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Distribution, habitat profile and genetic variability of Namibian succulent *Lithops ruschiorum*

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Background: The species-rich flora of southern Africa comprises a high number of endemics, including succulents such as the flowering stones in the genus *Lithops*, but conservation status for these species is not well underpinned because detailed field data and assessments of genetic diversity are lacking.

Objectives: We wanted to assess plant abundance and identify factors that may affect survival in *Lithops ruschiorum* through carefully conducted field surveys, and to determine amount and partitioning of genetic variation by amplified fragment length polymorphism (AFLP) analysis.

Method: Field surveys were carried out in nine populations in Namibia. The most meticulously studied population was divided into 51 sites, while another 43 sites were recognised in the remaining eight populations. At each site, occupied area and number of plants were recorded as well as altitude, aspect, slope, soil texture and substrate. Amplified fragment length polymorphism markers were employed to study 52 individuals from seven populations.

Results: In total, 8465 individuals were recorded. Plant density and/or plant number was associated with aspect, slope, soil texture, substrate and geographic distance from the coast. Analysis of molecular variation showed that 95% of the variability occurs within populations. Genetic and geographic distances among populations were correlated suggesting an isolation-by-distance pattern.

Conclusion: Results are concordant with a strong impact of fog-based precipitation on plant density in the coastal populations, whereas rain probably is more important at one population which is situated further inland. Within-population genetic variation was medium high as usually reported for perennial, outcrossing species, but the low population differentiation implies considerable gene flow and/or population fragmentation.

Keywords: abundance; AFLP; desert biome; molecular marker; plant conservation; plant inventory; population genetics.

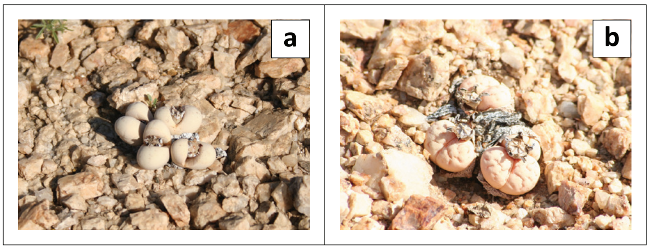
Introduction

Southern Africa harbours many endemic leaf succulents because of the unique environmental conditions (Young & Desmet 2016), but few of these taxa have been thoroughly studied in spite of imminent threats due to climate change and human activities such as illegal collecting of seeds and live plants, and habitat destruction resulting from prospecting, mining, off-road driving and, in recent years, the production of motion pictures in different parts of the Namib desert (Jainta 2017; Loots 2005, 2011). Decisions about conservation status and suitable protection measures are therefore not well underpinned. Methods for assessing plant abundance in sometimes cryptic species are needed, and factors that affect plant recruitment and survival should be determined. In addition, information on amount and partitioning of genetic variation is needed for determination of proper protection measures.

One of the insufficiently studied succulent genera is *Lithops* N.E.Br., which comprises 37 species (Cole 2006; Cole & Cole 2005) that are often referred to as stone plants or flowering stones because of their ability to blend in with their substrates on gravelly plains, rocky outcrops and slopes, which make them very difficult to detect and study. Individual plants can reach an age of at least 50 years (Schwantes 1957). Recruitment of new plants is erratic, with seed dispersal, germination and seedling establishment depending on unpredictable rainfall events. Many narrowly distributed plant species, such as *Lithops*, have a strong dependency on specific habitats, which, in combination with restricted gene flow, can lead to population fragmentation and subsequent extinction (IUCN 2001). Apart from a previous study on methods to determine plant density and habitat preferences in *L. pseudotruncatella* (Loots & Nybom 2017), in-depth field surveys in the genus are lacking. Studies on population genetics and intraspecific variation have also not been reported for the genus.

For this study, we focus on *L. ruschiorum* (Dinter & Schwantes) N.E.Br., which is endemic to Namibia and easily distinguishable from other *Lithops* species by its highly cordate profile, leaves that are often elevated above the soil surface and the very smooth, pale white to greyish or buff-coloured leaf face with few markings (Figure 1). Two varieties are acknowledged based mainly on leaf colouring and markings: the southerly distributed var. *ruschiorum* and the northerly distributed var. *lineata* (G.C.Nel) D.T.Cole. The yellow, 12–30-mm-wide flowers (Cole & Cole 2001) usually start to open in January, but flowering can be triggered by episodic rainfall events any time of the year. The broadly elliptic seed capsules are 5–6 locular and contain seeds without any features for dissemination enhancement (Cole & Cole 2005). *Lithops ruschiorum* has a shallow root system, drawing water from the uppermost 5 cm of the soil layer and must compete with other species in the same layer. Other challenges include herbivorous insects and larger herbivores such as domestic and wild ungulates. The current conservation status is Least Concern (IUCN 2001; Klaassen & Kwembeya 2013).

Populations of *L. ruschiorum* can be found in many, apparently different, microhabitats. Defining population boundaries is problematic because potentially suitable habitat can continue for several square kilometres, interrupted only by minor geographic boundaries that separate groups of plants, such as dry stream beds or unsuitable rock substrates. Within such an occupied area, aggregations of plants frequently occur at varying distances from one another. It is not clear whether these colonies are remnants of a once larger population that has become fragmented. The occurrence of separate small plant colonies can also stem from a close dependency on particular microhabitat features that are not immediately evident from the habitat at large, as previously demonstrated in *L. pseudotrunctella* which had a highly clumped distribution with more than 80% of the plants occurring on just 20% of an apparently suitable habitat (Loots & Nybom 2017). Plant density was positively correlated with a high percentage cover of gravel and pebbles.



Source: Photo courtesy of Sonja Loots

FIGURE 1: Plants of *Lithops ruschiorum* showing (a) a healthy plant in profile with the fissure clearly visible and developing capsules in a feldspar habitat and (b) a healthy plant with old leaves in the centre and three ripe, 6–7-locular seed capsules in a mixed gravel habitat.

Genetic variability and processes, such as isolation-by-distance and genetic drift, can be studied by deoxyribonucleic acid (DNA)-based markers, and appear to be closely associated with life form, breeding system, mode of seed dispersal and successional status (Nybom 2004). Amplified fragment length polymorphism (AFLP) markers were used to study interspecific variation among *Lithops* taxa, and to define nine clades within the genus (Kellner et al. 2011). Intraspecific variation has, however, as yet not been studied with molecular markers in *Lithops.*

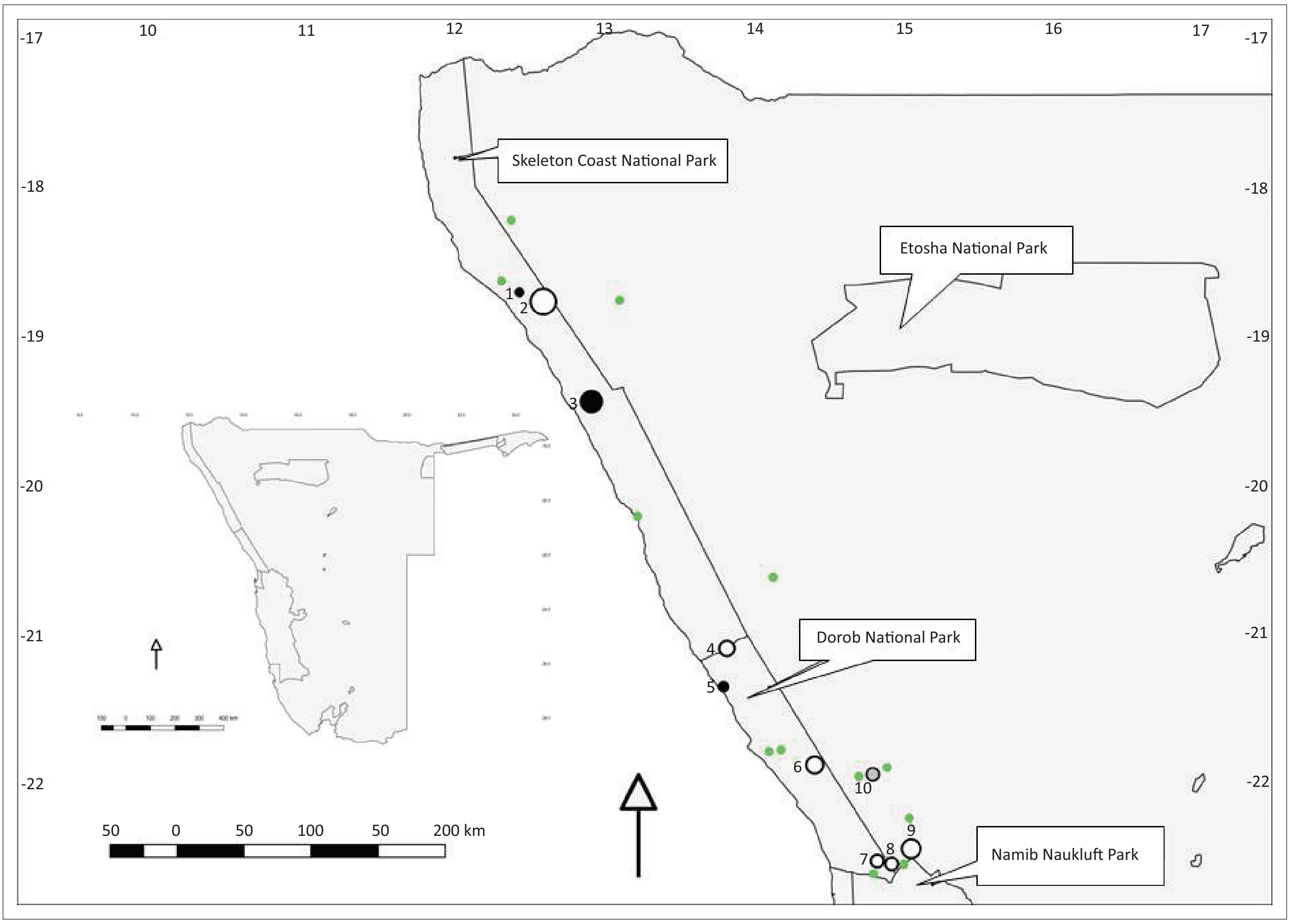
The current study was undertaken to estimate plant abundance in *L. ruschiorum* throughout its known distribution range in Namibia, including assessments of spatial population structure and the impact of habitat selection and climatic conditions. In addition, AFLP markers were applied to assess molecular variation between and within populations, and thus identify a possible isolation-by-distance pattern.

Material and methods

Study area and habitat parameters

Lithops ruschiorum grows in the central and northern desert biome of Namibia (Irish 1994), and has a distribution area that stretches more than 600 kilomtres (km) along the coast and approximately 75 km inland (Cole & Cole 2005). The central Namib desert is characterised by temperatures of -0.7 to 42 degrees Celsius (°C) close to the coast, while areas further inland vary between -1.2 and 43 °C. Annual rainfall is very sparse and unpredictable, but ranged from 17 millimetres (mm) closer to the coast to 87 mm further inland. Most of the precipitation is instead received as advective and high fog, caused by moist air flowing over the cold Benguela current along the Namibian coastline, reaching over 100 km inland and ranged from 183 mm near the coast to 3 mm further inland per year (Lancaster, Lancaster & Seely 1984). Average annual rainfall in the northern Namib ranges from 11 to 42 mm (Irish 1994). Other climate data for the northern Namib desert are still lacking. The terrain consists of vast gravel plains interrupted by inselbergs, low-lying, undulating hills, ridges and ephemeral river courses with riparian vegetation. Soils are usually sandy, but some silt, loam and clay occur. Most of the localities for *L. ruschiorum* are found within the Skeleton Coast National Park and Dorob National Park.

Lithops ruschiorum has the second largest distribution of all Namibian *Lithops* species, but still only 21 populations have been recorded (Cole & Cole 2005; Loots collection data 2006–2014; WIND 2006). Information on the precise location of these populations was obtained from the National Herbarium (WIND) specimen database, literature (Cole 1988a, 1988b) and local experts. Field trips were undertaken from October 2006 to September 2008 to locate the populations, and to survey nine populations from southeast of Rössing Mountain and Rössing Uranium Limited (RUL) license area in the central Namib desert, to Khumib River in the northern Namib (Figure 2). In 2011,



km, kilometres.

FIGURE 2: Distribution of surveyed *Lithops ruschiorum* populations. 1. View Point, 2. Khumib River, 3. Hoanib River, 4. Ugab River, 5. Ugab Salt Works, 6. Henties Bay–Uis Road, 7. Rössing Mountain, 8. Feldspar Ridge, 9. Rössing Uranium Limited (RUL) license area, 10. Henties Bay–Usakos Road. Circle size is roughly proportional to number of plants observed in each population. Populations used for collecting demographic data: black circle, populations used for collecting seed: grey circle, populations used for both purposes: unfilled circle. In addition, the total distributional area determined from all 21 recorded populations is shown (small green dots).

seeds were collected from the surveyed populations and the additional Henties Bay–Usakos Road population.

Boundaries are difficult to define for large populations that consist of many small and widely dispersed groups of plants. In this study, a site is defined as a group of plants that grow together on the same topographic feature such as a ridge, outcrop, slope or a gravel plain, and with no plants separated by unsuitable habitat. By contrast, sites within a population can be separated by unsuitable habitat although cross-pollination between sites should still be possible. A population is defined as a group of sites occurring at the same geographic location. Populations are separated by significant distances (minimum 10 km), unsuitable habitat and/or geographic barriers, making inter-populational cross-pollination unlikely. Only one site constitutes the entire population at View Point in the Skeleton Coast Park. For each population, number of sites, plants and occupied area are shown in Table 1.

One very large population, RUL, received special attention because of its size and previous plant and habitat analyses. Burke (2005) divided this population into 20 biotopes based on the number of endemic and Red List species in each biotope. Twelve of these contained *L. ruschiorum* and/or *Adenia pechuelii* (Engl.) Harms, another endemic succulent plant surveyed simultaneously. In the present study, a total of 68 sampling points, 1 km apart in grid square format, were drawn on a map to cover the 12 biotopes. Seven additional sampling points were placed in parts not covered by the grid, but known to contain dense clusters of *Lithops*. Each sampling point was marked with a set of Global Positioning System (GPS) coordinates (in WGS84 format) saved as waypoints in a GPS to enable

TABLE 1: Investigated populations with number of sites, number of plants and occupied area.

Population	Sites	Plants	Area (m²)
Feldspar Ridge	2	307	19 362
Henties Bay–Uis Road	11	1158	66 716
Hoanib River	4	1380	23 608
Khumib River	6	2213	16 004
Rössing Mountain	5	418	> 12 033
Rössing Uranium Limited (RUL)	51	2008	> 51 562
Ugab River	12	741	15 766
Ugab Salt Works	2	148	8979
View Point	1	92	500
Total	**94**	**8465**	**> 214 530**

subsequent retrievals. Each individual *Lithops* plant observed within 500 m of a sampling point was temporarily marked with a coloured marker (Mannheimer & Loots 2012). If more than one site could be identified after marking all the plants found within a 500 m radius, a set of GPS coordinates was recorded in the centre of each site. In addition, altitude, aspect (the compass direction that a slope faces), slope (steepness of a sloping surface), soil texture and rock substrate were recorded for each site (Appendix 1, Table 1a-A1). Number of mature plants (plants capable of reproduction), juveniles (plants that are flat on top and with as yet un-separated facial lobes) and damaged plants (with extensive predatory damage) was then determined for each site. The occupied area was measured for each site using the track log function of the GPS, recording one set of coordinates every second while walking along the outer boundary of detected plants. This parameter was then used to calculate the density of each site (number of plants/m²). A total of 22 soil samples were taken in different sites, and pH was determined for each sample using a Hanna microprocessor pH metre.

In each of the other eight successfully located populations, all *Lithops* plants were temporarily marked using coloured markers. When more than one site could be identified, the boundary for each site was demarcated with the GPS track log function. Data for habitat parameters were recorded as for RUL (Appendix 1, Table 1b-A1). Distance from each site to the sea coast was determined using the ruler function in Google Earth. One soil sample was taken from each population for pH determination.

Only one population (Khumib River) contained plants that were identified as var. *lineata*, but individuals of var. *ruschiorum* were growing in the same sites. No distinction was therefore made between sites or populations on the basis of intraspecific taxonomy. In populations with a sufficient number of plants, a single *L. ruschiorum* specimen was collected to ensure adequate representation in the National Herbarium (WIND) and for positive identification.

Molecular analyses

Seed capsules were collected from seven populations (Figure 2). Where possible, at least ten plants were sampled across the known geographical range of each population. A photograph was taken of each sampled plant for reference purposes. In populations where seed was scarce, but the plants produced many heads, a single fresh half leaf was collected from ten multi-headed plants and stored directly in silica gel for DNA extraction. Up to 50 seeds per sampled plant were sown in pots in a greenhouse in Alnarp in Sweden, with 14 h of light per day, and temperatures of 22–25 °C. Between 8 and 10 seedlings per population, each from a different mother plant, were sampled for DNA extraction after one year of growth. Deoxyribonucleic acid extraction was performed using the DNeasy Qiagen DNA Plant Mini Kit, according to manufacturer's instructions, with two modifications: all centrifugations were run at 14 000 revolutions per minute (rpm), and in the last step, the DNA was eluted in only 2 × 50 microlitres (µL) to prevent excessive dilution of samples with low DNA concentration.

Genetic variation was investigated using standard AFLP procedure (Vos et al. 1995) except that restriction and ligation were performed simultaneously at 37 °C overnight. Based on an extensive primer screening in a previous analysis of the genus *Lithops* (Kellner et al. 2011), three selective primer pair combinations were chosen: E32/M48 (AAC/CAC), E35/M51 (ACA/CCA) and E46/M51 (ATT/CCA). In addition, the primer combination E33/M47 (AAG/CAA) was also used. To avoid direct labelling of specific primers, we used the 'poor man's approach' according to Schuelke (2000). Polymerase chain reactions (PCR) were performed using an Eppendorf Mastercycler gradient S (Eppendorf AG, Hamburg, Germany). Automated detection of AFLP fragments was carried out by the Senckenberg Biodiversitäts- und Klimaforschungszentrum (Frankfurt am Main, Germany), using an ABI 3730 sequencing machine and the size standard LIZ-600 (ABI Life Technologies, Darmstadt, Germany). To ensure unambiguous scoring of AFLP fragments, ten positive controls were repeated in each run; thus, each of the positive controls was run three to five times in total. Amplified fragment length polymorphism profiles were scored as presence (1) and absence (0) of fragments ranging from 80 to 300 base pairs, using the software Genographer 2.1.4 (Banks & Benham 2008). Bands that were detected in < 75% of repetitions of the positive controls were deleted.

Statistical analyses

The numerical plant and habitat parameters (altitude, slope, distance from coast, occupied area, plant number and plant density) were tested for normality using the Anderson Darling test and transformed into natural logarithms as needed. Statistical analyses of plant and habitat parameters were carried out on two separate data sets: (1) sites in the RUL population and (2) sites in the other eight populations. Firstly, the relationships between occupied area, plant number and plant density were estimated by Spearman rank correlation analyses. Subsequently, associations between plant parameters and habitat parameters were estimated using univariate (including all data, i.e. 51 and 43 sites, respectively) and multivariate methods (including only 29 and 26 sites, respectively, because of list-wise deletion of missing values). Spearman rank correlation analyses were performed to study associations between the numerical habitat parameters (altitude, slope and coastal distance) and plant number and plant density. Several one-way analyses of variance (ANOVA) were performed to study the possible impact of category habitat parameters (aspect, substrate and soil texture) on plant number and plant density, followed by Tukey pairwise comparisons. Because of the unbalanced number of sites for especially aspect, some alternatives were merged into wider groups (e.g. NE+N+NW). A general linear mixed model (GLM) was used to analyse the effects of all parameters (altitude, slope, coastal distance, aspect, substrate and soil texture) simultaneously and their interactions as fixed effects, and sites per populations as random effects, on plant density and total plant number. The analyses were performed under the R environment (R Core Team 2018) using the *lmer* function implemented in the *lme4* package (Bates et al. 2015) with *p* values obtained from *lemeTest* package (Kuznetsova, Brockhoff & Christensen 2016). Best fitting models were discovered by model simplification procedures starting with a full model containing all factors and their interactions, and a subsequent stepwise reduction of the full model. An ANOVA comparing all models was used to select the best fitting one.

Principal component analyses (PCAs) were performed to explore the relationships between the habitat parameters, using indicator (dummy) variables for aspect, substrate and soil texture. In addition, plant number and occupied area were entered into the analysis to highlight the co-occurrences (but not the causality) between these parameters and the habitat parameters.

The AFLP data were used to estimate genetic variance within and among populations by an analysis of molecular variance (AMOVA) using GenAlEx v.6.5 (Peakall & Smouse 2012). Genetic diversity within populations was estimated as percentage of polymorphic loci and as expected heterozygosity H_E, which is equivalent to Nei's unbiased gene diversity (Nei 1978) when calculations are based on polymorphic, biallelic loci and when number of samples are equal among populations. Genetic structuring within and among populations was evaluated with principal coordinate analysis (PCoA) with Sørensen distances using PC-ORD v.6.07. Finally, an association between genetic and geographic distances among samples was investigated with a Mantel test, computed in PC-ORD.

Ethical considerations

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

Results

Populations and plant counts

During the field work in 2006–2008, only nine *L. ruschiorum* populations were located out of the 21 previously recorded (Figure 2). These populations were found on gravel plains, rocky ridges and outcrops, gentle to steep slopes and hill tops, and occasionally on mountain slopes. The plants were mostly growing in very gravelly soil, but occasionally in rock crevices with almost no soil, and usually in fully sun-exposed positions but sometimes in half shade.

A total of 8465 *L. ruschiorum* plants were recorded at the 94 sites identified in the nine populations (Table 1). The Skeleton Coast Park with populations View Point, Khumib River, Hoanib River and Ugab River contained 51% of the total number of recorded plants. The largest population was Khumib River in the northern part of this park, with over 2200 plants, and the second largest was RUL in the southern part of the distribution area, with just over 2000 plants. Because of the considerable challenges in spotting *Lithops* plants in their natural habitat, plant numbers reported here are certainly too low, but relative differences between populations and sites should still be accurate.

Percentage of mature plants out of the total number was 90.3%, while 8.6% were damaged and 1.1% were juveniles (Appendix 1, Table 1a-A1 and 1b-A1). Juveniles are exceptionally hard to spot and many are likely to have been overlooked. The Khumib River population had the highest percentage of juvenile plants (4.8%), while RUL had only 0.6% and View Point and Ugab Salt Works none at all. The highest percentage of damaged plants was encountered at View Point (55.4%) followed by RUL (17.3%) and Hoanib River (7.6%). Plant number differed considerably between sites in the same population; for example, the 12 sites at the Ugab River ranged from just 2 to 161 plants, the eight sites at Henties Bay–Uis Road from 5 to 621 plants and the six sites at Khumib River from 49 to 692 plants. Similarly, the occupied areas differed: the four sites in Hoanib River ranged from 751 to 12 397 m², the five Rössing Mountain sites ranged from below 5000 m² to almost 10 000 m² and the eight Henties Bay–Uis Road sites from just 200 m² to above 20 000 m².

Plant number, occupied area and plant density

As *Lithops* have a very patchy distribution also on apparently suitable habitats, and as there can be significant stretches of unsuitable habitat between occupied areas within a population, all parameters related to number and density of plants were based on the sites instead of entire populations. Information about plant number and occupied area for each site is presented in Appendix 1, Tables 1a-A1 and 1b-A1.

The survey of the RUL population initially made use of 75 sampling points. While no *Lithops* plants could be observed in the neighbourhood of 42 of these points, the remaining 33 sampling points contained plants and could be divided into one to four sites, yielding a total of 51 sites for RUL. Occupied area ranged among the different sites from 10 to 12 000 m², with a mean of 1101 m² (not measured for less than three plants), while plant number per site ranged from 1 to 440 with a mean of 39.4. Plant density was calculated as plant number divided by occupied area, and ranged from 0.003 to 0.797 with a mean of 0.014 plants per m².

In the other eight populations, *Lithops* plants occurred in 1–12 sites per population, and a total of 43 sites were analysed. Occupied area for these sites varied from 200 to 22 684 m² with an average of 3885 m², and plant number from 3 to 692 with a mean of 150.2. Plant density varied from 0.002 to 0.265 with a mean of 0.064 plants per m².

The boundaries of a site (i.e. occupied area) were determined by the number of plants as well as the distances between these plants. As expected, positive correlations were obtained between occupied area and number of plants, both in RUL and in the other eight populations (Table 2). Occupied area and plant density were instead negatively correlated in both these data sets with the closest association found in RUL.

TABLE 2: Spearman rank correlation values for associations among occupied area, plant number and plant density, and two numerical habitat parameters (altitude and slope) at 51 sites in the Rössing Uranium Limited population, and at 43 sites in the other eight populations.

Parameter 1	Parameter 2	*r*	*N*	*p*
RUL				
Plant number	Occupied area	0.585	43	< 0.001***
Plant number	Plant density	-0.137	43	0.369
Occupied area	Plant density	-0.732	43	< 0.001***
Plant number	Altitude	0.084	51	0.593
Plant number	Slope	0.127	42	0.454
Plant density	Altitude	-0.223	43	0.150
Plant density	Slope	0.411	37	0.012*
Other eight populations				
Plant number	Occupied area	0.676	41	< 0.001***
Plant number	Plant density	0.418	41	0.006**
Occupied area	Plant density	-0.363	41	0.020*
Plant number	Altitude	0.195	40	0.222
Plant number	Slope	0.445	37	0.006**
Plant number	Coastal distance	-0.157	43	0.314
Plant density	Altitude	0.098	39	0.546
Plant density	Slope	0.504	36	0.002**
Plant density	Coastal distance	-0.308	41	0.047*

RUL, Rössing Uranium Limited.
*, $0.05 > p > 0.01$; **, $0.01 > p > 0.001$; ***, $p < 0.001$.

Number of plants and density were positively correlated in the data set with eight populations, whereas no association was found between these parameters in RUL. Both data sets have a high percentage of sites with large occupied areas that have low densities (39% for RUL; 50% for the other eight populations), but RUL has the highest percentage of small sites with high densities (33% for RUL; 10% for the other eight populations), while the other eight populations have the highest percentage of sites with large occupied areas that have high densities (2% for RUL; 19% for the other eight populations). When plants were divided into mature, damaged and juvenile, number of mature plants had the strongest correlation with occupied area, while number of juvenile plants had the weakest correlation (data not shown).

Impact of habitat characteristics

Information about habitat variables for each site is presented in Appendix 1, Tables 1a-A1 and 1b-A1. Each site was represented by the predominant aspect or substrate when several alternatives had been scored. In some cases, it was not possible to determine predominant aspect or substrate because these varied too much within site. Principal component analyses were performed on all the habitat variables together with occupied area and number of

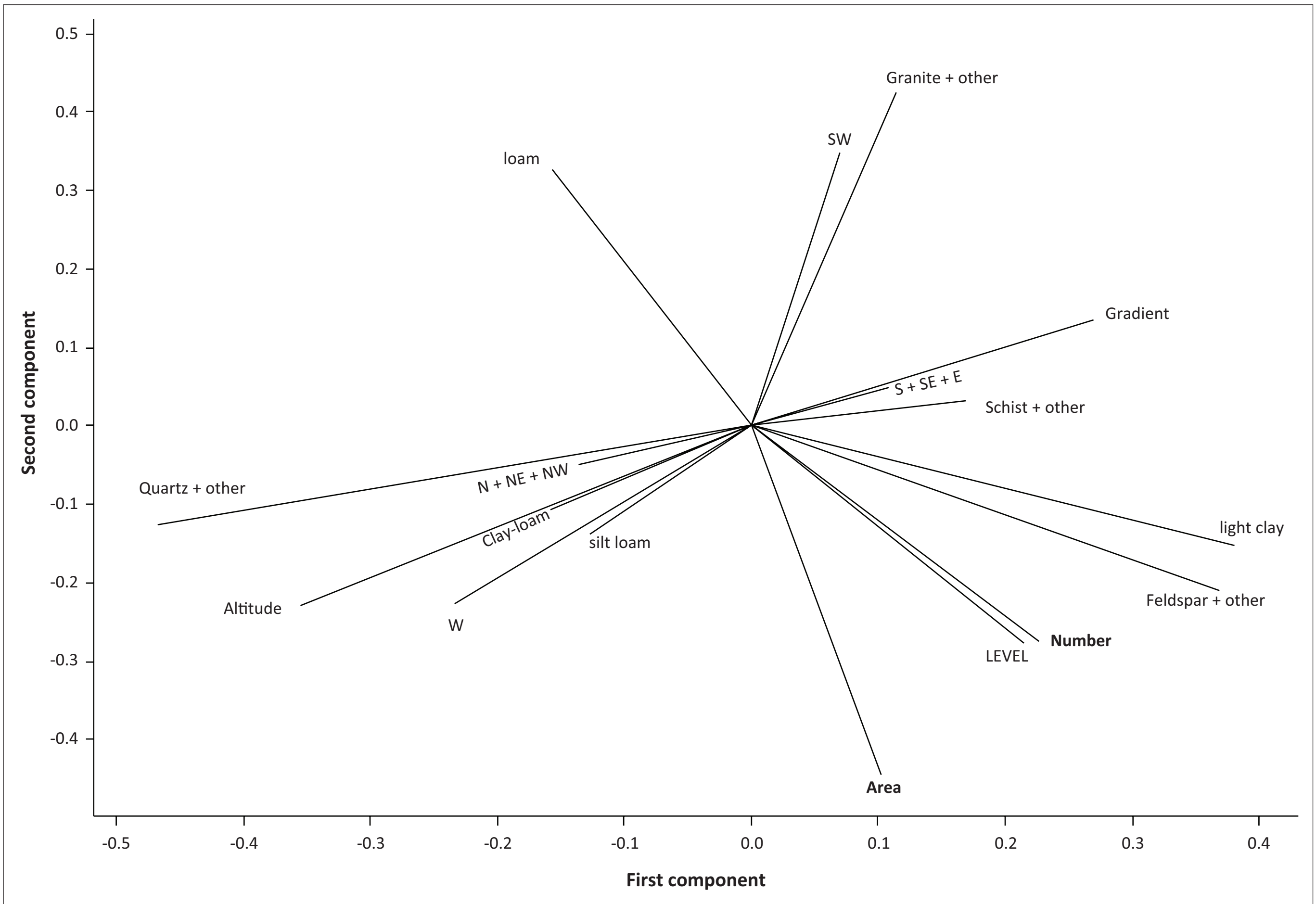


FIGURE 3: Principal component analysis showing the relationship between number of plants (Number), occupied area (Area) and habitat parameters: altitude, aspect (N+NE+NW, SW, S+SE+E, LEVEL), gradient (degree of slope), soil texture (loam, light clay, silt loam, clay loam) and substrate (Quartz + other, Granite + other, Schist + other, Feldspar + other) in the 51 sites at Rössing Uranium Limited.

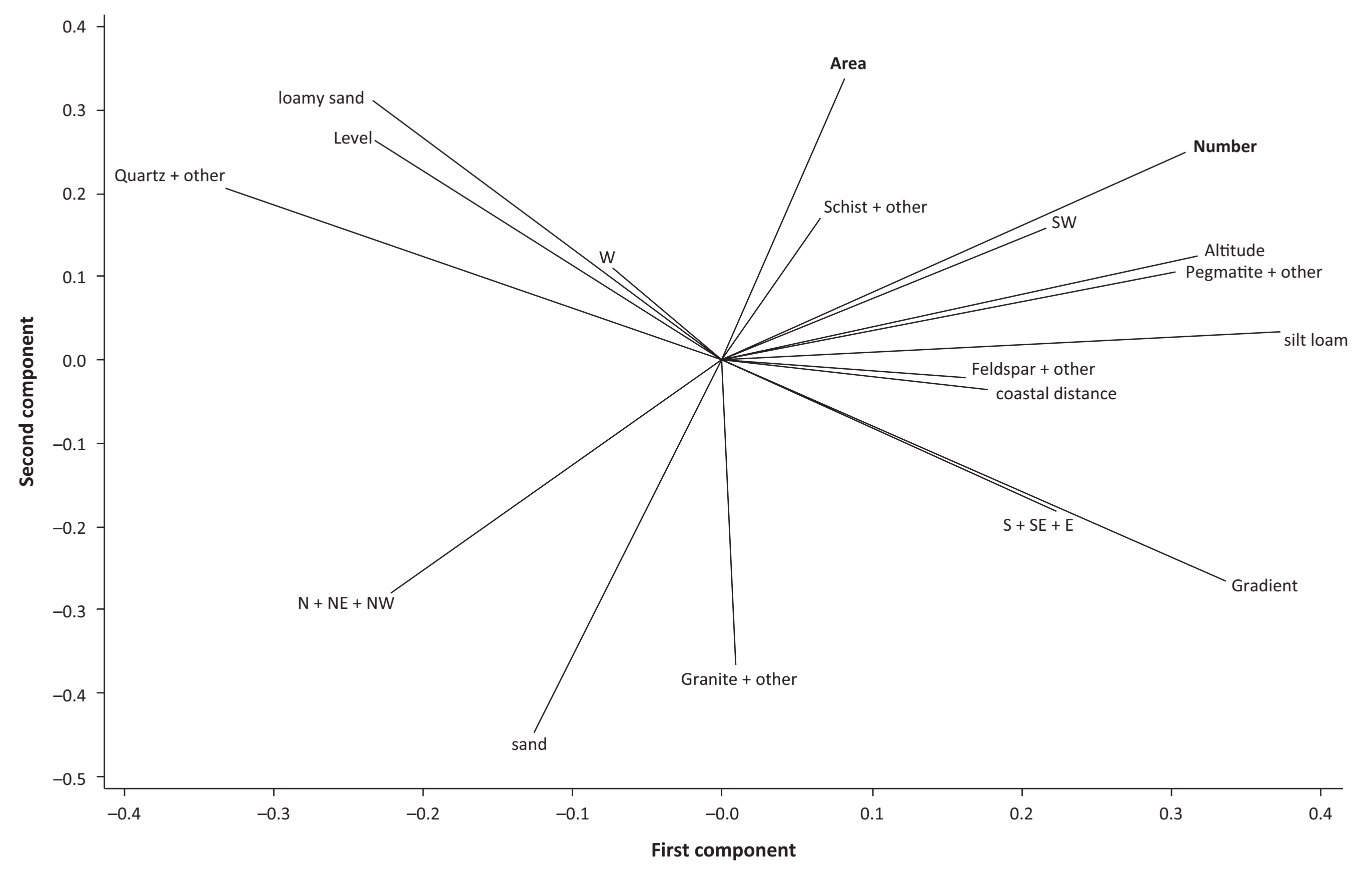


FIGURE 4: Principal component analysis showing the relationship between number of plants (Number), occupied area (Area) and habitat parameters: altitude, aspect (LEVEL, W, SW, S+SE+E, N+NE+NW), coastal distance, gradient (degree of slope), soil texture (loamy sand, silt loam, sand) and substrate (Quartz + other, Schist + other, Pegmatite + other, Feldspar + other, Granite + other) in the 43 sites of the other eight populations.

plants for RUL (Figure 3) and the eight populations (Figure 4). These descriptive analyses provide an overview of the observed associations between different habitat variables and plant abundance for each site determined in two ways: as occupied area and plant number.

At RUL, all sites were found within a range of 527–704 metres above sea level (masl). The steeper sites occurred mostly at the lower altitudes, usually with a southern–eastern aspect, on feldspar, granite or schist, with light clay. Sites at higher altitudes tended to have a northern or western aspect, and were situated mainly on quartz with loam or silt loam. By contrast, altitude varied considerably among the eight populations (18–617 masl), with sites at higher altitudes overall steeper with an eastern–southern–southwestern aspect, an overrepresentation of the substrates feldspar and pegmatite, and silt loam. Sites at lower altitudes instead tended to be level or to have a northern aspect, and to have granite or quartz, and sand or loamy sand. Associations between the habitat parameters and the plant abundance parameters are consistent with the statistical analyses described below.

At RUL, the most common aspect group was S+SE+E (23 sites), while the other aspect groups occurred in only three to six sites each (Appendix 1, Table 2-A1). Level sites had the highest number of plants followed by SW-facing sites. Although not associated according to ANOVA (Table 3), aspect had a significant effect on plant number

TABLE 3: Importance of three habitat parameters (aspect, soil texture and substrate) for plant number and plant density determined with analysis of variance and Tukey pairwise comparisons, for 51 sites at Rössing Uranium Limited and for 43 sites at the other eight populations.

Parameter	df	*F*	*p*
RUL			
Aspect (Plant number)	4/33	1.41	0.251
Aspect (Plant density)	4/29	3.34	0.022*
Soil texture (Plant number)	3/47	0.99	0.407
Soil texture (Plant density)	3/39	1.35	0.272
Substrate (Plant number)	3/45	0.49	0.691
Substrate (Plant density)	3/38	0.30	0.827
Other eight populations			
Aspect (Plant number)	4/28	3.64	0.016*
Aspect (Plant density)	4/26	3.12	0.032*
Soil texture (Plant number)	2/40	4.00	0.026*
Soil texture (Plant density)	2/37	2.34	0.111
Substrate (Plant number)	4/36	1.52	0.218
Substrate (Plant density)	4/33	4.16	0.008**

For an extended version, see Appendix 1, Table 2-A1.
*, $0.05 > p > 0.01$; **, $0.01 > p > 0.001$.
RUL, Rössing Uranium Limited; df, degrees of freedom.

according to GLM (Table 4). Aspect interacted with slope, due mainly to an overrepresentation of sites with an S+SE+E aspect on steeper slopes, while the flatter sites instead had more variable aspects. In the eight populations data set, the most common aspect was W (eight sites) followed by SW and S+SE+E (seven sites each) and NE+N+NW (six sites). According to ANOVA (Table 3), aspect had a significant impact ($F = 3.64$, $p = 0.016$) with the highest

TABLE 4: Factors and co-variables selected by a general linear mixed model to explain variation in plant density and total plant number for 29 sites at Rössing Uranium Limited.

Source of variation	Sum of squares	Mean squares	df	*F*-value	Pr(> *F*)
Plant density					
Aspect	0.0499	0.0125	4/15	00.29	0.8807
Soil type	0.0457	0.0152	3/15	00.35	0.7880
Substrate	0.0083	0.0028	3/15	00.06	0.9780
Soil type: Substrate	0.2233	0.0745	3/15	01.72	0.2051
Total plant number					
Altitude	119	119	1/8	00.15	0.7072
Slope	548	548	1/8	00.70	0.4277
Aspect	35 502	8876	4/8	11.30	0.0023**
Soil type	1029	343	3/8	00.44	0.7327
Substrate	2050	683	3/8	00.87	0.4956
Altitude: Slope	610	610	1/8	00.78	0.4037
Altitude: Aspect	1428	476	3/8	00.61	0.6294
Slope: Aspect	26 143	8714	3/8	11.10	0.0032**
Altitude: Slope: Aspect	1606	1606	1/8	02.05	0.1906

df, degrees of freedom.
**, 0.01 > p > 0.001.

TABLE 5: Factors and co-variables selected by a general linear mixed model to explain variation in plant density and total plant number for 26 sites at the other eight populations.

Source of variation	Sum of squares	Mean squares	df	*F*-value	Pr(> *F*)
Plant density					
Altitude	0.0000	0.0000	1/0.00	34.30	0.9999
Coastal distance	0.0005	0.0005	1/0.00	745.89	1.0000
Soil type	0.0092	0.0046	2/2.00	7569.77	0.0001**
Substrate	0.0120	0.0030	4/2.00	4933.73	0.0002**
Aspect	0.0052	0.0013	4/2.00	2143.36	0.0005**
Slope	0.0082	0.0082	1/0.00	13 502.79	1.0000
Soil type: Substrate	0.0022	0.0011	2/2.00	1836.04	0.0005**
Soil type: Aspect	0.0012	0.0004	3/2.00	663.83	0.0015*
Substrate: Aspect	0.0029	0.0010	3/2.00	1592.39	0.0006**
Soil type: Slope	0.0048	0.0048	1/0.00	7851.67	1.0000
Substrate: Slope	0.0027	0.0027	1/0.00	4367.92	1.0000
Aspect: Slope	0.0026	0.0026	1/0.00	4259.93	1.0000
Total plant number					
Altitude	373	373.5	1/0.70	0.1669	0.7738
Coastal distance	140	139.8	1/0.64	0.0625	0.8593
Soil type	25 166	12 582.9	2/3.47	5.6234	0.0815
Substrate	77 027	19 256.7	4/2.00	8.6059	0.1068
Aspect	5597	1399.3	4/2.00	0.6254	0.6912
Slope	18 965	18 965.1	1/47.15	8.4756	0.0055*
Soil type: Substrate	8218	4108.8	2/4.89	1.8362	0.2541
Soil type: Aspect	13 534	4511.2	3/2.00	2.0161	0.3485
Substrate: Aspect	24 999	8333.1	3/2.00	3.7241	0.2189
Soil type: Slope	21 597	21 596.9	1/1979.22	9.6518	0.0020*
Substrate: Slope	3029	3028.9	1/7.67	1.3536	0.2796
Aspect: Slope	47	46.5	1/5.76	0.0208	0.8903

df, degrees of freedom.
*, 0.01 > p > 0.001; **, p < 0.001

number of plants in sites on SW-facing slopes, whereas GLM did not detect any association (Table 5).

Soil texture differed considerably between the two data sets, with only loamy sand (27 sites), silt loam (nine sites) and sand (seven sites) recorded in the eight populations, whereas clay loam, light clay, loam and silt loam were almost equally common (11–15 sites) at RUL (Appendix 1, Table 2-A1). Soil texture did not affect plant number at RUL and there was significant impact in the eight populations ($F = 4.00$, $p = 0.026$; Table 3) only in ANOVA, with silt loam being the most beneficial.

The most common substrate at RUL was quartz + other (28 sites) followed by feldspar + other (13 sites) and granite + other (six sites), while quartz + other (17 sites), granite + other (15 sites) and pegmatite + other (four sites) were most common in the eight populations data set (Appendix 1, Table 2-A1). Substrate was not significant for plant number in either data set.

When instead plant density was used as the dependent variable in ANOVA, aspect had a significant impact both at RUL ($F = 3.34$, $p = 0.022$) and in the eight populations ($F = 3.12$, $p = 0.032$), with the highest densities on slopes facing SW or S+SE+E (Table 3). General linear mixed model did not detect any impact of aspect at RUL but a strong impact in the eight populations in spite of interactions with both soil texture and substrate (Table 5). Soil texture did not affect plant density in either data set according to ANOVA, while a significant association was found in the eight populations with GLM. Substrate had a significant impact for the eight populations with both ANOVA ($F = 4.16$, $p = 0.008$) and GLM (Table 5) with the highest density on pegmatite + other.

Spearman rank correlation coefficients and GLM were used to detect associations between the two quantitative habitat parameters altitude and slope, on the one hand, and plant number and plant density, on the other hand (Tables 2, 4 and 5). Altitude was not associated with either plant number or plant density. Slope was positively correlated with density in RUL ($r = 0.411$, $p = 0.012$) and in the eight populations ($r = 0.504$, $p = 0.002$) as well as with plant number in the eight populations ($r = 0.445$, $p = 0.006$). The latter was confirmed with GLM, while no association was found with plant density. In addition, impact of the distance between the site and the sea coast was investigated for the eight populations data set, and showed a negative correlation with plant density ($r = -0.308$, $p = 0.047$) but none with number of plants. A corresponding effect could not be shown with GLM, possibly because of the heavily reduced number (26 instead of 43) of sites included in this analysis.

The pH ranged between 7.7 and 9.6 in all the 30 soil samples, indicating that *L. ruschiorum* grows in neutral to slightly alkaline soils. The mean for RUL was 9.0, while the eight populations had a mean of 8.6. There was no correlation when pH was compared with plant number or plant density, but these results need to be treated with caution as only one soil sample had been taken for each site.

Genetic differentiation

A total of 52 individuals from seven populations were analysed with four primer pairs yielding 102 polymorphic

TABLE 6: Amplified fragment length polymorphism (AFLP)-based estimates of genetic variation, within each of the seven sampled *Lithops ruschiorum* populations, estimated as percentage of polymorphic loci and mean expected heterozygosity (H_E).

Population	Number of plants	PPL	H_E
Rössing Uranium Mine (RUL)	6	60.78	0.221
Khumib River	7	75.49	0.279
Ugab River	6	53.92	0.199
Feldspar Ridge	7	68.63	0.253
Rössing Mountain	8	73.53	0.260
Henties Bay–Uis Road	9	64.71	0.216
Henties Bay–Usakos Road	9	64.71	0.244

PPL, percentage of polymorphic loci; RUL, Rössing Uranium Limited.

TABLE 7: Distribution of molecular variance among and within seven populations and six populations (without Khumib River) of *L. ruschiorum*, based on AFLP data with all results highly significant according to permutation tests ($p_{\text{random} \geq \text{data}} < 0.001$).

Source of variation	df	Sum of squares	Estimated variance	Percentage of variance
Seven populations				
Among populations	6	120.2	0.80	5
Within populations	45	635.6	14.12	95
Six populations (without Khumib River)				
Among populations	5	86.0	0.46	3
Within populations	39	536.1	13.74	97

df, degrees of freedom.

AFLP bands. Mean percentage of polymorphic loci was 65.97 and mean expected heterozygosity was 0.239 (Table 6). As expected, the two parameters co-varied, with Khumib River having the highest values followed by Rössing Mountain, whereas Ugab River had the lowest. An AMOVA showed that 95% of the variability resided within populations and 5% between populations (Table 7). Variation between populations declined to less than 3% when repeating the analysis without the Khumib River population which is situated approximately 300 km from the remainder.

The Mantel test showed a correlation between geographic and genetic distances ($r = 0.4179$; $p = 0.001$) when the analysis was performed on all seven populations. There was, however, no correlation ($r = 0.0054$, $p = 0.421$) when the test was repeated without the Khumib River population.

The PCoA is a multivariate test that reveals amount of similarity among samples without presuming any group structure. The first three dimensions explained a total of 30.3% of the variance when all samples were analysed together, and showed that the Khumib River samples are widely dispersed and that many occur as outliers in the right hand and lower parts of the plots (Figures 5a and 5b). Repeating the analysis without the Khumib River samples resulted in 32.6% explained variance on the first three dimensions, and samples from all populations were intermingled without any group structure (Figure 5c and 5d).

Discussion

In the largest and most detailed field inventory ever published on a single *Lithops* species, we were able to locate nine previously recorded populations of *L. ruschiorum*, and estimate occupied area as well as plant number in one to several (up to 51) sites in each population. Although additional populations exist, we have probably targeted most of the larger populations of this species. We have also collected carefully quantified data in the field for five habitat variables (altitude, aspect, slope, soil texture and substrate) for each of the 94 sites, and can thus provide information on where one can expect the largest populations, evaluated as occupied area and plant number. The co-occurrence of the habitat variables and plant abundance are illustrated by statistical analyses, and the relationships determined in our study should be very valuable as a basis for further investigations on factors that impact the distribution of *Lithops* in the field. Molecular analysis was applied on a population level for the first time in *Lithops*, and revealed expected levels of within-population variation, whereas differentiation between populations was very low, and mainly because of the divergence of the geographically most distant population.

Population number and population size

The main reasons that only nine out of the 21 recorded populations were located are probably: (1) locality descriptions on herbarium specimens and in publications lack sufficient detail or are deliberately vague so as to prevent illegal collecting, and (2) the cryptic nature of the plants: in the absence of rain for a prolonged period of time, plants shrink and become concealed by their substrate. Despite spending hours looking for the population near Cape Cross, for example, we were unable to find it although it was subsequently located (Jainta 2017), and despite GPS coordinates being available another population in the central Namib could not be found. Together, the nine located populations are, however, spread over a large geographic area and should be able to provide valuable information about factors determining plant abundance in this species.

Because of the clumped distribution of plants within a population, previous attempts to apply plant density estimation methods in *L. pseudotruncatella* resulted in large over- or under-estimations except when using the time-consuming adaptive cluster sampling and belt transect methods (Loots & Nybom 2017). As *L. ruschiorum* plants usually appear in clumped patches, efforts were made to instead obtain absolute plant counts in the present study. A number of sites were defined within each population, and plant number as well as habitat parameters were recorded. The count was probably relatively accurate for View Point, which is a small and very isolated population. In many of the other populations, our plant counts most likely grossly underestimated true plant number. Especially the Khumib River population is most likely larger than implied in this study; Google Earth images show that similar habitat extends over several square kilometres and therefore may contain many more plants. The second largest count was obtained within RUL license area where the species grows, at varying densities, on approximately 52 km².

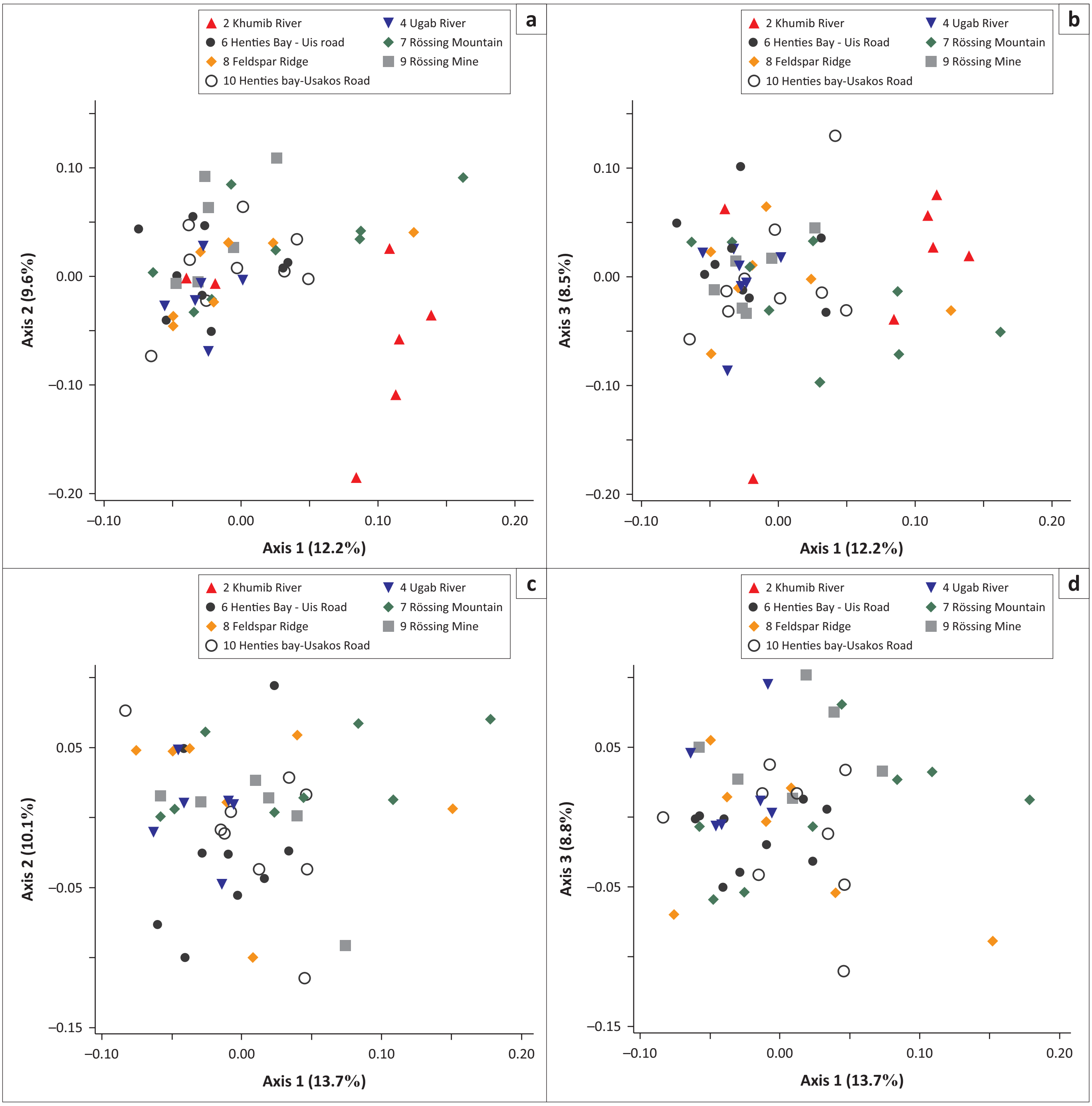


FIGURE 5: Principal coordinate analysis of amplified fragment length polymorphism data for 52 plants derived from seven populations of *Lithops ruschiorum.* (a) and (c) show dimensions 1 and 2, while (b) and (d) show dimensions 1 and 3. (a) and (b) include all populations, whereas (c) and (d) exclude the somewhat deviating population Khumib River.

Plant number, occupied area and plant density

A clumped distribution has been described for many species in the family Aizoaceae (Burke & Mannheimer 2003; Ihlenfeldt 1983), and was thoroughly investigated in *L. pseudotruncatella* (Loots & Nybom 2017). Designation of several separately analysed sites within populations in the present study allowed detailed description of the occurrence of *Lithops* plants and their habitat preferences. Occupied area as well as plant number varied strongly among sites in both data sets, but, on average, sites were almost four times larger in the eight populations and contained almost four times as many plants compared to sites in RUL. Calculated across the individual sites within each data set, mean density was more than four times higher in the eight populations. Occupied area was positively correlated with plant number, and negatively correlated with plant density in both data sets. Plant number and density were positively correlated in the eight populations, where some large sites were densely populated. By contrast, large sites at RUL usually contained relatively few plants per square metre.

Impact of habitat characteristics

Fog has a crucial impact on all vegetation in the Namib desert. The high fog zone extends up to 60 km inland from the Atlantic Ocean and produces fog-derived precipitation 60–120 days per year (Mendelsohn et al. 2002; Robertson et al. 2012). Here, fog may produce five times as much precipitation as rain, and fog is also more predictable, thus affecting the distribution of many plant species in the Namib desert (Hachfeld & Jürgens 2000; Lancaster et al. 1984; Olivier 1995; Seely & Henschel 1998; Seely & Pallet 2008). In a study on present and projected impacts on the distribution of the genus *Lithops* in Namibia, Guo et al. (2017) quantified the effects of several environmental variables scored from climatic and topographic maps. The percentual contribution was highest for terrain morphology (roughly equivalent to slope in our study) with 24%, followed by soil texture with 21%, number of fog days (roughly equivalent to coastal distance in our study) with 20%, geology (rock substrate in our study) with 18% and altitude with 6%. In addition, relative humidity (not measured in our study) accounted for 11%. A close dependency on specific habitats, especially for taxa inhabiting the Namib desert region, has also been described for another dwarf succulent, namely *Conophytum* N.E.Br. (Young & Desmet 2016). Climate projections for the 2070s suggest that *Lithops* is likely to suffer from a severe range contraction caused mainly by a reduction in the number of fog days (Guo et al. 2017).

Although it takes rainfall events to trigger germination and initial establishment of *Lithops* plants, regular fog precipitation may be more important for plant survival and growth, especially in species like *L. ruschiorum*. A combination of the amount of fog precipitation and occurrence of an otherwise suitable habitat may thus have great impact on plant abundance in this species. Eight of our studied populations are located within the high fog zone, where lower air temperatures and higher humidity allow them to benefit from the various types of fog (Seely & Henschel 1998). By contrast, the RUL population is situated approximately 60 km inland, in the outskirts of the high fog zone. Here, higher air temperatures and lower air humidity may overcome the effect of fog precipitation (Hachfeld & Jürgens 2000) and plants growing here are probably more dependent on rain (Hachfeld 2000).

Estimation of plant abundance is not straightforward in species with a patchy or heavily clumped distribution. Plant number and occupied area provide different estimates of the size of a plant site, but plant density was more closely associated with most of the habitat variables and therefore appears to be superior for determination of habitat preferences. It should also be stressed that the associations reported in this study are tentative only, as a larger set of more balanced data is needed for exploration of the true magnitude of impact from each habitat parameter.

Altitude most likely affects the ability of the sites to retain fog precipitation, but the range was very small at RUL (527–704 m) and no associations were found. The larger range recorded in the eight populations data set (18–617 m) possibly helped to detect a positive association with plant number as indicated in the PCA (Figure 4) although no significant impact could be established with correlation analysis or GLM.

Slope ranged from 0° to 40° at RUL and between 0° and 25° in the eight populations. In the latter data set, positive correlations were found between slope, on the one hand, and plant number and density, on the other hand. These associations are most likely because of an increased interception of fog precipitation at the steeper slopes found on inselbergs and rocky ridges. Interestingly, some of the largest and densest populations were found on overall steeper terrain, such as Khumib River, Hoanib River, Rössing Mountain and Feldspar Ridge. As RUL is located further inland, steeper slopes will not have the same beneficial effect. The weaker but still significant positive correlation between plant density and slope at RUL would instead be because the hilly terrain harbours overall smaller sites with high plant densities. By contrast, the level terrain at RUL consists of gravel plains interrupted by dry sandy water courses and holds fewer plants albeit often on large surfaces.

Distance from the coast may have an impact on plant abundance, given that fog-derived precipitation plays an important role although surface winds, carrying fog from the ocean, as well as the often variable mountain-plain winds, should not be underestimated (Seely & Henschel 1998). As expected, coastal distance was negatively correlated with plant density in the eight populations, but there were some exceptions: although close to the ocean, the slope on which the small View Point population grows is unable to retain much fog. By contrast, the large Khumib River population is further inland but situated on slopes in fog-trapping valleys.

Aspect had considerable influence, with plant number being the highest on SW-facing slopes in the eight populations. SW- and S–E-facing slopes also harboured sites with the highest density in both data sets. The beneficial impact of SW-facing slopes is consistent with the fog arriving with coastal winds (Seely & Henschel 1988). In the putatively less fog-dependent RUL population, the majority of the analysed sites were, however, found on S–E-facing slopes. In Namibia, rain usually arrives with eastern winds, and the rain-dependent *L. pseudotruncatella* had its highest plant density in S–E- or S-facing plots (Loots & Nybom 2017).

Substrate had no significant impact on plant number or density at RUL, while a positive influence was demonstrated in the eight populations with pegmatite+other doing best. Moreover, most of the *L. ruschiorum* sites were recorded on quartz, feldspar or granite which are light-coloured in the distribution area of this species. Two of the sites in Rössing Mountain occurred on a darker substrate, commonly found on this inselberg characterised by dark mottled and banded gneiss of the Khan formation. Here, the darker colour is perhaps outweighed by other positive factors such as the short distance from the sea and suitable elevation and aspect.

An impact of soil texture was noted only in the eight populations where silt loam appears to be more beneficial than sandy loam or sand. Sand had a negative impact also on *L. pseudotruncatella* (Loots & Nybom 2017), possibly because sand cannot provide necessary stabilisation for the small root systems of *Lithops*.

Genetic structure

Amplified fragment length polymorphism markers have been used to successfully investigate differentiation at population level of species in the sub-family Ruschioideae (Buys et al. 2008; Ellis, Weis & Gaut 2006), and nine clades were defined within the genus *Lithops* (Kellner et al. 2011). To our knowledge, the present study is, however, the first to use DNA markers to study genetic diversity between and within populations of a single *Lithops* species.

Within-population diversity estimated as expected heterozygosity had a mean of 0.24, which is similar to previously reported values and also in keeping with random amplified polymorphism DNA (RAPD)-derived estimates for short-lived perennials (0.20), with narrow-range distribution (0.28), outcrossing breeding system (0.27), water-dispersed seeds (0.27) and growing in early-successional vegetation (0.17; Nybom 2004). The fact that the large Khumib River population is also the most diverse (0.28) could suggest that recognition of two varieties of *L. ruschiorum* (both of which were found in this population) is associated with increased diversity but more sampling is required to investigate possible differentiation between these taxa.

Only 5% of the genetic variability occurred among populations, indicating very low differentiation. *Lithops* is outcrossing, and probably pollinated by a variety of insects (Cole & Cole 2005; Smith et al. 1998), suggesting that gene flow could be prominent. Still, values around 25% – 35% are usually found in outcrossing species sampled from populations within a restricted distribution (Nybom 2004). The low differentiation in our study may be indicative of a relatively recent fragmentation of a previously larger population. In addition, there are almost certainly populations that are still unrecorded and that contribute to gene flow among populations. According to both the PCoA and the Mantel test, the only genetically divergent population is Khumib River which is situated at least 300 km from the other populations.

Conservation status of *Lithops ruschiorum*

In the assessment for the Red Data Book (Loots 2005), the largest population of *L. ruschiorum* was inferred to contain no more than 1100 mature plants. The present study shows that there are at least two populations with over 2000 plants, with the largest at Khumib River in the Skeleton Coast Park. The second largest, at RUL, should also be conserved, especially considering its distance from the Skeleton Coast Park, the long-term monitoring programme for *L. ruschiorum* that is based there and the fact that the mine has recently changed ownership.

Number of juveniles is likely to be grossly underestimated, and recruitment probably takes place in most populations. Witkowski and Liston (1997) report that population dynamics in *Haworthia koelmaniorum* Oberm. & D.S.Hardy are characterised by adult persistence because seedling establishment is most likely episodic. This may also be true for *Lithops*, emphasising the importance of conserving adults in their habitat.

The current status of Least Concern remains valid for *L. ruschiorum*, but many threats from habitat destruction prevail. Bulldozer tracks across some of the habitat at RUL have not been re-colonised since the inception of the mine 30 years ago. In other populations with off-road driving, no *L. ruschiorum* were recorded in the vehicle tracks but age of the tracks cannot be determined. Some sites are vulnerable to both off-road driving, for example the ones between Henties Bay and Uis, where there is no protection. Anecdotal evidence from the last ten years suggests that poaching of *Lithops* may be on the rise. Herbivory of *Lithops* plant bodies by animals (possibly springbok, hares, grasshoppers, armoured crickets, birds and rodents) often result in the death of plants, but it is not known how this affects the overall mortality rate. Prolonged dry periods can increase grazing pressure as demonstrated at RUL, which, in turn, can lead to increased desertification thus threatening dwarf succulents in arid environments.

Conclusions

Results are concordant with a strong impact of fog-based precipitation on especially plant density in populations closer to the coast, whereas rain is probably more important at RUL which is situated further inland. Within-population genetic variation was medium high but the low population differentiation implies considerable gene flow and/or recent population fragmentation and holds clues for a conservation strategy for the species.

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Competing interests

The authors declare that they have no financial or personal relationships that may have influenced them in writing this article.

Authors' contributions

S.L. designed the study, performed the field work and most of the statistical evaluations, carried out most of the laboratory work and had a leading role in writing this manuscript. C.M.R. supervised the laboratory work, performed the AFLP data evaluations and contributed to this manuscript. M.S. assisted with the laboratory work. J.S. performed the AFLP band scoring. V.H. conducted some of the statistical analyses. L.G.-G. contributed to this manuscript. H.E.N. supervised the study and assisted in writing this manuscript.

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Data availability statement

Data created during this study belong to the Government of Namibia and the authors. Data sharing is possible with the application of information request forms, available on the website of the National Botanical Research Institute of Namibia (http://www.nbri.org/) or by contacting the authors.

Disclaimer

The views expressed in the submitted article are our own, and do not represent anyone else.

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Appendix 1

TABLE 1a-A1: Field data, including habitat parameters, plant number, occupied area and plant density, collected for 51 *Lithops ruschiorum* sites at the RUL population.

Site	Altitude (masl)	Soil texture	Substrate	Aspect	Slope	Mature plants	Damaged plants	Juveniles	Total number of plants	Occupied area (m^2)	Density (number of plants/occupied area)
1	648	light clay	feldspar, granite	S, W, E / undetermined*	7.0	136	40	6	182	6355	0.029
2	672	loam	quartz	S, W, E, N / undetermined	1.0	144	31	3	178	12000	0.015
3	632	clay loam	quartz, schist, feldspar	E, S / grouped with S+SE+E	1.0	21	1	0	22	1384	0.016
4	594	loamy sand	quartz, feldspar	S, W, E, N / undetermined	5.0	19	19	0	38	1555	0.024
5 a	633	silt loam	granite	level	0.0	42	11	0	53	1500	0.035
5 b	636	not recorded	not recorded	S, W, E, N / undetermined	2.3	38	4	2	44	340	0.129
6 a	612	silt loam	quartz, feldspar	not recorded	not recorded	11	1	0	12	1371	0.009
6 b	681	not recorded	quartz, feldspar	not recorded	not recorded	15	5	0	20	not measured	
6 c	647	not recorded	quartz, feldspar	not recorded	not recorded	18	6	0	24	not measured	
6 d	618	not recorded	quartz, feldspar, granite	level, N, SW, W / undetermined	7.2	40	11	0	51	not measured	
7	664	light clay	granite	SW	10.5	20	3	2	25	952	0.026
8	669	clay loam	quartz, feldspar	W, SW / grouped with W	20.0	54	10	0	64	1097	0.058
9a	635	loam	quartz, feldspar	SE	13.0	4	1	0	5	10	0.500
9 b	639	not recorded	quartz, feldspar	SE	10.0	7	2	0	9	60	0.150
9 c	641	not recorded	quartz, feldspar	SE	13.0	5	3	0	8	10	0.800
9 d	636	not recorded	quartz, feldspar	S	18.0	33	9	0	42	120	0.350
10	667	light clay	quartz, feldspar	SW, SE, W / undetermined	3.0	21	3	0	24	817	0.029
11	682	light clay	quartz, feldspar	NW, SE / undetermined	3.0	3	3	0	6	1785	0.003
12	679	clay loam	quartz, feldspar	S	10.0	6	1	0	7	525	0.013
13 a	700	clay loam	quartz, feldspar	W	7.0	5	2	0	7	948	0.007
13 b	704	not recorded	quartz, feldspar	E	8.0	31	11	0	42	453	0.093
14	623	silt loam	quartz	W	6.0	8	0	0	8	1102	0.007
15 a	611	clay loam	quartz, feldspar	S	not recorded	15	2	0	17	130	0.131
15 b	619	not recorded	quartz, feldspar	E	6.0	35	4	0	39	not measured	
15 c	620	not recorded	quartz, feldspar	S	9.0	10	3	0	13	253	0.051
16	624	clay loam	quartz, feldspar	S	4.0	1	0	0	1	not measured	
17 a	611	clay loam	feldspar	S, E / grouped with S+SE+E	14.0	78	19	0	97	495	0.196
17 b	612	not recorded	feldspar, quartz	S	20.0	5	1	0	6	200	0.030

Table 1a-A1 continues on the next page →

TABLE 1a-A1 (Continues...): Field data, including habitat parameters, plant number, occupied area and plant density, collected for 51 *Lithops ruschiorum* sites at the RUL population.

Site	Altitude (masl)	Soil texture	Substrate	Aspect	Slope	Mature plants	Damaged plants	Juveniles	Total number of plants	Occupied area (m^2)	Density (number of plants/occupied area)
17 c	609	clay loam	quartz, feldspar	W	14.	5	0	0	5	200	0.025
17 d	606	not recorded	quartz, granite	SE	3.0	3	0	0	3	not measured	
18 a	578	light clay	feldspar	S	15.0	4	7	0	11	1059	0.010
18 b	573	not recorded	feldspar	S	17.5	23	1	0	24	500	0.048
18 c	577	not recorded	feldspar	level	0.0	5	1	0	6	400	0.015
18 d	567	not recorded	feldspar	E	10.0	5	2	0	7	10	0.700
19 a	556	silt loam	feldspar	S, SE, SW / grouped with S+SE+E	not recorded	32	4	0	36	100	0.360
19 b	560	not recorded	feldspar	not recorded	not recorded	19	1	0	20	not measured	
19 c	560	not recorded	feldspar	not recorded	not recorded	12	0	0	12	500	0.024
19 d	561	not recorded	feldspar	not recorded	not recorded	9	0	0	9	200	0.045
20	588	light clay	schist	W	not recorded	10	1	0	11	232	0.047
21	562	loam	quartz, granite, feldspar	S	35.0	43	2	0	45	2135	0.021
22	546	light clay	schist, quartz, feldspar	S	25.0	32	1	0	33	327	0.101
41	652	loam	marble	SW	5.0	7	1	0	8	170	0.047
44	542	clay loam	not recorded	S	3.0	1	0	0	1	not measured	
45	527	clay loam	granite	SW	20.0	4	0	0	4	10	0.400
46	657	light clay	quartz	NW	10.0	9	4	0	13	852	0.015
47	608	loam	granite	SW	7.0	10	6	0	16	100	0.160
48 a	693	loam	quartz, feldspar	N, S / grouped with N+NE+NW	6.5	6	0	0	6	200	0.030
48 b	703	not recorded	quartz, feldspar	E	4.0	42	18	0	60	340	0.177
49	608	light clay	feldspar	level, S / grouped with level	15	383	57	0	440	6676	0.066
50	630	light clay	feldspar	SE	7.5	39	17	0	56	3016	0.019
68	599	clay loam	quartz, feldspar	SW	20.0	137	0	0	137	1073	0.128
Total	-	-	-	-	-	**1666**	**329**	**13**	**2008**	-	-

TABLE 1b-A1: Field data, including habitat parameters, plant number, occupied area and plant density, collected for 43 *Lithops ruschiorum* sites in the other eight surveyed populations. Sites 51 a–e are in Rössing Mountain (for geographic location, see Figure 1), sites 52 a–b in Feldspar Ridge, sites 53 a–b in Ugab Salt Works, sites 83a to 87b in Ugab River, sites 88a to 95 in Henties Bay–Uis Road, sites 96a–f in Khumib River, site 97 in View Point and sites 98a–d in Hoanib River.

Site	Altitude (masl)	Coastal distance (km)	Soil texture	Substrate	Aspect	Slope	Mature plants	Damaged plants	Juveniles	Total number of plants	Occupied area (m²)	Density (number of plants / occupied area)
51 a	492	35.0	loamy sand	quartz	W	5	26	0	0	26	1470	0.018
51 b	617	35.0	loamy sand	schist	W	25	168	0	2	170	1233	0.138
51 c	592	34.7	loamy sand	quartz, schist	not recorded	12.5	142	1	1	144	9130	0.016
51 d	617	35.1	loamy sand	quartz	W	12.5	71	0	0	71	not measured	
51 e	604	33.5	loamy sand	schist, quartz	not recorded	not recorded	7	0	0	7	200	0.035
52 a	470	42.7	silt loam	feldspar, granite, quartz	SW	15	208	0	0	208	10154	0.021
52 b	470	44.2	silt loam	feldspar, granite	S	20	98	0	1	99	9208	0.110
53 a	18	4.6	loamy sand	quartz, pegmatite	NW, W / grouped with N+NE+NW	8	72	0	0	72	5712	0.013
53 b	18	4.8	sand	quartz, pegmatite	level	2.5	76	0	0	76	3267	0.023
83 a	133	26.5	sand	granite	N	20	7	0	0	7	200	0.035
83 b	not recorded	26.5	loamy sand	granite, shale	not recorded	not recorded	2	0	0	2	not measured	
84 a	103	26.2	loamy sand	granite	NE	18	83	0	0	83	1339	0.062
84 b	105	26.2	sand	granite, shale	W	11	61	1	1	63	439	0.144
84 c	110	26.2	loamy sand	granite	not recorded	not recorded	123	1	5	129	5278	0.024
84 d	108	26.2	sand	not recorded	W	10	33	6	0	39	1272	0.031
85 a	100	26.8	loamy sand	granite, shale	SW	10	69	1	1	71	1144	0.062
85 b	97	26.8	loamy sand	granite, shale	SE	10	161	0	0	161	3290	0.049
86 a	99	26.1	loamy sand	granite, shale	level	0	8	0	0	8	200	0.040
86 b	105	26.1	sand	granite, shale	SE	5	4	0	0	4	200	0.020
87 a	103	26.4	sand	granite, shale	SE	15	113	0	0	113	607	0.186
87 b	108	26.4	loamy sand	granite, shale	All / undetermined*	12.5	60	1	0	61	1797	0.034
88 a	180	35.0	loamy sand	quartz, feldspar, others	W	5	65	0	0	65	7160	0.009

Table 1b-A1 continues on the next page →

TABLE 1b-A1: Field data, including habitat parameters, plant number, occupied area and plant density, collected for 43 *Lithops ruschiorum* sites in the other eight surveyed populations. Sites 51 a–e are in Rössing Mountain (for geographic location, see Figure 1), sites 52 a–b in Feldspar Ridge, sites 53 a–b in Ugab Salt Works, sites 83a to 87b in Ugab River, sites 88a to 95 in Henties Bay–Uis Road, sites 96a–f in Khumib River, site 97 in View Point and sites 98a–d in Hoanib River.

Site	Altitude (masl)	Coastal distance (km)	Soil texture	Substrate	Aspect	Slope	Mature plants	Damaged plants	Juveniles	Total number of plants	Occupied area (m²)	Density (number of plants /occupied area)
88 b	177	35.0	loamy sand	quartz, feldspar, others	NE	8	20	1	0	21	11068	0.002
89	190	44.1	loamy sand	quartz, feldspar, pegmatite	level	0	151	5	0	156	22684	0.007
90	201	34.1	loamy sand	quartz, feldspar	All / undetermined	5	139	1	0	140	8840	0.016
91	203	36.3	loamy sand	quartz, feldspar	All / undetermined	10	586	32	3	621	13729	0.045
92 a	309	50.9	loamy sand	quartz	All / undetermined	5	20	1	0	21	200	0.105
92 b	305	50.9	loamy sand	quartz	All / undetermined	5	5	0	0	5	200	0.025
93	320	41.48	loamy sand	quartz, others	level	0	9	0	0	9	1185	0.008
94 a	228	38.0	loamy sand	quartz, feldspar	N	16	5	0	0	5	200	0.025
94 b	not recorded	38.0	loamy sand	quartz, feldspar	not recorded	not recorded	8	0	0	8	200	0.040
95	217	37.8	loamy sand	quartz	level, SW / grouped with level	2.5	103	3	1	107	1250	0.086
96 a	343	26.5	silt loam	pegmatite, granite, schist	SE, SW / grouped with SW	17.5	641	19	32	692	4738	0.146
96 b	368	26.3	silt loam	granite, schist, pegmatite, feldspar	SE	15	120	7	6	133	1118	0.119
96 c	383	26.4	silt loam	not recorded	SE	13	359	6	13	378	1815	0.208
96 d	325	26.4	silt loam	pegmatite, granite, schist	SE, SW / grouped with S+SE+E	17.5	468	1	24	493	4247	0.116
96 e	378	26.5	silt loam	pegmatite, schist, granite	SW	18	436	2	30	468	1766	0.265
96 f	371	26.4	silt loam	pegmatite	NW	not recorded	47	1	1	49	2320	0.021
97	242	14.5	silt loam	quartz, basalt	SW	15	41	51	0	92	500	0.184
98 a	221	18.8	loamy sand	schist, shale, feldspar, pegmatite, granite	SW	18	283	51	12	346	6314	0.055
98 b	222	18.6	loamy sand	granite, pegmatite, schist	W	not recorded	53	2	1	56	751	0.075
98 c	233	18.6	loamy sand	granite, pegmatite, schist	SW	25	442	33	14	489	4146	0.118
98 d	225	18.8	loamy sand	granite, pegmatite	W	10.5	455	19	15	489	12397	0.039
Total	-	-	-	-	-	-	**6039**	**246**	**163**	**6448**	-	-

TABLE 2-A1: Details of ANOVAs performed to investigate the effect of aspect, substrate and soil texture on plant number and density at RUL and the other 8 populations, accompanied by Tukey test grouping (TTG): entries with identical letters do not differ at $P < 0.05$. Mean values are based on natural logarithm transformations.

Parameter	Categories	*N*	Mean	DF	*F*	*P*	TTG
RUL							
Aspect (Number)	level	3	4.0	4/33	1.41	0.251	A
	SW	6	3.1				A
	S+SE+E	23	2.7				A
	NE+N+NW	3	2.1				A
	W	4	2.0				A
Aspect (Density)	SW	6	-2.4	4/29	3.34	0.022	A
	S+SE+E	19	-2.4				A
	level	3	-3.4				A
	W	4	-4.1				A
	NE+N+NW	3	-4.5				A
Substrate (Number)	feldspar + other	13	3.3	3/45	0.49	0.691	A
	granite + other	6	3.2				A
	schist + other	2	3.0				A
	quartz + other	28	2.8				A
Substrate (Density)	granite + other	6	-2.6	3/38	0.30	0.827	A
	schist + other	2	-2.7				A
	feldspar + other	12	-3.0				A
	quartz + other	22	-3.2				A
Soil texture (Number)	light clay	14	3.2	3/47	0.99	0.407	A
	silt loam	11	3.1				A
	loam	11	3.0				A
	clay loam	15	2.4				A
Soil texture (Density)	loam	11	-2.3	3/39	1.35	0.272	A
	clay loam	11	-3.0				A
	silt loam	7	-3.3				A
	light clay	14	-3.4				A
Other 8 populations							
Aspect (Number)	SW	7	2.4	4/28	3.64	0.016	A
	S+SE+E	7	2.0				A
	W	8	1.9				A
	level	5	1.6				A
	NE+N+NW	6	1.4				A
Aspect (Density)	SW	7	-2.4	4/26	3.12	0.032	A
	S+SE+E	6	-2.5				A
	W	7	-3.1				A
	level	5	-3.9				A
	NE+N+NW	6	-4.1				A
Substrate (Number)	pegmatite + other	4	2.5	4/36	1.52	0.218	A
	feldspar + other	2	2.2				A
	schist + other	3	1.9				A
	granite + other	15	1.7				A
	quartz + other	17	1.7				A
Substrate (Density)	pegmatite + other	4	-2.3	4/33	4.16	0.008	A
	schist + other	3	-2.7				AB
	granite + other	13	-2.8				A
	quartz + other	16	-3.8				B
	feldspar + other	2	-4.2				AB
Soil texture (Number)	silt loam	9	2.3	2/40	4.00	0.026	A
	loamy sand	27	1.7				B
	sand	7	1.6				AB
Soil texture (Density)	silt loam	9	-2.6	2/37	2.34	0.111	A
	sand	6	-3.2				A
	loamy sand	25	-3.5				A