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Towards better risk assessment for conservation of flowering stones: Plant density, spatial pattern and habitat preference of *Lithops pseudotruncatella* in Namibia

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ABSTRACT

To provide a better understanding of spatial pattern and habitat preferences for a cryptic xerophyte, a number of variables were investigated in a 10,000 m^2 (1 ha) study area in a population of *L. pseudotruncatella*; plant density, spatial arrangement of plants, and habitat specificity. Two censuses, carried out in dry season and in rainy season, revealed a total of 448 and 860 plants, respectively, with a severely clumped distribution. Five out of seven plant density estimation methods, applied in dry season, produced data that vastly over-or under-estimated plant number while Adaptive Cluster Sampling and the Belt Transect method were more accurate, with 557 and 540 plants, respectively. Plant number in 10 × 10 m test plots within the study area was positively associated with a high percentage cover of gravel and pebbles as opposed to sand or stones, and with a gentle rise as opposed to a slope in the topography of the plots. A significant association was found between the occurrence of *Lithops* and *Avonia albissima* in the test plots suggesting that the latter, which is more visible, can be used as an indicator of *Lithops* in the field.

1. Introduction

The genus *Lithops* N.E. Br. (Aizoaceae) belongs to a group of dwarf succulent plants commonly known as "flowering stones" due to their ability to blend in with their environment in the semi-arid to arid parts of southern Africa. A single plant consists of one or more reduced pairs of opposite, highly succulent leaves that are fused at the base, and are either flush with the ground or partially elevated (Cole and Cole, 2005). Their reduced morphology and likeness to the surrounding stones help to protect them against herbivory, while a tough epidermis prevents excessive water loss during dry periods. The plants are naturally slow-growing and some species can reach 50 years or more in nature (Schwantes, 1957). Although most species grow in arid biomes, they generally avoid dunes and other forms of shifting sands (Cole and Cole, 2005) and instead prefer gravel plains, rocky outcrops and hillsides.

Illegal harvesting of live plants and seeds, together with habitat alteration, are the main factors believed to currently impact the survival of *Lithops* in Namibia, and several taxa are now listed as threatened with extinction in Namibia (Loots, 2005) and South Africa (Raimondo et al., 2009). Namibia uses the IUCN Red List Categories and Criteria (IUCN, 2001, 2013) to assign conservation categories to indigenous plant species. Well-informed conservation decisions regarding *Lithops* populations are much needed but these are difficult to formulate without detailed information on population parameters such as the number of mature individuals, extent of occurrence, area of occupancy, recruitment and threats. The most accurate way of determining population size is to count every plant detected. Unfortunately, in genera such as *Lithops* where individual plants are extremely cryptic when not in flower, particularly in the dry season, such undertakings become very time-consuming and there can be considerable degree of error. Various plant density estimators can provide valid data with less efforts but their efficiency is, to a high degree, dependent on the spatial arrangement of plants in the studied population. Some *Lithops* species have been reported to have a severely clumped distribution (Loots, 2011).

In Namibia, *Lithops pseudotruncatella* (Berg.) N.E. Br. is the only *Lithops* species growing in the savanna biome. The current conservation status is Least Concern, and although quantitative population data is lacking in many populations, anecdotal evidence indicate that some of its populations are in decline. Using *Lithops pseudotruncatella* as a model species, the current study was undertaken to determine habitat

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preferences and to investigate which plant density estimator method is most accurate for this species and possibly also for other species with a similar spatial distribution.

2. Material and methods

2.1. Study site

The targeted population of *L. pseudotruncatella* subsp. *pseudotruncatella* covers about 2.5 ha in total, and is situated approximately 45 km south-east of Windhoek, on the northern outskirts of the town Groot Aub, Namibia, on the Khomas Hochland Plateau at an altitude of 1693 m. This plateau belongs to the highland savanna vegetation zone (Giess, 1998), in the savanna biome (Irish, 1994) and receives summer rainfall. Data recorded for the meteorological station in Windhoek show a mean of 300–360 mm annual rainfall while temperatures reach an average annual maximum of 31 °C for December/January and an average annual minimum of 7.5 °C for June/July (Meteorological Service Division, 2012). The area is characterized by the Hakos Group sandstones (Miller, 2008) and eutric leptosols (De Pauw et al., 1998, 1999). The overall topography consists of a very gentle east-facing slope towards an ephemeral river. Within the area, there are gentle quartz rises. There is no formal grazing management regime but small and large stock are continuously present in relatively small numbers.

A square of 100 × 100 m (1 ha) was laid out to cover part of the total population, with corners marked with a GPS using the Universal Transverse Mercator (UTM) system and the WGS 84 Datum. Iron droppers were used to demarcate each 50 m point in the hectare to facilitate field work. Exact coordinates are omitted to protect the population from unscrupulous collecting. Three parameters were studied: plant density, spatial distribution pattern and habitat characteristics.

2.2. Density assessment methods

Estimations of plant density were carried out in the dry season of June and July, 2012. In addition to conducting an initial census of all *Lithops* plants marked temporarily with numbered plastic markers (Mannheimer and Loots, 2012) in the whole 1 ha study area as a control, seven density estimation methods were applied using the census-detected plants; (1) Nearest Neighbour (Cottam and Curtis, 1956) using 250 randomly chosen plants from the initial census as a starting point, (2) Closest Individual (Cottam et al., 1953) using 150 measurements from a random point to the closest plant, (3) Kendall-Moran (Kendall and Moran, 1963) using the same data as in method 2 plus an additional 150 measurements to the next neighbour, (4) Ordered Distance Third Closest Individual (Morisita, 1957) using the same random points as in methods 2 and 3and taking measurements to 3rd closest plant, (5) Variable Quadrant Plot (VQP) (Coetzee and Gertenbach, 1977), using four quadrats of 10 m × 10 m as starting points and ending up with a final size of 50 m × 50 m thus covering ¼ of the hectare, (6) Belt Transect (Elzinga et al., 1998) using ten 100 m × 1 m rectangular quadrats placed systematically at 10 m intervals along an E-W gradient, and (7) Adaptive Cluster Sampling (Philippi, 2005) using 200 initial 1 m × 1 m random quadrats. Density from Distances software (Henderson and Seaby, 1999) was used to calculate plant densities for the data collected with the first four methods. Density for the Adaptive Cluster Sampling method was calculated in MS Excel 2010 according to Krebs (1999).

2.3. Census and determination of spatial pattern

In addition to the initial census conducted in 2012 (see above), another census of all *Lithops* plants was carried out in the rainy season of February 2013. The 1 ha study area was divided into 100 test plots of 10 × 10 m each. The locations of all detected *Lithops* plants were marked temporarily with plastic markers and the number of *Lithops* in each test plot was counted. Since multi-headed plants are common in this species, leaf pairs occurring in close proximity were checked for facial patterns and colours in order to determine whether they were more likely to derive from a single plant or from separate plants.

2.4. Habitat characteristics

In each of the 100 10 × 10 m test plots, the following variables were recorded in the rainy season of February 2013: (1) the topography was categorized as flat, slope, depression, rise or undulating; (2) aspect was determined with a compass; (3) gradient was measured with a clinometer; and (4) substrate was categorized as follows: sand (<0.2 cm), gravel (0.2–2 cm), pebbles (2–6 cm), medium stones (6–20 cm), large stones (20–60 cm) and rock (>60 cm) (Strohbach, 2001) and then a 'substrate cover' was subjectively estimated as the percentage of the total area in the test plot that is covered by each of the six substrate types. Since patches of habitat appear to be unavailable for *Lithops*, due to the presence of trees, thorn shrubs or other shrubbery, dense patches of grass, large stones, game trails etc., 'available habitat' was subjectively estimated as the percentage habitat in each test plot that was available for *Lithops*. Since *Avonia albissima* appears to co-occur with *Lithops* to a large degree, presence/absence of this species was noted for each of the 100 test plots.

Twelve soil samples of approximately 1 L each were collected from the top 4–5 cm in the study area. Three samples each were collected from test plots with zero, few (1–9), medium (30–46) and a high (55–90) number of *Lithops* plants, respectively. A 1000 μm sieve was used to separate the soil from the stone particles, and the percentage of stone particles in each sample was determined.

2.5. Statistical analyses

Based on the census data, a Goodness of Fit test for a Poisson distribution was carried out to determine if the observed distribution of plants in the 100 test plots differs significantly from the distribution that would be expected from a population with individuals occurring at random. A Principal Component Analysis (PCA) was performed to determine the relationship between the number of plants per plot and the percentage cover of the different substrates in the test plots as well as gradient, topography and aspect. Associations between the percentage cover of the different substrates and plant number was also investigated with Spearman's rank correlation coefficient using Bonferroni corrections for multiple analyses. For the 12 test plots where soil samples were taken, Spearman's rank correlation coefficient was used to compare the number of plants with the percentage stone particles in the soil to determine if there is an association between the number of plants and the percentage stone particles at root level. A chi-square test of association in the form of a 2 × 2 contingency table was used to detect a possible co-occurrence of *Lithops* and *Avonia* in the 100 test plots. All statistical analyses were carried out using Minitab17.1® Statistical Software (2013) and Microsoft Excel 2010.

3. Results and discussion

Although the work was carried out on a single population and therefore is relevant only to this population, our results are likely to be applicable to other *Lithops* species, since overall habit, life cycle, spatial patterns and habitat preferences are very similar.

3.1. Estimation of plant density

The two censuses carried out in 2012 and 2013 detected 448 and 860 plants, respectively. The additional plants found in 2013 were too large in size to have developed within the seven-month interval between the two census occasions. Instead, the 48% increase in plant number is most likely in part due to the division of the 1 ha area into

Table 1
Results of two censuses and data obtained with seven methods of estimating plant density.

Density estimation method	Number of plants in ha	% of Census 2012
Census 2012 (dry season	448	100
Census 2013 (rainy season)	860	
Nearest neighbour	1711	382
Closest individual	36	8
Kendall-Moran	55	12
Ordered distance third closest individual	70	15
Variable quadrant plot (VPQ)	292	65
Belt transect	540	120
Adaptive cluster sampling (ACS)	557	124

more easily monitored 10 × 10 m test plots, and in part to plants being more detectable after the rains that fell from January up until the second census. Whenever possible, fieldwork involving counts of *Lithops* should therefore be conducted during or just after a rain event, and small plot sizes should be used. This is feasible for species such as *L. pseudotruncatella*, which occurs in the savanna biome where rainfall is relatively predictable. However, in desert biomes, where rainfall is both unpredictable and erratic, this becomes more difficult.

All of the density estimation methods proved exceedingly time consuming, except the Belt Transect method. The results obtained with the Adaptive Cluster Sampling method (557 plants) and the Belt Transect method (540 plants) are the closest to the number of plants obtained in the two censuses and therefore the most accurate (Table 1). The Belt Transects took only 2.3 h for two persons to complete, whereas the Adaptive Cluster Sampling Method took 8.9 h. It should be noted that most of the recorded plants had already been marked during the preceding census. If carried out from the start, each of the tested methods is likely to require 3–5 times longer when applied to *Lithops* as it takes longer to find the plants.

3.2. Spatial pattern

The 2013 census revealed an extremely clumped distribution; almost 92% of the total population occurred in just 20% of the test plots, while 80% of the test plots contained fewer than 10 plants, accounting for only 8% of the total population in the study area. A clumped distribution was also confirmed by the Goodness of Fit test for a Poisson distribution: $\chi^2 = 1959$, df = 10, P < 0.001. The gross over-estimation calculated with the Nearest Neighbour method, and the gross under-estimation calculated with the Closest Individual method and its variants, also confirm a clumped pattern (Table 1). Analysing a smaller part of a population of a cryptic plant species using these two methods together with the Belt Transect method, could serve as an indicator as to whether the targeted species has a clumped distribution.

A clumped distribution has been reported for many species in the family Aizoaceae (Ihlenfeldt, 1983; Burke and Mannheimer, 2003). For *Lithops* there are probably two main reasons: firstly, the morphology of the *Lithops* seed capsule suggests a wash-out mechanism similar to that of the subtribe Dracophilinae (Mannheimer, 2006) mainly resulting in short-distance seed dispersal (Ihlenfeldt, 1983; Cole and Cole, 2005) and seedling establishment close to other individuals, secondly, the plants inhabit only those small pockets that offer a highly suitable habitat.

3.3. Habitat characteristics

The PCA shows that mainly southeast- or south-facing plots situated on a rise, with a large percentage of pebbles and gravel, are likely to harbour a high number of *Lithops* plants (Fig. 1). A high percentage of sand or medium-sized stones is instead negatively associated with plant number as are also slopes, and north- and east-facing aspects. The mean available habitat was 45.7% (std 25.4) in the 44 test plots where *Lithops* were found. This parameter was highly correlated with the number of *Lithops* plants found in the same plots (Table 2). Number of *Lithops* plants was positively correlated with percentage of pebbles and gravel and negatively with percentage of sand (Table 2). Local adaptation to different edaphic micro-environments has been reported also for other succulents, and apparently plays a major role in the divergence between *Argyroderma* species in potentially functional morphological traits but may also be important for the diversification of the Aizoaceae in southern Africa (Ihlenfeldt, 1994; Schmiedel and Jürgens, 1999; Ellis and Weis, 2006; Ellis et al., 2006; Hartmann, 2006).

For those test plots where soil samples had been taken, there was no correlation between plant number and percentage stone particles in the soil (Rs = 0.19, p = 0.55; $\bar{x}$stone particles = 61%, s = 8.04). This could be due to sampling error. Possibly, the samples should instead have been taken along a plant density gradient.

Lithops seem to prefer a habitat with pebbles and gravel as neither larger stones nor sand can provide the stabilization required by the

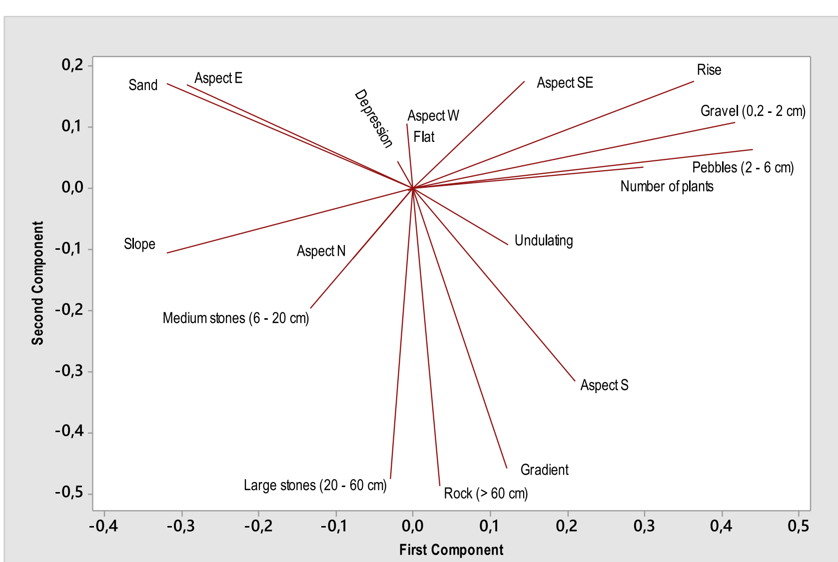

Fig. 1. Principal Component Analysis showing the relationship between number of *Lithops* plants, substrate cover, gradient, topography and aspect in 100 10 × 10 m test plots.

Table 2
Spearman rank correlation coefficient (Rs) calculated for associations between number of *Lithops* plants and the percentage cover of each of six different substrates as well as available habitat and gradient in 100 10 × 10 m test plots. Using Bonferroni correction for eight tests, p < 0.005 can be regarded as significant at the 5% level. Also indicated is the range of substrate cover for the 44 test plots containing *Lithops*.

Substrate (ø) in cm	Rs	P-value	Min–max range of % substrate cover for 44 plots with *Lithops*
Sand (<0.2)	−0.389	<0.0001	0–61
Gravel (0.2–2)	0.519	<0.0001	10–60
Pebbles (2–6)	0.635	<0.0001	10–60
Medium stones (6–20)	−0.303	0.0022	3.5–60
Large (20–60)	−0.121	0.2318	1–10
Rock (>60)	−0.137	0.1726	1–10
Available habitat	0.577	0.000	
Gradient	0.083	0.431	

relatively weak root system of the *Lithops* plants. Presence of more soil in the medium- to large-sized stone habitat instead increases water retention and may lead to *Lithops* plants rotting and dying in the rainy season. In addition, the more sparse larger stones cannot effectively protect *Lithops* plants from being detected by predators. The plants instead obtain the required stability and protection in a substrate consisting of gravel or pebbles or, more commonly, combinations thereof since pebbles and gravel usually occur together in patches of various sizes. Such a combination remains relatively stable even when the soil is soaked through after a thunderstorm and prevents trampling of the plants in a habitat that is frequented by large and small stock. It also affords seedlings protection by shading them from prolonged exposure to sunlight and this helps them to become established.

Avonia albissima was shown to have a significant association with *Lithops* in this population but may be less habitat-specific since it was recorded in 76 test plots while *Lithops* was recorded in only 44. Nevertheless, this somewhat less cryptic species can be a useful indicator of *Lithops* in the field.

To our knowledge, this is the first plant census and habitat preference study performed on a *Lithops* population. Due to their cryptic habit (with most adults reaching only between 2 and 8 mm above soil and with an average diameter of 1.05 cm for juveniles and 1.7 cm for mature plants in this population), the two censuses and plant density estimations required about 5 months of field work for two persons in total, while the habitat preference study (using pre-marked plants) required an additional month for two persons. We believe that the outcome of this study has determined the most accurate methods to use when determining plant density and can serve as a model for future research on *Lithops* and other cryptic plant species, and consequently assist in developing a basis for better conservation assessments and protection policies. The *L. pseudotruncatella* complex consists of five subspecies and three varieties (Cole and Cole, 2005) and a total of some 37 known populations (Plant Red List Database of Namibia, 2016), with 25 belonging to subsp. *pseudotruncatella*. Given a recent 60% reduction in the habitat of the population of the study site (the largest known population of this subspecies) due to habitat destruction (Loots, pers. obs), combined with the projected steady decline of other populations of this subspecies as a result of illegal collecting, and the effect of extreme weather patterns etc. observed in some populations, the status of subsp. *pseudotruncatella* may have to be revised.

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