km^2 (Von Staden and Kremer-Köhne, 2015).

3.2. Plant size and life history stage demographics

Size class distribution frequencies for the number of leaves (L) and leaf layers (LL), as well as for leaf rosette height (RH) and diameter (RD), for all populations combined and for individual populations, appeared fairly consistent (Supplementary Fig. 2). However, we identified variable L as the most accurate predictor of *A. lettyae* life history stage and fecundity. Life history stage classes spanned several size classes based on the number of leaves (L), with slight differences between life history stage classes and size classes at the transitions from juvenile to subadult ($L>7$ versus $L>10$), and from subadult to adult ($L>14$ versus $L>15$) (Fig. 2). Across all *A. lettyae* populations, 10% of individuals were juveniles, 8% were subadults and 82% adults (Supplementary Fig. 1 LH a), and approximately 80% of *A. lettyae* individuals occurred in the bigger size classes $14<L<35$ comprising adult, mainly reproductive individuals (Fig. 2). Non-reproductive adult plants occurred in the same size classes as reproductive adults, and the proportion of non-reproductive adults decreased with an increase in size class, reaching a very low proportion (<1%) in the largest size classes $25<L<35$ (Fig. 2). During our surveys, we found few dead adult *A. lettyae* individuals, which had likely died following a fire, suggesting a very low mortality rate. Overall, the surveyed *A.*

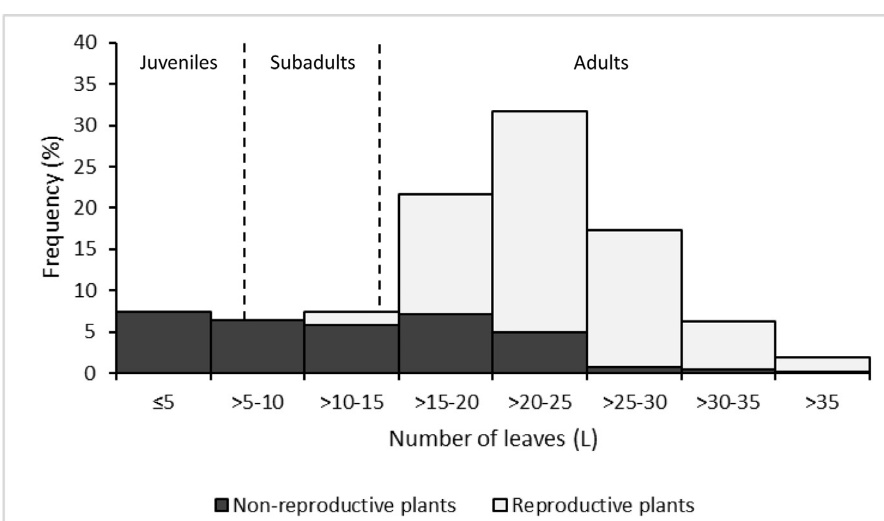

Fig. 2. *Aloe lettyae* size class distribution in terms of the number of leaves (L) for all surveyed plants combined (n = 809). The percentage of non-reproductive and reproductive individuals, as determined by CHAID (Chi-square Automatic Interaction Detection) analyses, was used to establish the approximate size at which the transition from one life history stage class to the next occurred (from juvenile to subadult, and from subadult to adult). Stage class transitions are indicated by dashed lines.

Table 2
Known *Aloe lettyae* populations in Limpopo Province, South Africa, in order of Woodbush Granite Grassland (WGG) fragment size (ha). The population's initial estimate (number of flowering plants), distance to nearest neighbouring population (km), identity code (ID) of nearest neighbouring population, and the altitudinal range (m) of the locality are shown.

A. lettyae population				WGG fragment	
Population[a]	Initial estimate[b] (number of flowering plants)	Distance to nearest neighbouring population (km)	ID of nearest neighbouring population	Altitudinal range (m)	Size (ha)[c]
HB*	1240	2.0	EB	1368–1534	192
DR	35	2.3	RE	1505–1618	86
SK	40	6.0	VE	1750–1874	76
EB*	925	1.4	LE	1365–1413	60
VE	150	1.5	RE	1430–1520	40
RE&	120	1.5	VE	1470–1530	25
FG*	145	1.1	FH	1614–1746	14
O1	20	3.4	O2	1327–1398	10
O2	200	3.4	O1	1356–1369	10nn
LE&	195	1.4	EB	1374–1402	9
BL&	150	0.9	BU	1490–1607	9
BU	15	0.9	BL	1611–1721	6
FH	50	1.1	FG	1616–1685	6
RR	135	1.0	PA	1473	<1nn
G	140	4.7	RR	1196	<1
PA	20	1.0	RR	1478	<1nn
PR	10	1.2	FH	1555–1577	<1nn
W1&	150	2.9	W2	863	<1nn
W2	20	2.9	W1	820	<1nn

[a] population sampled in 2016&; population sampled in 2017*.
[b] at the start of field work in summer 2016.
[c] nn=non-natural habitat.

Table 3
Area of occupied habitat (ha), number of *Aloe lettyae* plants and their density (plants/ha) as determined by Nearest-neighbour (NN) and the Point-centred quarter (PCQ) methods, respectively, for *A. lettyae* clumps sampled along transects in the large populations HB and EB, and the smaller population FG. For each clump, the aggregation of plants was determined by calculating the non-randomness index (a) (Pielou 1959) using the PCQ plant density, with an aggregated distribution indicated when $a > (n-1)/n$.

			NN		PCQ		
Population	Clump	Area of occupied habitat (ha)	Number of plants	Plants/ha	Number of plants	Plants/ha	Aggregation ($a > (n-1)/n$ =aggregated)
HB	A1	0.40	228	576	214	541	4.46 > 0.94
HB	A2	0.66	433	655	313	473	5.73 > 0.97
HB	B	0.38	326	864	283	751	6.66 > 0.89
HB	D	4.40	2405	546	2850	647	5.10 > 0.98
HB	G	0.30	204	672	243	800	4.00 > 0.86
HB	L	0.59	570	960	608	1025	3.77 > 0.94
HB	O	0.15	263	1778	587	3965	3.50 > 0.88
HB	R	0.48	856	1780	762	1584	4.00 > 0.93
	Overall	7.36	5285	979	5860	1133	
EB	1	0.63	983	1564	411	655	4.35 > 0.96
EB	3	0.15	182	1463	188	1004	4.18 > 0.93
EB	7	0.24	200	835	241	1009	5.07 > 0.94
EB	9	0.44	544	1227	459	1036	4.34 > 0.96
EB	10	0.20	339	1670	153	756	4.47 > 0.94
EB	14	0.21	314	1510	110	529	4.18 > 0.95
EB	16	0.17	121	709	113	661	5.10 > 0.94
	Overall	2.04	2683	1283	1675	807	
FG	1	0.35	140	523	336	1253	4.28 > 0.93

lettyae populations appeared healthy with low disease incidence (rust and aloe cancer affected 6% and 1% of all surveyed plants, respectively) and severity (rust 0.7 ± 0.1%, aloe cancer 0.2 ± 0.1%), and very low incidences of the pests white scale and aphids (<0.2% of all surveyed plants) (Kremer-Köhne, 2018).

The L SCDs for all *A. lettyae* populations combined ($n = 809$) and for the big populations EB ($n = 329$) and HB ($n = 454$) individually, exhibited a unimodal, approximately bell-shaped distribution (Supplementary Fig. 2a–c). For the small population FG ($n = 26$), the L SCD was also unimodal with one missing size class ($5 < L \leq 10$) (Supplementary Fig. 2d). Patterns in the structure of the seven *A. lettyae* populations surveyed (HB, EB, FG, RE, BL, LE, W1) were revealed by the CHAID model which split the *A. lettyae* life history stage class data (juvenile, subadult, adult) into four population nodes with significantly different proportions of juvenile, subadult and adult individuals, respectively, with a 78% correct prediction probability (χ^2 = 191.450; df = 6; $p < 0.0001$)

Table 4
The national *Aloe lettyae* population: area of occupied habitat (ha) and number of plants as established by the PCQ method along transects (Transects), counts for clumps in which all plants were sampled (All plants), and estimates for both clumps in larger populations and small populations which were not sampled (Non-sampled) as well as for populations in which plants were sampled randomly (Random).

Population	Sampling	Area of occupied habitat (ha)	Number of plants
HB	Transects	7.36	5860
	All plants	0.17	187
	Non-sampled	3.21	500
	Total HB	10.74	6547
EB	Transects	2.04	1675
	All plants	<0.01	21
	Random (clump 21)	0.04	100
	Non-sampled	0.39	445
	Total EB	2.47	2241
FG	Transect	0.35	336
RE	Random	1.31	165
LE	Random	0.16	200
BL	Random	0.52	330
W1	Random	0.07	150
Other small populations	Non-sampled	1.87	770
National population		17.49	10,739

(Fig. 3). Node 1 contained only population EB which had the lowest percentage of juveniles (3%) and the highest percentage of adults (89%). Node 2 comprised populations HB, FG, RE and BL with 14% juveniles and 77% adults. Node 3 was occupied by population LE with 25% juveniles and 63% adults, while Node 4 contained population W1 with the highest percentage (58%) of juveniles and lowest percentage of adults (32%). The percentage of subadults varied little across the four CHAID nodes (8–13%) (Fig. 3).

In the respective study seasons, the percentage of adult plants (comprising both flowering and non-flowering individuals) differed between *A. lettyae* populations, and there were also differences in the percentage of flowering adults (Table 5). For the populations sampled in 2016 (RE, BL, LE and W1) and those sampled in 2017 (EB, HB, FG), the percentage of flowering plants ranged from 73% to 90%, and from 71% to 87%, respectively (Table 5).

4. Discussion

4.1. Population size and plant density

Aloe lettyae populations are aggregated and occur in tall, dense grassland vegetation in which plants are difficult to detect. This impedes the ability to locate populations in the landscape, as well as individuals within a population. Although the PCQ method developed for sampling tree composition in forests (Cottam and Curtis, 1956) is considered least biased by plant clumping (Krebs, 2014), this method has been shown to underestimate forb and grass species densities in grassland populations, which often exhibit aggregation (Risser and Zedler, 1968; Good and Good, 1971; Mitchell, 2007). In this study, *A. lettyae* plant densities calculated by the PCQ method differed to some extent from those calculated by the NN method. Notwithstanding, our estimates put the national *A. lettyae* population at ~10,800 individuals. Differences between estimates obtained with these two distance sampling methods have also been observed in succulent *Delosperma* species (Marom, 2006) and the tree aloe *Kumara plicatilis* (=*Aloe plicatilis*) (Cousins et al., 2014). Phama et al. (2014) surveyed *A. peglerae* populations using another method of distance sampling, the line transect sampling technique, and concluded that the technique needed some refinement. Comparing initial estimates of flowering *A. lettyae* plants obtained at the start of field work in summer 2016 with

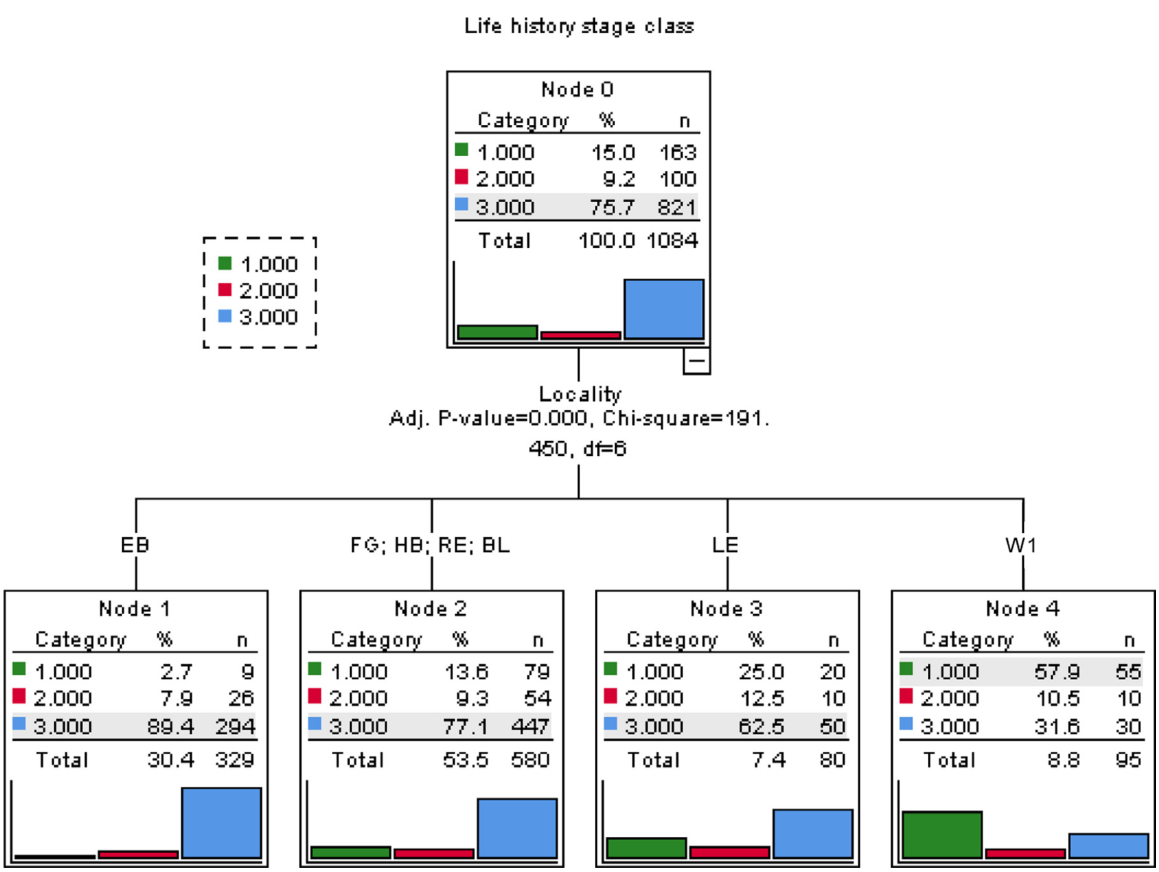

Fig. 3. Classification tree model for *Aloe lettyae* life history stage classes (1=juvenile, 2=subadult, 3=adult) as determined by CHAID (Chi-square Automatic Interaction Detection) analyses when using the independent variable locality (=population HB, EB, FG, RE, BL, LE, W1).

Table 5
Percentage of adults (%, comprising both flowering and non-flowering individuals) in sampled *Aloe lettyae* populations split into flowering and non-flowering adults (%). Populations grouped into four nodes according to life history stage using the classification tree technique CHAID (Chi-square Automatic Interaction Detection) (Fig. 3). The number of plants sampled (*n*) and the year of sampling is indicated.

Population	Node	Sampling			Adults	
		n	Year	Adults in population (%)	Flowering (%)	Non-flowering (%)
EB	1	329	2017	89	87	13
HB	2	454	2017	77	77	23
FG	2	26	2017	81	71	29
RE	2	60	2016	77	80	20
BL	2	40	2016	73	90	10
LE	3	80	2016	63	82	18
W1	4	95	2016	32	73	27
Total		1084				

the actual plant counts in small clumps, showed that actual abundance may be underestimated on average by as much as 53%. Population sizes have also been underestimated for *A. peglerae* (Arena et al., 2015) and for the dwarf Asphodelaceae taxon *Haworthiopsis koelmaniorum* (Witkowski and Liston, 1997) due to the difficulty of finding small cryptic plants in dense vegetation, as well as for *Euphorbia barnardii* (Knowles and Witkowski, 2000). Although the clumped distribution of *A. lettyae* plants within populations appears to have affected the plant density estimations, we recommend using the PCQ method, in combination with the NN method, for a follow up survey to be consistent with this study.

4.2. Plant size and life history stage demographics

Aloe species grow slowly and are long-lived, with some tree aloes having been estimated to be over 200 years old (Smith and Van Wyk, 2008). For stemless aloes, long-term population structure monitoring data have only been published for the slow-growing *A. peglerae* (Scholes, 1988; Pfab and Scholes, 2004; Phama et al., 2014). In *A. peglerae*, population size structure was based on the leaf rosette diameter (Arena et al., 2015). However, population size structures have also been investigated in the stemless *Aloe petricola* using the height of the leaf rosette (Weisser and Deall, 1989) and in the stemless *A. ortholopha* by measuring the circumference of the leaf rosette (Kunonga et al., 2019). In this study, the plant size metrics L, LL, RH and RD were evaluated for *A. lettyae*, and the number of leaves (L) was found to be the most accurate predictor of life history stages. To save on time and sampling effort by eliminating unnecessary measurements, we propose that only the number of leaves, as a proxy for life history stage, be counted during future *A. lettyae* demographic surveys.

The classification tree technique CHAID is occasionally used for ecological modelling (Menéndez et al., 2006; Moreno-Fernández et al., 2015; Nishio et al., 2015). However, to our knowledge, CHAID is a novel approach to population structure assessment. As indicated by the CHAID model, *A. lettyae* populations EB, HB, FG, RE and BL occurring in relatively undisturbed WGG fragments comprise high proportions of adult individuals, while only few *A. lettyae* juveniles were found, although more may have been present, as they were difficult to detect in the dense vegetation. Differences in the habitat profiles of these populations (Kremer-Köhne, 2018), fire occurrence and other disturbances may be possible causes for the differences in population structure. The high percentages of juveniles and low percentages of adults in the small populations LE and W1 may be considered unusual as population LE occurs in a degraded WGG fragment with patches of bare soil and population W1 occurs in non-natural habitat on a fire break between timber plantations.

In *A. petricola* and *A. peglerae*, the population size structure was also characterised by the dominance of individuals in the intermediate, and the intermediate and large size classes, respectively, while very few seedlings and juveniles were observed (Weisser and Deall, 1989; Pfab and Scholes, 2004; Arena et al., 2015). This size class distribution is considered typical of aloes, which generally reach reproductive maturity relatively quickly (± 5 years), and then continue growing slowly for many years before dying (Weisser and Deall, 1989). Recruitment levels in the relatively long-lived *A. peglerae* (average adult lifespan of 60 years) were found to be low, and on average, seedlings grew into juveniles after four years, and juveniles matured into adults within six years (Pfab and Scholes, 2004). This developmental history is likely similar in *A. lettyae* (Kremer-Köhne, *pers. obs.*). Flowering of individual *A. peglerae* plants has been shown to be highly variable between years (Scholes, 1988; Arena et al., 2015; Payne et al., 2019), and there was an increase in the percentage of flowering *A. peglerae* plants as plant size increased (Arena et al., 2015). In this study, we found that overall 80% of adult *A. lettyae* plants were flowering across all size classes over the two sampling years (2016 and 2017). As in *A. peglerae*, the proportion of flowering *A. lettyae* plants increased with increasing plant size, with >99% of *A. lettyae* plants in the largest size class flowering. This finding suggests that even when an *A. lettyae* plant reaches reproductive size, it does not necessarily flower every year. Although long-term data are not yet available for *A. lettyae*, preliminary observations seem to indicate that flowering of individual *A. lettyae* plants is not as variable between years as that of *A. peglerae*.

Furthermore, a dominance of middle size class individuals, with fewer small and large class sizes (bell-shaped SCD) was found in the stemless *A. ortholopha* (Kunonga et al., 2019) and the slow-growing tree aloe *Kumara plicatilis* (Cousins et al., 2014). Cousins et al. (2014) suggest that the survival of long-lived adult plants such as *K. plicatilis* determines population persistence and assume that recruitment occurs through the addition of small numbers of juveniles over long periods, continually replacing senescing adults. If populations are consistently dominated by adults with a very high probability of survival, minimal recruitment is not a cause for concern. Therefore, bell-shaped SCDs observed in slow growing, long-lived species such as *A. lettyae* may be considered as indicative of healthy, stable populations (Cousins et al., 2014).

5. Conclusion

Our study provided snapshot information on the size structure and life history demographics of extant *A. lettyae* populations, and baseline *A. lettyae* population data for rigorous follow-up studies to aid management and conservation of this range restricted, Endangered aloe. To assess if *A. lettyae* populations are increasing or declining in number of individuals, long-term population size and structure monitoring is required, using consistent methodology and implementing regular data assessment (Witkowski et al., 1997; Van der Merwe and Geldenhuys, 2017). While other populations might yet be located, the results from this study indicated that *A. lettyae* is restricted to 19 populations, 13 of which occur in Critically Endangered WGG remnants; the other six small populations occur in non-natural habitat. *Aloe lettyae* can therefore be used as a flagship species in raising support for the conservation of its highly threatened habitat. The findings of our study suggest that *A. lettyae* remains in the Endangered category, owing to its restricted, fragmented and declining EOO and AOO (Von Staden and Kremer-Köhne, 2015). In terms of the number of plants, 49% of the national *A. lettyae* population is protected in the Haenertsburg Nature Reserve in which the persistence of the species will likely be affected by management practices.

Declarations of Competing Interest

None

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.sajb.2020.01.029.

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