Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2019:28
Faculty of Landscape Architecture, Horticulture
and Crop Production Science

# Habitat characteristics, genetic diversity and conservation concerns for the genus Lithops in Namibia

Sonja Loots

Doctoral Thesis No. 2019:28 • Habitat characteristics, genetic diversity and conservation concerns for the genus Lithops in Namibia • Sonja Loots

# Habitat characteristics, genetic diversity and conservation concerns for the genus *Lithops* in Namibia

Sonja Loots
*Faculty of Landscape Architecture, Horticulture and Crop Production Science*
*Department of Plant Breeding*
*Alnarp*

Doctoral thesis
Swedish University of Agricultural Sciences
Alnarp 2019

Acta Universitatis agriculturae Sueciae
2019:28

Cover: The landscape photograph shows the plain to the south of the Tssaus Mountain with the habitat of *Lithops hermetica* after some rains in 2011, i.e. the last rains this area would get for 7 years; top left to right: *L. ruschiorum* in flower; *L. julii* in flower; *L. karasmontana* subsp. *eberlanzii* ( now merged with subsp. *bella*) of the rare "avocado cream" form. Bottom left to right: *L. ruschiorum* in feldspar substrate; the monitoring plot of *L. vallis-mariae* with temporary markers, from which all the plants were illegally removed some time after this photograph was taken ; contractors at the Rössing Uranium mine helping to find *L. ruschiorum* after the poles of the monitoring plots were secured with cement. (photos: S. Loots)

ISSN 1652-6880
ISBN (print version) 978-91-7760-374-0
ISBN (electronic version) 978-91-7760-375-7

Print: SLU Service/Repro, Uppsala/Alnarp 2019

# Habitat characteristics, genetic diversity and conservation concerns for the genus *Lithops* in Namibia

## Abstract

The dwarf succulent genus *Lithops* (Aizoaceae) is endemic to southern Africa, with 16 species in Namibia. *Lithops* are vulnerable to a variety of threats and assignment of accurate conservation status is difficult due to insufficient information about population parameters and influence of habitat variables. In addition, taxa are defined according to leaf characters that may be adapted to plant habitat, resulting in over-classification. Plant density, spatial arrangement of plants and habitat profile was investigated in a 1 ha study area in a population of *L. pseudotruncatella*. Five out of seven plant density estimation methods vastly over- or under-estimated plant number as determined in a census. Plant number in 100 10×10 m test plots was positively associated with a higher percentage cover of gravel and pebbles as opposed to sand or stones, and with a gentle rise as opposed to slopes and depressions. Plant abundance and habitat variables were also evaluated in 9 populations of L. *ruschiorum.* Evaluation of the 51 and 43 sites of the meticulously studied RUL population, and the remaining 8 populations, respectively, showed that plant density and/or plant number was associated with aspect, slope, soil texture, substrate and geographic distance from the coast. Results are concordant with a strong impact of fog-based precipitation on plant density in the coastal populations, whereas rain is probably more important at RUL, situated further inland. Plant number dropped by 50% in 21 10×10 m plots monitored at RUL over nine years, most likely due to a prolonged drought in 2012–2016. Plant number declined also in some of the 15 plots in the other populations. AFLP markers were employed to study 52 individuals from 7 populations of *L. ruschiorum*, and 223 individuals from 44 populations representing 15 *Lithops* species and 23 taxa in total. An AMOVA demonstrated low levels of genetic differentiation between populations of *L. ruschiorum*, and a significant but rather weak isolation-by-distance pattern. Analyses of genetic structuring and phylogenetic relationships identified several groups of closely related species, while subspecific taxa often overlapped considerably. Main reason for the overall low variability in *Lithops* is probably a rapid and recent diversification of succulents after the global expansion of arid environments in the late Miocene. Three nomenclatural changes were made, affecting: (1) *L. optica* and *L. herrei*, (2) *L. amicorum* and *L. karasmontana*, and (3) subspecies *bella* and *eberlanzii* of *L. karasmontana.*

*Keywords:* AFLP, Aizoaceae, ecology, microhabitat, molecular marker, phylogenetics, plant abundance, spatial distribution, species distribution, succulent, taxonomy

*Author’s address:* Sonja Loots, SLU, Department of Plant Breeding, P.O. Box 103,
23053 Alnarp, Sweden

# Habitat characteristics, genetic diversity and conservation concerns for the genus *Lithops* in Namibia

## Sammanfattning

Suckulentsläktet *Lithops* (Aizoaceae) är endemiskt i södra Afrika, med 16 arter i Namibia. *Lithops* hotas av växtsamlare, habitatdegradering inklusive ökenspridning samt predation. Fastställande av skyddsbehov försvåras av bristande information om populationsdynamiken samt påverkan av olika habitatvariabler. Dessutom baseras avgränsningen av olika taxa i huvudsak på bladkaraktärer som kan vara adapterade till habitatet. Antal växtindivid och deras spatiella placering undersöktes i en 1 hektar stor observationsyta i en population av *L. pseudotruncatella*. Fem av sju metoder för uppskattning av antal växtindivid under- eller överskattade grovt det faktiska antalet som fastställts genom räkning av alla växtindivid. Antal växtindivid i 100 10×10 m försöksrutor var positivt korrelerat med en hög procent grus och småsten istället för sand eller större stenar, och med en liten höjning istället för sluttningar och sänkor. Antal växtindivid och habitatvariabler undersöktes även i 9 populationer av *L. ruschiorum*. Den mest undersökta populationen, RUL, delades upp i 51 växtytor, medan ytterligare 43 växtytor undersöktes i de 8 andra populationerna. Utvärdering av dessa växtytor visade att växttäthet och/eller antal växtindivid påverkades av väderstreck, lutning, jordart, bergart samt geografiskt avstånd från kusten men inte av höjden över havet. Resultaten tyder på att nederbörd i form av dimma har stor påverkan på växttätheten i kustnära populationer medan regn troligen är viktigare i RUL som ligger längre in i landet. Antal växtindivid minskade med 50% i 21 10×10 m försöksrutor i RUL under en 9-årsperiod, troligen på grund av långvarig torka under 2012–2016. Antal växtindivid minskade även i några av de 15 försöksrutorna i de andra populationerna, särskilt mellan 2012 och 2016. AFLP-markörer användes för att studera (1) 52 individ ur 7 populationer av *L. ruschiorum*, och (2) 223 individ ur 44 populationer som representerade 15 *Lithops* arter och totalt 23 olika taxa. Analys av markörvariationen visade på låga nivåer av genetisk differentiering mellan populationer av *L. ruschiorum*, och en signifikant men ändå ganska svag effekt av avståndsrelaterad isolering. Analys av genetisk struktur och fylogenetiska samband identifierade flera grupper av närbesläktade arter medan underarter oftast överlappade kraftigt. Huvudanledning till den låga variationen hos *Lithops* är troligen en snabb diversifiering av suckulenter som inträffat nyligen, efter den globala expansionen av torra områden i slutet av Miocen. Tre nomenklatoriska förändringar gjordes, vilka berör: (1) *L. optica* och *L. herrei*, (2) *L. amicorum* och *L. karasmontana*, samt (3) underarterna *bella* och *eberlanzii* av *L. karasmontana*.

*Nyckelord:* AFLP, Aizoaceae, ekologi, molekylär markör, fylogenetik, växttäthet, växtutbredning, suckulent, taxonomi

*Author's address:* Sonja Loots, SLU, Department of Plant Breeding,
P.O. Box 101, 23053 Alnarp, Sweden

# Dedication

To Jeshua, the Son of God, my Lord and Saviour,
and to my family, for their patient support

*Success is stumbling from failure to failure with no loss of enthusiasm*
Winston Churchill

# Contents

# List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Loots, S. and Nybom, H. (2017). Towards better risk assessment for conservation of flowering stones: plant density, spatial pattern and habitat preference of *Lithops pseudotruncatella* in Namibia. *South-Afr. J. Bot.* 109: 112–115.

II Loots, S., Ritz, C.M., Schwager, M., Sehic, J., Garkava-Gustavsson, L., Herklotz, V. and Nybom, H. Distribution, habitat profile and genetic variability of Namibian succulent *Lithops ruschiorum*. *Bothalia ABC*, manuscript in press.

III Loots, S., Nybom, H., Schwager, M., Sehic, J. and Ritz, C.M. Genetic variation among and within *Lithops* species in Namibia. Manuscript submitted.

IV Loots, S. and Nybom, H. Changes in plant abundance for the endemic succulent *Lithops ruschiorum* (Aizoaceae). Manuscript.

Paper I reproduced with the permission of the publisher.

The contribution of Sonja Loots to the papers included in this thesis was as follows:

I Planned the study. Conducted the field work. Evaluated the data with some assistance from co-author. Had a leading role in writing the manuscript together with co-author.

II Planned the study. Conducted the field work. Evaluated the field-collected data with some assistance from HN. Conducted the AFLP analyses and data evaluations in co-operation with CR, MS, JS and LGG. Produced the first draft and wrote the manuscript together with co-authors.

III Planned the study in co-operation with HN and CR. Conducted the field work. Conducted the AFLP analyses in co-operation with CR, MS and JS. Assisted in the data evaluations. Wrote the manuscript together with co-authors.

IV Planned the study. Conducted the field work. Evaluated the data with some assistance from co-author. Had a leading role in writing the manuscript together with co-author.

# Acronyms and abbreviations

| | |
|---|---|
| ACF | Aberrant Colour Form |
| ACS | Adaptive Cluster Sampling |
| AFLP | Amplified Fragment Length Polymorphism |
| AMOVA | Analysis of Molecular Variance |
| ANOVA | Analysis of Variance |
| AOO | Area of Occupancy |
| CAM | Crassulacean Acid Metabolism |
| CBD | Convention on Biological Diversity |
| CITES | Convention on International Trade in Endangered Species of fauna and flora |
| CREW | Custodians of Rare and Endangered Wildflowers |
| DAPC | Discriminant Analysis of Principal Components |
| DD | IUCN Red List Category “Data Deficient” |
| DNA | Deoxyribonucleic Acid |
| E | *Eco*RI adapter |
| EIS | Environmental Information System of Namibia |
| EN | IUCN Red List and threatened Category “Endangered” |
| EOO | Extent of Occurrence |
| GIS | Geographic Information System |
| GLM | General Linear Model |
| GPS | Geographic Positioning System |
| $H_e$ | Expected Heterozygosity |
| IPAs | Important Plant Areas |
| IUCN | International Union for the Conservation of Nature |
| LC | IUCN Red List Category “Least Concern” |
| M | *Mse*I adapter |
| MAWF | Ministry of Agriculture, Water and Forestry of Namibia |
| NBRI | National Botanical Research Institute |

| | |
|---|---|
| NBSAP | National Biodiversity Strategy and Action Plan |
| NCE | Namibia Chamber of the Environment |
| NCO | Nature Conservation Ordinance |
| NE | IUCN Red List Category “Not Evaluated” |
| NGO | Non-Governmental Organisation |
| NJ | Neighbour-Joining phylogenetic analysis |
| NPGRC | National Plant Genetic Resources Centre (Genebank) |
| NT | IUCN Red List Category “Near Threatened” |
| PCA | Principal Component Analysis |
| PCoA | Principal Coordinate Analysis |
| PCR | Polymerase Chain Reaction |
| PGA | Phosphoglyceric acid |
| PPL | Percentage Polymorphic Loci |
| PRE | The National Herbarium of South Africa |
| R | Rare (a regional conservation status category used by South Africa and adopted by Namibia) |
| RH | Relative air Humidity |
| RUL | Rössing Uranium Limited |
| SADC | Southern Africa Development Community |
| SANBI | Southern African National Biodiversity Institute |
| SD | Standard Deviation |
| UK | United Kingdom |
| USA | United States of America |
| UV | Ultra Violet |
| VQP | Variable Quadrant Plot |
| VU | IUCN Red List and threatened Category “Vulnerable” |
| WGS 84 | World Geodetic System 1984 |
| WIND | National Herbarium of Namibia |

# 1 Introduction

## 1.1 A preamble to the identity, growth habits and proclivities of *Lithops*

*Lithops* belong to a group of dwarf succulent plants in the Aizoaceae family, most commonly known in English as “stone plants”, “flowering stones” and “pebble plants”. The vernacular names derive from their ability to take on the appearance of the stony substrates in the specialised habitats that they occupy in southern Africa and where they can be virtually undetectable. In native languages they are known by a whole host of other names (Cole, 2005). The Latin name *Lithops* is derived from “*Lithos*”, which means stone and “*-ops*” referring to “face” in ancient Greek and was first published by N.E. Brown in 1922 (Cole, 2005).

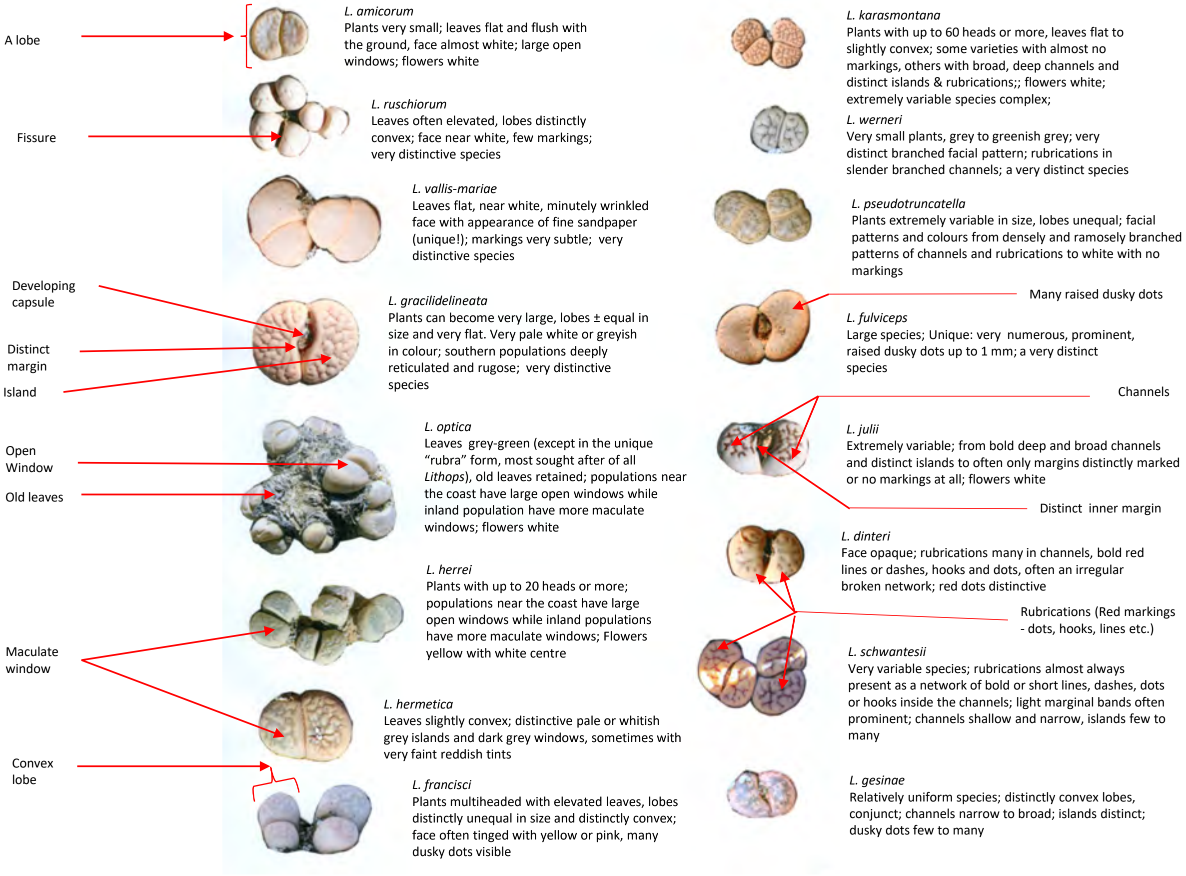
The ability of *Lithops* plants to remain undetected for a significant part of the season serves to defend them against herbivory. Being partially buried in the soil together with their tough epidermis prevents excessive water loss during dry periods. They are slow-growing in nature and can become 50 years and older (Schwantes, 1957).

They have considerable value for the international horticulture trade and apart from this, *Lithops* may have a very important ecological role, often being one of only a few plant species blooming outside the main flowering season in very harsh environmental conditions within their natural range that is South Africa, Namibia and Botswana. The plants are often confined to isolated quartz outcrops or other specialised habitats. In very dry years and in habitats that receive very little rain precipitation, observations suggest that they may constitute an important supplementary food source at times for small and larger herbivores.

### 1.1.1 Plant morphology

Differences in leaf shape is one of the most remarkable features of the subfamily Ruschiodeae, to which *Lithops* belongs, with trigonous and terete leaves being the most common in the Ruschieae tribe, resulting in a reduction in leaf surface area, which assists in preventing loss of water in dry conditions (Klak *et al.*, 2004, 2009).

*Lithops* have an exceptionally limited number of morphological features, essentially consisting of a pair of small (up to 5 cm in diameter), fused, water-storing succulent leaves, partially buried in the soil. Remarkably, this single feature and the variation that exists within it, is used to distinguish between taxa in the genus. A plant can consist of a single leaf pair, fused at the base and cone-shaped in appearance, or have up to 20 or more leaf pairs, depending on the species. Some species, such as *L. gracilidelineata* and *L. pseudotruncatella* subsp. *groendrayensis*, rarely produce more than one leaf pair (Cole and Cole, 2005), but most taxa produce double, triple or multiple-headed specimens that can be either flush with the ground such as *L. gracilidelineata* or more elevated, such as *L. fransisci*, and *L. ruschiorum*, which can have up to 35 leaf pairs. The vertical profile and size of the leaves also vary, with *Lithops werneri* being one of the smallest species, its facial diameters reaching a maximum of 24×18 mm, while *L. pseudotruncatella* subsp. *groendrayensis* is one of the largest with facial diameters reaching 53×44 mm (Cole and Cole, 2005). Their cryptic nature, sunken growth and the fact that they never completely shed their old leaves, are characters shared with 37 species in 11 other genera in the family Aizoaceae (Hartman, 2006). A fissure divides the top of the leaf into two more or less equal halves. The top surface of the leaf, or face, is variously marked and coloured to mimic the substrate in which the plants grow, and vary widely among taxa.



*L. amicorum*
Plants very small; leaves flat and flush with the ground, face almost white; large open windows; flowers white

*L. ruschiorum*
Leaves often elevated, lobes distinctly convex; face near white, few markings; very distinctive species

*L. vallis-mariae*
Leaves flat, near white, minutely wrinkled face with appearance of fine sandpaper (unique!); markings very subtle; very distinctive species

*L. gracilidelineata*
Plants can become very large, lobes ± equal in size and very flat. Very pale white or greyish in colour; southern populations deeply reticulated and rugose; very distinctive species

*L. optica*
Leaves grey-green (except in the unique "rubra" form, most sought after of all *Lithops*), old leaves retained; populations near the coast have large open windows while inland population have more maculate windows; flowers white

*L. herrei*
Plants with up to 20 heads or more; populations near the coast have large open windows while inland populations have more maculate windows; Flowers yellow with white centre

*L. hermetica*
Leaves slightly convex; distinctive pale or whitish grey islands and dark grey windows, sometimes with very faint reddish tints

*L. francisci*
Plants multiheaded with elevated leaves, lobes distinctly unequal in size and distinctly convex; face often tinged with yellow or pink, many dusky dots visible

*L. karasmontana*
Plants with up to 60 heads or more, leaves flat to slightly convex; some varieties with almost no markings, others with broad, deep channels and distinct islands & rubrications;; flowers white; extremely variable species complex;

*L. werneri*
Very small plants, grey to greenish grey; very distinct branched facial pattern; rubrications in slender branched channels; a very distinct species

*L. pseudotruncatella*
Plants extremely variable in size, lobes unequal; facial patterns and colours from densely and ramosely branched patterns of channels and rubrications to white with no markings

*L. fulviceps*
Large species; Unique: very numerous, prominent, raised dusky dots up to 1 mm; a very distinct species

*L. julii*
Extremely variable; from bold deep and broad channels and distinct islands to often only margins distinctly marked or no markings at all; flowers white

*L. dinteri*
Face opaque; rubrications many in channels, bold red lines or dashes, hooks and dots, often an irregular broken network; red dots distinctive

*L. schwantesii*
Very variable species; rubrications almost always present as a network of bold or short lines, dashes, dots or hooks inside the channels; light marginal bands often prominent; channels shallow and narrow, islands few to many

*L. gesinae*
Relatively uniform species; distinctly convex lobes, conjunct; channels narrow to broad; islands distinct; dusky dots few to many

*Figure 1.* An overview of Namibian *Lithops* indicating mainly facial features used to distinguish between taxa (terminology after Cole and Cole, 1988).

*Lithops* can be divided into a yellow-flowering group and a white-flowering group. In Namibia, only 4 of the 16 recognised species have white flowers and only one has a yellow flower with a white centre, while 11 have pure yellow flowers. In South Africa, only 5 species have white flowers, 11 have yellow with a white centre and 9 have pure yellow flowers (Cole and Cole, 2005) in addition to the extremely variable flower colour of *L. verruculosa*. The most diagnostic characters in the genus are therefore the shape and size of the leaves in addition to the windows, channels, fissures, islands, margins, rubrications, dusky dots, tanniniferous idioblast patterns and colours on the top surface of the leaf (normally referred to as the face). Part of what makes *Lithops* a unique group of plants is the often astonishing amount of variation in these facial features, sometimes within a single population. However, this same diversity has contributed significantly to the confusion that resulted from attempts to classify the plants.

The most detailed, and currently most widely accepted descriptions and classification of the morphology of all the *Lithops* taxa are given by Cole and Cole (1988, 2005, 2006, 2012). These descriptions are largely based on the size and shape of the leaves, the facial features, the colour and size of the flowers and the shape and size of the capsules and seeds, underpinning the importance of morphological characters in this group, which are still used to identify taxa in the field.

Hartmann (2006) compared the anatomy of the outer cell walls of the leaves of some *Lithops* taxa to that of some other sunken species in the Aizoaceae family and noted the uniformity of epidermal outer wall construction in *Lithops,* as opposed to the wide variation in one of its closest relatives, *Conophytum* N.E.Br.

Ehler and Barthlott (1978) analysed the seed coats of some Ruschioideae taxa in detail, including two South African *Lithops* species, and found that the outer wall surface of the testa cells of these species are smooth and without any micro-sculpturing. Hassan *et al*. (2005) analysed seed coat morphology of 26 species of the family Aizoaceae, but their work did not include any species from the subfamily Ruschioideae to which *Lithops* belong.

As with most other members of the Aizoaceae family, *Lithops* possess loculicidal, hygrochastic capsules. After having absorbed water, the expanding keels on the inside of the capsule forces open the valves that form the lid of the capsule to expose the (predominantly 5) locules containing the seeds. This mechanism is a commonly occurring adaptation to arid areas where rainfall is erratic (Hartmann, 1988). The type of *Lithops* seed capsule belongs to the *Delosperma* N.E.Br. type of fruit, which has no closing bodies or covering membranes (Hartmann, 1988) and is also rather fragile, and therefore considered unspecialized within the Ruschieae tribe as opposed to the more woody capsules that are more persistent (Klak *et al.*, 2013). *Lithops* seeds are simply washed out by rain drops falling on the open seed capsule as opposed to hydroballochory, where the capsules instead use the kinetic energy of raindrops and the more specialised morphological adaptations of the capsules to form a kind of nozzle to expel the seeds by means of a jet action mechanism (Parolin, 2005), ensuring that all the seeds are not dispersed in a single rainfall event. It is possible that *Lithops* seed employ other mechanisms to ensure that all the seeds do not germinate at the same time, but this has not been investigated.

Landrum (2001) studied wide-band tracheids that occur almost exclusively in the core Ruschioideae. These are tracheids that have wide secondary walls and are hypothesized to prevent cell collapse under severe water stress situations. This in turn helped the species in this sub-family to colonise new micro-environments all over arid southern Africa.

### 1.1.2 Breeding system and genetic diversity

Landrum (2001) and Klak *et al.* (2003) report x = 9 as the basic chromosome number for all Mesembryanthemoideae and Ruschioideae, the latter including *Lithops*. Cole and Cole (2005) report that chromosome counts were carried out for a dozen *Lithops* taxa more than 40 years ago and that two of these taxa deviated but no further information is given about this study. Klak *et al.* (2013) illuminated ploidy levels in Aizoaceae, reporting that most taxa in the Ruschioideae are diploids ($2n = 2x = 18$) but tetraploids such as *Cephalophyllum* Haw. and *Conophytum* N.E.Br. are also found as well as hexaploids such as *Leipoldtia* L.Bolus on rare occasions.

The showy white and yellow flowers of *Lithops* suggest that they are insect-pollinated. No population genetics research has been published on any taxon of *Lithops* to date. Alexander (2003) found that threatened species in general have larger genomes compared to more secure relatives. Whether this is true also for *Lithops* could perhaps be investigated by comparing taxa with widely different distribution areas. It is possible, however, that the recent radiation suggested for Aizoaceae (Klak *et al.*, 2004) is not yet reflected in differentiated genome sizes.

## 1.2 Systematics

### 1.2.1 A short history of traditional and molecular taxonomy of the family Aizoaceae

The genus *Lithops* N.E.Br. belongs to the family Aizoaceae (previously Mesembryanthemaceae, also known as the ice plant family), which is part of the core of the families of the order Caryophyllales. Aizoaceae is the world's largest succulent plant family (Van Jaarsveld, 1987) and of the four subfamilies currently recognized, subfamily Ruschioideae is the largest, with 111 genera and some 1600 species (Klak *et al.*, 2013). Of these, 36 genera with 144 species, of which 57 are endemic, occur in Namibia (Klaassen and Kwembeya, 2013).

Schwantes (1947, 1957, 1971) classified the Ruschioideae by means of a tribal system. Bittrich and Hartmann (1988) provided descriptions of each of their five proposed subfamilies, namely Aizooideae, Tetragonioideae, Sesuvioideae, Mesembryanthemoideae and Ruschioideae. They also suggested three possible synapomorphic characters for this family, namely bladder cells in the epidermis, the hygrochastic capsule and the perianth stamen tube. Mannheimer (2006) noted that taxonomic work in this family is complicated by the succulent nature of the plants and phenotypic plasticity which is most noticible in their morphology, and varies greatly in response to environmental variables. Chesselet *et al.* (2002) divided the family into four tribes based on the floral nectaries, but Klak *et al.* (2003) and Thiede ( 2004) could only uphold three of these tribes following their work on genetic divergence. Finding a low level of divergence among members of this sub-group, they suggested that, in addition to ecological and climatological factors, several special morphological traits such as leaf shape, the hygrochastic capsules and the wide band tracheids found almost exclusively in the core Ruschioideae, have facilitated a major and recent radiation in this group (Klak *et al.*, 2003). Klak *et al.* (2003) based their classification for the family Aizoaceae on four chloroplast DNA regions, found three major clades and reduced the subfamilies from five to four, defining the Sesuvioideae as a sister group to the rest of the Aizoaceae. Three tribes for the Ruschioidiae sub-family, Apatesieae, Dorotheantheae, and Ruschieae, the largest tribe which includes the genus *Lithops,* were also identified then. Chesselet (2002) divided the subfamily Ruschioideae into four tribes based on distinct nectary types, but genetic divergence was too low to support both Delospermae and Rushieae and subsequently Delospermae was included in Ruschieae (Klak *et al.*, 2003; Thiede, 2004). Thiede later used chloroplast DNA sequencing to show how the lack of a particular intron in the two tribes of the Ruschioideae subfamily suggest that they are monophyletic (Thiede, 2007). Using 10 chloroplast gene regions, Klak *et al*. (2013), presented a phylogenetic hypothesis for the species included in the Ruschieae and Drosanthemae groups of the subfamily in an attempt to sort out the many unresolved relationships in this group.

Some of the closest relatives of *Lithops* include other dwarf succulents in the tribe Ruschieae such as the genus *Dinteranthus* Schwantes, with at least one species with sunken growth, well camouflaged in their natural habitat of quartz stones, similar epidermal cell anatomy (Hartmann, 2006), and the absence of bracteoles on the flower stalks, a character also shared with *Titanopsis* Schwantes and *Gibbaeum* NE.Br. (Hartmann, 2004). The thick, cobble-stone-shaped epidermal cells that *Lithops* possesses, consist of apical groups of erect wax platelets and this characteristic is shared with *Schwantesia* Dinter, *Dinteranthus* and *Lapidaria* (Dinter & Schwantes) N.E.Br, in addition to the apparent absence of bracteoles in the inflorescences and the lack of covering membranes and broad valve wings in the capsules (Hartmann, 2006). *Lapidaria margaretae* shares the complicated epidermal cell walls of *Lithops*, is also relatively well camouflaged and often grows in association with *L. julii* but always has a multi-bodied plant, is more elevated and produces a larger, more spectacular yellow flower than *Lithops*.

The first *Lithops* species was described by A.H. Haworth as *Mesembryanthemum turbiniforme* after William John Burchell discovered it in South Africa in 1811 (Nel, 1946). Almost 100 years later, in 1908, the second *Lithops* species was described by A. Berger as *Mesembryanthemum pseudotruncatellum*, from the vicinity of Windhoek (Nel, 1946). Nicholas E. Brown renamed *Mesembryanthemum turbiniforme* as *Lithops turbiniformis* in 1922. During the following decades, dozens of species were published by Brown, Bolus, Schwantes, Dinter and Nel, but there was no clear concept of the relationship between species or which populations should be grouped with which species.

Extensive research has been published on the systematics of *Lithops* (Nel, 1946; Fearn, 1968; Cole, 1988, 2001, 2005; Clark, 1996), of which the most comprehensive is that of Cole in 1988, after about 30 years of studying the genus. The first identification key was published by Clark (1996). A taxonomic review of the genus *Lithops* was published as part of the circumscription and delimitation of the family Aizoaceae by Cole and Cole (2001). The book by Cole (1988) was later revised to incorporate new taxa and to present an updated key to the species (Cole and Cole, 2005). Recently described taxa include *L. hermetica* (Cole, 2000), *L. amicorum* together with a variety of *L. fulviceps* (Cole, 2006) and *L. karasmontana* subsp. *karasmontana* var.

*immaculata* (Cole, 2012). This brings the number of *Lithops* species validly published in taxonomic literature (Cole and Cole in Hartmann, 2001) to 37. The total number of accepted taxa (species and subspecies) described for Namibia is 32, but with varieties included, the number of taxa published to date rises to 40 for Namibia, out of a total of 93 taxa based on Cole and Cole in Hartmann (2001), Cole and Cole (2005, 2009) and Cole (2006, 2012) (Table 1). The National Herbarium (PRE) of the South African National Biodiversity Institute (SANBI) recognizes only 29 taxa for South Africa, namely 24 species with 5 subspecies (Germishuizen and Meyer, 2003). Figures 2 to 17 (Photographs taken *in situ*) show one example of each of the 16 species occurring in Namibia.

Table 1. *Lithops taxa currently recognized to occur in Namibia according to Cole and Cole (2005, 2009) and Cole (2006, 2012) and accepted to subspecies level by the National Herbarium of Namibia (*WIND*, 2006; Klaassen and Kwembeya, 2013).*

| Species | Subspecies | Variety | Endemic status |
|---|---|---|---|
| *L. amicorum* D.T.Cole | | | Endemic |
| *L. dinteri* Schwantes | *dinteri* | *dinteri* | Endemic |
| *L. dinteri* Schwantes | *dinteri* | *brevis* (L.Bolus) B.Fearn | Namibia / South Africa |
| *L. dinteri* Schwantes | *multipunctata* (de Boer) D.T.Cole | | |
| *L. francisci* (Dinter & Schwantes) N.E.Br. | | | Endemic |
| *L. fulviceps* (N.E.Br.) N.E.Br. | | *fulviceps* | Namibia / South Africa |
| *L. fulviceps* (N.E.Br.) N.E.Br. | | *lactinea* D.T.Cole | Endemic |
| *L. gesinae* de Boer | | *annae* (de Boer) D.T.Cole | Endemic |
| *L. gesinae* de Boer | | *gesinae* | Endemic |
| *L. gracilidelineata* Dinter | *gracilidelineata* | *gracilidelineata* | Endemic |
| *L. gracilidelineata* Dinter | *gracilidelineata* | *waldroniae* de Boer | Endemic |
| *L. gracilidelineata* Dinter | *brandbergensis* (de Boer) D.T.Cole | | Endemic |
| *L. hermetica* D.T.Cole | | | Endemic |
| *L. herrei* L.Bolus | | | Namibia / South Africa |

| Species | Subspecies | Variety | Endemic status |
|---|---|---|---|
| *L. julii* (Dinter & Schwantes) N.E.Br. | *julii* | | Endemic |
| *L. julii* (Dinter & Schwantes) N.E.Br. | *fulleri* (N.E.Br.) B.Fearn | *rouxii* (de Boer) D.T.Cole | Endemic |
| *L. karasmontana* (Dinter & Schwantes) N.E.Br. | *karasmontana* | *karasmontana* | Endemic |
| *L. karasmontana* (Dinter & Schwantes) N.E.Br. | *karasmontana* | *aiaisensis* (de Boer) D.T.Cole | Endemic |
| *L. karasmontana* (Dinter & Schwantes) N.E.Br. | *karasmontana* | *lericheana* (Dinter & Schwantes) D.T.Cole | Endemic |
| *L. karasmontana* (Dinter & Schwantes) N.E.Br. | *karasmontana* | *tischeri* D.T.Cole | Endemic |
| *L. karasmontana* (Dinter & Schwantes) N.E.Br. | *karasmontana* | *immaculata* D.T.Cole | Endemic |
| *L. karasmontana* (Dinter & Schwantes) N.E.Br. | *bella* (N.E.Br.) D.T.Cole | | Endemic |
| *L. karasmontana* (Dinter & Schwantes) N.E.Br. | *eberlanzii* (Dinter & Schwantes) D.T.Cole | | Endemic |
| *L. optica* (Marloth) N.E.Br. | | | Endemic |
| *L. pseudotruncatella* (A.Berger) N.E.Br. | *pseudotruncatella* | *pseudotruncatella* | Endemic |
| *L. pseudotruncatella* (A.Berger) N.E.Br. | *pseudotruncatella* | *elisabethiae* (Dinter) de Boer & Boom | Endemic |
| *L. pseudotruncatella* (A.Berger) N.E.Br. | *pseudotruncatella* | *riehmerae* D.T.Cole | Endemic |
| *L. pseudotruncatella* (A.Berger) N.E.Br. | *archerae* (de Boer) D.T.Cole | | Endemic |
| *L. pseudotruncatella* (A.Berger) N.E.Br. | *dendritica* (G.C.Nel) D.T.Cole | | Endemic |
| *L. pseudotruncatella* (A.Berger) N.E.Br. | *groendrayensis* (H.Jacobsen) D.T.Cole | | Endemic |
| *L. pseudotruncatella* (A.Berger) N.E.Br. | *volkii* (Schwantes ex. de Boer & Boom) D.T.Cole | | Endemic |
| *L. ruschiorum* (Dinter & Schwantes) N.E.Br. | | *ruschiorum* | Endemic |
| *L. ruschiorum* (Dinter & Schwantes) N.E.Br. | | *lineata* (G.C. Nel) D.T.Cole | Endemic |
| *L. schwantesii* Dinter | *gebseri* (de Boer) D.T.Cole | | Endemic |
| *L. schwantesii* Dinter | *schwantesii* | *schwantesii* | Endemic |

| Species | Subspecies | Variety | Endemic status |
|---|---|---|---|
| *L. schwantesii* Dinter | *schwantesii* | *marthae* (Loesch & Tischer) D.T.Cole | Endemic |
| *L. schwantesii* Dinter | *schwantesii* | *rugosa* (Dinter) de Boer & Boom | Endemic |
| *L. schwantesii* Dinter | *schwantesii* | *urikosensis* (Dinter) de Boer & Boom | Endemic |
| *L. vallis-mariae* (Dinter & Schwantes) N.E.Br. | | | Endemic |
| *L. werneri* Schwantes & H.Jacobsen | | | Endemic |

Figure 2. *Lithops amicorum*

Figure 3. *Lithops dinteri*

Figure 4. *Lithops francisci*

Figure 5. *Lithops fulviceps*

*Figure 6. Lithops gesinae*

*Figure 7. Lithops gracilidelineata*

*Figure 8. Lithops hermetica*

*Figure 9. Lithops herrei*

Figure 10. Lithops julii

Figure 11. Lithops karasmontana

Figure 12. Lithops optica

Figure 13. Lithops pseudotruncatella

Figure 14. Lithops ruschiorum

Figure 15. Lithops schwantesii

Figure 16. Lithops vallis-mariae

Figure 17. Lithops werneri

Wallace (1985, 1988) suggests that the idioblasts [large isolated cells that differ from neighbouring tissues and contain non-living substances, such as tannin (in the case of *Lithops*) beneath the epidermis of the leaves] may be of taxonomic use as they remain constant within a species and that they may be of particular use in deciding whether to include or exclude problematic taxa within a species group. Wallace and Fairbrothers (1985) suggested a multidisciplinary biosystematic approach in order to address the confusion in *Lithops* taxonomy, and used scanning electron microscopy and histology to identify systematically useful characters in the seed and epidermis, with specific mentioning of a highly specialised epidermal surface (Wallace and Fairbrothers, 1986).

Korn (2011) attempted to classify window (translucent area on the leaf surface that allows light to enter) patterns in *Lithops*, and defined two basic types of window pattern, namely (1) a reticulated (geometric) network similar to a Voronoi diagram, and (2) an array of tannin idioblasts. This classification can only be useful if applied in combination with a multidisciplinary taxonomic approach, and results should perhaps be treated with caution as only three taxa were examined by Korn (2011) of which at least one had an invalid name.

Some taxa overlap in morphology to such an extent that they are not easily identifiable without information about their geographic origin. This may be an indication of over-classification as demonstrated in earlier reports (e.g., Nel, 1946) and by the large number of synonyms existing for many taxa in the genus. Even within populations, morphology of *Lithops* can be extremely variable, not only in patterns and colours of the facial features, but also in plant size.

It should be noted that a number of taxa, such as *L. fulviceps*, *L. gracilidelineata*, *L. ruschiorum*, *L. vallis-mariae* and *L. werneri*, are easily identified as their morphology is distinct from that of any other species. Cole and Cole (2005), however, mentioned several pairs of taxa that may derive from convergent evolution; these taxa have very similar leaf colour, pattern and markings but different flower colour. Until now, the species in each pair have not been regarded as particularly closely related as they do not appear to be inter-fertile.

### 1.2.2 Molecular taxonomy of *Lithops*

Scogin (1972) compared total protein band patterns for three *Lithops* taxa and found them to be affected by developmental stage and thus not taxonomically useful. However, he also investigated individual isozyme patterns which were species-specific and therefore more informative.

Wallace (1990) used allozyme variation in *Lithops* to assess genetic variation between taxa. His results suggested a high degree of genetic similarity within *Lithops*, which he explains with a high degree of inter-fertility among yellow-flowered species and a similarly high degree of inter-fertility among white-flowered species. This view was not supported by Cole and Cole (2012), who found only some 12 colonies out of 450 to be of possible hybrid origin. Wallace further suggests that *Lithops* must have evolved recently and undergone a rapid morphological diversification which was not accompanied by a similar genetic divergence. A dendrogram of all the investigated *Lithops* taxa resulted in three major groups with four subgroups (Wallace, 1990).

The four sub-groups of Wallace (1990) are in partial agreement with those in Kellner *et al*. (2011), who used AFLP to investigate genetic diversity in the genus. In both these assessments, *L. optica* and *L. herrei* grouped together as well as *L. gracilidelineata* and *L. werneri*, *L. dinteri* and *L. dorotheae*, *L. pseudotruncatella* and *L. steineckiana*, *L. julii* and *L. hallii*, *L. otzeniana* and *L. olivacea* and finally *L. helmutii* and *L. divergens*. In Cole and Cole (2005), *L. steineckiana* is suggested to be a nursery hybrid between *L. pseudotruncatella* and *L. ruschiorum*, which is strengthened by the fact that it grouped with the putative parental species in Kellner *et al.* (2011).

Kellner *et al*. (2011) compared their AFLP data with DNA sequence data and found that the AFLP data yielded appreciably more polymorphism than DNA sequencing. More importantly, they found that the nine clades revealed by the AFLP data do not fit the current morphology-based taxonomy. They were, however, not able to determine whether the genus is monophyletic, but this could be due to what they call the pitfalls of AFLP -based phylogenies and low-sequence divergence. Their Bayesian phylogenetic analysis confirmed the close relationship between *Lithops* and *Lapidaria*, *Schwantesia* and *Dinteranthus*, which was discussed already by Hammer (1999) and Hartman (2006), based on the structure of the epidermal cells, absence of bracteoles and lack of covering membranes within the capsules.

## 1.3 Distribution and ecological aspects

### 1.3.1 Geographical distribution and origin of *Lithops*

Bittrich and Hartmann (1988) reported that most species (96%) in Aizoaceae are endemic to arid and semi-arid parts of southern Africa while only a few occur in Australia and the central Pacific. This would make southern Africa, and more specifically South Africa, the centre of diversity (Klak *et al*., 2003) and origin for this family. More importantly, within southern Africa, the greatest number of species in this family occur in the Succulent Karoo Biome (Fig. 18), which is one of the world's 25 biodiversity hotspots (Hilton-Taylor, 1996). Botswana, which is also largely an arid country, has only four recorded colonies of *Lithops*, namely *L. leslei* in a south-eastern corner of the country (Cole and Cole, 2005). This may be explained by the relatively low number of plant species in Botswana (Maggs *et al.*, 1998). However, there is also the possibility that the flora in this country has not been sufficiently well studied.

Four species occur in both South Africa and Namibia, namely *L. herrei, L. fulviceps, L. dinteri* and *L. julii* (Cole and Cole, 2005). *Lithops ruschiorum*, for which there are unconfirmed records as far north as just south of the Kunene River in Namibia, may also occur in southern Angola. A number of taxa have very restricted distributions, namely *L. amicorum, L. werneri, L. pseudotruncatella* subsp. *volkii* and subsp. *archerae, L. francisci* and *L. hermetica,* while other taxa have much larger distribution areas, such as *L. ruschiorum, L. gracilidelineata* and *L. vallis-mariae.*

Of all the plants with a sunken growth in the Aizoaceae family, *Lithops* has colonized the widest range of climatic conditions (Hartmann, 2006). According to the distribution map in Cole and Cole (2005), some 14 *Lithops* taxa occur within the Succulent Karoo Biome (Fig. 18), which is also one of the two centres of endemism in Namibia, with the Kaokoveld centre of plant endemism being the largest in terms of geographical coverage (Maggs *et al*., 1998). However, unlike most other members of the family Aizoaceae, which radiated within the Succulent Karoo (Klak *et al*., 2003), more than half of the species in the genus *Lithops* occur outside the Succulent Karoo region (Fig. 18), suggesting that the succulent Karoo may not be the centre of diversity for *Lithops*. The reason is probably that *Lithops* are adapted to a wide range of edaphic habitats in areas with both large-scale and small-scale habitat heterogeneity, which are repeated many times at a smaller scale over the entire distribution area. However, species richness for *Lithops* is highest along the lower Orange River, or the Gariep Centre (Kellner *et al*., 2011), which is included in part of the Succulent Karoo and the Nama Karoo biomes and hosts at least 11 *Lithops* taxa on either side of the Orange River.

The distribution ranges of a number of taxa are relatively discrete, such as *L. optica* and *L. herrei.* There are, however, many species with overlapping ranges in both Namibia and South Africa. In Namibia the ranges of *L. ruschiorum* and *L. gracilidelineata* overlap, as well as *L. karasmontana* and *L. schwantesii, L. gesinae* and *L. schwantesii, L. karasmontana* and *L. francisci,* and *L. julii* and *L. dinteri*. Nonetheless, different species seldom grow together in mixed colonies. There are instances where colonies of different species or subspecies grow in very close proximity to one another, with no evidence of hybridisation. Examples include *L. francisci* and *L. karasmontana*, *L. gesinae* and *L. schwantesii*, *L. karasmontana* and *L. schwantesii.* These observations are supported by herbarium specimens and photographs and observations by researchers (Cole and Cole, 2005).

The centre of diversity for *Lithops* appears to be the combined area of the Succulent Karoo and Nama Karoo Biomes in Namibia and South Africa but the genus has a much larger geographic distribution area, equalling approximately 1.3 million km² (Cole and Cole, 2005). Hartmann (2006) reports that the genus is difficult to handle in a hierarchical taxonomic system because all states of speciation are present as a result of the large distribution area and the widely different climatic conditions.

*Figure 18a.* Large map of African countries indicating Namibia (from wikitravel.com, 2018)

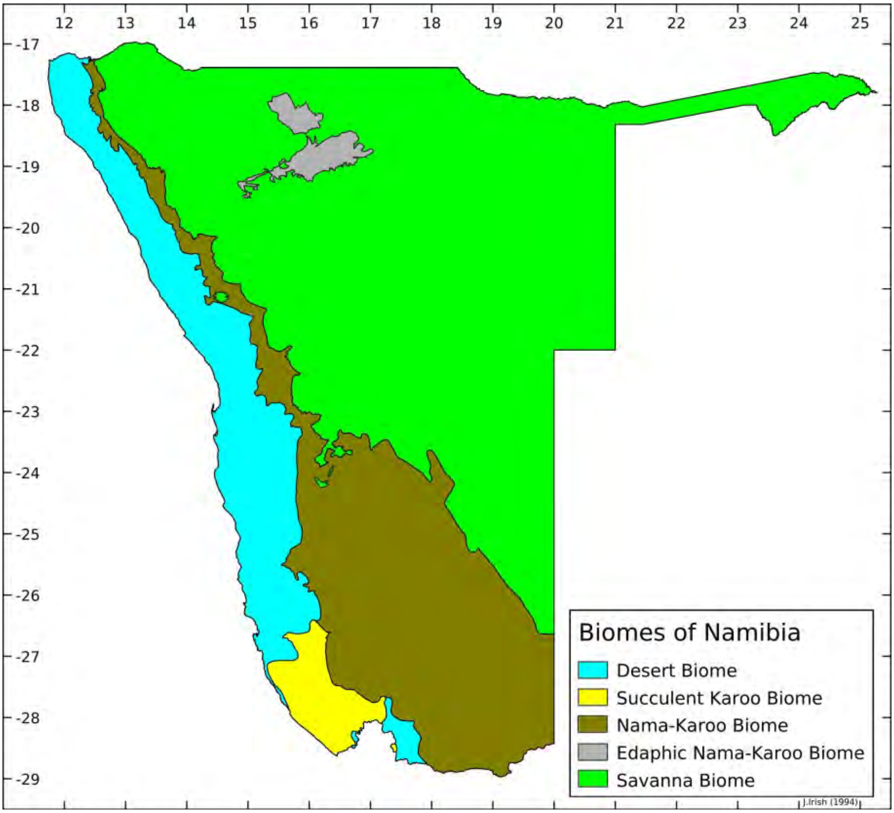


*Figure 18b.* Biomes of Namibia (Irish, 1994).

*Figure 18c.* The Nama Karoo Biome.

*Figure 18d.* The Succulent Karroo Biome.

*Figure 18e.* The Desert Biome.

*Figure 18f.* The Savanna Biome.

### 1.3.2 Ecology and habitats

*Lithops* inhabit a wide range of micro-habitats that are often characterised by a high proportion of gravel to soil. A substrate with at least 50% gravel and pebbles allows the plant bodies and shallow roots to become safely embedded. A high gravel to soil ratio also lowers the competition from many other plant species. In addition, leaves of *Lithops* plants are adapted to mimic the surrounding stones. However, many of these microhabitats are extremely fragile and often occur on scree slopes. Some contain dense communities of various lichens or of other succulent genera such as *Anacampseros* L. (Portulacaceae), *Crassula* L. and *Tylecodon* H.R.Tölken (Crassulaceae). It is conceivable that *Anacampseros albissima,* another remarkable desert plant, may be a competitor to *Lithops* in these habitats, since they usually occur in higher densities compared to *Lithops*, with leaves reduced to scales and a large underground storage organ. This enables them to withstand extended dry periods, whereas preliminary research suggests that *Lithops* populations start to decline after 3 or 4 years of poor rainfall (Paper IV). Unpublished data from closely monitored populations of *L. herrei* by staff from the Richtersveld National Park in South Africa seems to support these findings.

Eller and Ruess (1982) investigated the role that soil plays in preventing water loss and found that plants of *L. leslei* and *L. karasmontana* that are embedded into the soil, have a reduced rate of transpirational water loss. Not surprisingly, the plants are protected by the soil that surrounds the cone of the leaf, and many of the soils that *Lithops* inhabits in Namibia have a reasonably high loam or clay content.

*Lithops* do not always survive in soil that was removed from their habitat (Cole and Cole, 2005), and this may be an indication that at least in certain habitats, they do not colonise disturbed soil. This seems to be confirmed in at least one habitat where the topsoil was disturbed at a mine site by bulldozers some 30 years ago, and although the site is inhabited by a very dense population of *L. ruschiorum,* they have not re-colonised the bulldozer tracks (Loots, 2011).

There is no doubt that habitat and microhabitat are crucial in determining where *Lithops* are able to survive and thrive. A combination of factors make a habitat suitable for *Lithops*. For example in the case of *L. pseudotruncatella,* which occurs in the highland savannah vegetation where there is a relatively high annual rainfall, the plants require dense gravel habitats on rises or slopes and will not survive in depressions where water can collect and stand for several days.

By far, most of the recorded *Lithops* species in Namibia occur in white quartz gravel, followed by other kinds of gravel with a light colour, such as pegmatite, feldspar or quartz. The reason for this may be that white quartz does not gain as much heat during the day compared to rock types with a darker colour, and therefore cools off faster after sunset. This in turn would allow any moisture in the air to condensate during the night. Von Willert *et al*. (1992) as cited by Schmiedel *et al*. (1999), showed that the reflectivity of quartz was considerably lower than that of brown shale or red soil environments, and that this was correlated with differences in leaf temperature of plants growing inside and outside the quartz fields, respectively. On Rössing Mountain, situated some 40 km inland from the coast, *L. ruschiorum* grows in darker substrates (gneiss). Most likely, this is possible only because this population occurs within the high fog zone that receives fog precipitation on a regular basis, i.e., between 37 and 87 days per year, from cool fog-bearing winds from the southwest in the morning and the NNE in the late afternoon (Seely and Henschel, 1998) and the air humidity is still relatively high here (Hachveld and Jürgens, 2000). Fog and fog drizzle precipitation in 1999 was measured at more than 60 mm for this part of the desert. Furthermore, the cool south-west winds blowing against the mountain, may also help to prevent excessive evaporation of moisture from plants because the gravelly substrate does not heat up as much as it would in the absence of these winds. Populations of *L. ruschiorum* recorded further away from the coast, occur predominantly in light-coloured substrates like feldspar and quartz but can thrive in a variety of rock types provided that the climate is suitable, i.e., as long as there is enough moisture to sustain the plants.

Conversely, *Lithops hermetica* grows in pockets of off-white calcrete, grey limestone and calcrete gravel on dark grey limestone tables, some 90 km from the coast in extremely dry conditions. This is apparently one of the harshest environments, where fog does not reach, and rainfall is extremely low, being part of the Succulent Karoo. So what makes this habitat suitable for *Lithops*? It may be the ultimate test of survival.

There seems to be a threshold for associations with grass species. *Lithops* can co-exist with a certain density of grass cover and sometimes grow at the bases of perennial grasses. This could be beneficial because perennial grasses have a more extensive root system that penetrates deeper into the soil than that of *Lithops*. However, if the density of annual and perennial grasses becomes excessive, *Lithops* can no longer compete for space and other resources especially if the habitat is suitable for a wider range of vegetation. The depth of the roots almost certainly plays a role. Annual grasses may be more important as competitors to *Lithops* since they also have a shallow root system.

Dwarf succulents do not inhabit all the potentially suitable habitats that are available to them (Ihlenfeldt, 1983) and *Lithops* is no exception. This is partially a result of their wash-out capsules that prevents long-distance seed dispersal. Possibly species with this mechanism may fit the model for metapopulation dynamics, in which there are many patches within the species distribution area, some of which are still inhabited, some of which were inhabited but where the species has become extinct, and some of which are still vacant (Hanski and Gilpin, 1997). A mere patchy distribution does not qualify, and it has not yet been investigated whether *Lithops* or any other species in Aizoaceae may fit this ecological concept.

## 1.4 Plant physiology

Cockburn (1974) found strong evidence of Crassulacean Acid Metabolism (CAM) in *Lithops bromfieldii* var. *insularis.* At night, when it is cooler and the relative air humidity (RH) is higher, stomata are opened in CAM plants and $CO_2$ is fixed into malic acid. During the day, malic acid is decarboxylated and the released $CO_2$ becomes re-fixed without photorespiration by ribulose-1,5-bisphosphate-carboxylase (rubisco) into glycerate 3-phosphate (3-PGA) of the Calvin cycle, which then leads to formation of sucrose, starch, and other photosynthetic products (Salisbuy and Ross, 1992).

Eller and Ruess (1982) performed controlled experiments on transpiration and water uptake to study the effects of embedding of *L. karasmontana* plants into the soil. They were able to show that embedded plants are indeed protected from desication through the cone mantle surface and also by the soil around the mantle. Plants that were exposed to free air increased their transpiration rates with decreasing RH but water uptake did not take place at the same rate. By contrast, plants that were embedded took up water at the same rate as transpiration with decreased RH. Similar results were obtained when the experiments were repeated with *L. leslei.*

Nobel (1989) tested the thermal tolerance of leaf tissue of two *Lithops* (*L. hookeri* and *L. leslei*) and two *Haworthia* Duval species using a simulation model and found that the chlorenchyma cells were slightly more tolerant of temperature extremes than parenchyma and could withstand simulated temperatures of up to 68 °C for an hour. According to Nobel (1989, 1988 cited in 1989) the only vascular plants that can tolerate higher chlorenchyma temperatures than the tested *Lithops* and *Haworthia*, are agaves and cacti, where nearly half of the 32 species tested could withstand 70 °C for 1 hour.

Turner and Picker (1993) investigated the thermal environment of two *Lithops* species, namely *L. comptonii* and *L. gracilidelineata,* to determine whether leaf and soil temperatures are related, how window clarity influences leaf temperature and if variation in leaf colour has any effect on leaf temperature. They concluded that soil properties, such as a change in conductivity and absorbance of radiation, have a very strong influence on *Lithops* mantle temperature. There was no correlation between the substrate reflectivity and leaf temperature but for *L. gracilidelineata,* the leaves took longer to cool down than the surrounding soil. However, soil surface, leaf surface and leaf interior temperatures were never significantly different. High soil temperature will therefore lead to high leaf temperature on the surface and because there is a steep vertical gradient in soil temperature, the leaf interior will also be cooler with increasing soil depth. From their experiments, it appears that the variation in facial colours has no significant effect on the temperature of the leaf face or mantle. Lastly, the clarity of the window does affect the temperature of the interior part of the leaf. When the window is opaque such as for *L. gracilidelineata* and therefore transmittance of light is limited, the surface temperatures are higher than those of the leaf interior. When the window is open and transparent, such as for *L. comptonii,* the leaf interior becomes warmer than the surface. It must be pointed out that the assumption by Turner and Picker (1993) that all *Lithops* leaves are completely embedded in the soil is wrong. At least seven species have a significant proportion of plants that are noticeably elevated at least some of the time (Cole and Cole, 2005; pers. obs).

Egbert *et al*. (2008) analysed the influence of epidermal windows on the light environment inside the leaves of six succulents, including *L. olivacea,* using an omnidirectional fibre optic micro probe. The amount of light was expressed as the ratio of internal to external (incident) light. They found that the quantity of light at various depths below the windows on the adaxial surfaces of the leaves are highly variable, and that light quality and quantity inside the leaves did not correlate with the growth habit of the plants, the size of the window (as a proportion of the total leaf area) or the location inside the leaf; the spectral quality of light inside the leaves reflected the absorption properties of chlorophyll with most of the photons in the green wavelengths. The incident light at nearly all wavelengths decreased with increasing depth inside leaves of all species but the degree of reduction with depth vary among species. The maximum incident light of primarily green wavelengths around 550 nm, inside the leaves of the subterranean species ranged from 0.7 to nearly 1.5 at 0.2 cm, immediately below the window margin, indicating that nearly all the incident light penetrated into the leaf tissue adjacent to the window margin. Relative to the irradiance incident on the leaves, reduction in light levels of the hydrenchyma in *L. olivacea* was over 70%. Also, very little light penetrated to the deepest chlorenchyma of the leaves in *L. olivacea* as well as *Haworthia truncata*, another submerged dwarf succulent. Amounts of near-infrared radiation just beneath the windows in the leaves as well as in the central hydrenchyma always exceeded incident levels of infrared radiation impacting on the leaves. Near infrared radiation levels decreased at increasing tissue depths in *L. olivacea* and four other species, which could be the result of absorption or progressive loss of radiation through light scattering. Also, values of incident light in the near infrared region of the spectrum well above 1 in these succulents, and up to nearly 10 below the window, emphasize the high degree of light focusing and absorption of thermal energy by the succulent leaf tissue of these species. When they applied reflective tape to cover the windows, no differences in quantity or quality of light was observed just inside the window margin or at the chlorenchyma at the sides of the leaf, although light levels were reduced in the central hydrenchyma (water-storage parenchyma) of these leaves in *L. olivacea*.

Field *et al*. (2013) explored the availability of light in various parts of the plant body of *L. aucampiae* and discovered that accumulation of flavonoids in the upper parts of the leaves blocks sunlight and aids in protection from harmful UV radiation. This effect is mediated by the windows which allow sunlight to penetrate to the photosynthetic tissues deeper in the plant body. They also discovered that an increased concentration of chlorophyll a, and a greater chlorophyll a:b ratio in above-ground leaf parts, enable maximum use of incoming light for photosynthesis. By contrast, inverted conical epidermal cells in the below-ground parts of the leaf increased chlorophyll b and reduced chlorophyll a:b to ensure maximum absorption under low light levels. These findings were described as an unprecedented physiological flexibility in a xerophytic plant and also appears to be unique to Aizoaceae.

## 1.5 Domestication, cultivation and uses of *Lithops*

Among the dwarf succulents, *Lithops* constitutes a unique group of plants and their special morphological features give them unusual horticultural appeal among succulent enthusiasts. This appeal has been evident since the discovery of the first species (Cole and Cole, 2005; Hammer, 1999).

Most botanical gardens, that have a succulent plant section, often display a number of *Lithops* for educational purposes, e.g., the Royal Botanic Gardens Kew, UK, Botanic gardens in Kirstenbosch, South Africa, the Botanical Garden in Lund, Sweden, the National Botanic Garden in Windhoek, Namibia etc. Most *Lithops* in cultivation are found in commercial nurseries and private collections, however. Medicinal uses have been recorded for *L. lesliei* (Smith and Crouch, 1999) and it was listed as Near Threatened (NT) for this reason (Raimondo *et al.*, 2009). *Lithops* is also known by several tribes in South Africa to be edible (Cole and Cole, 2005) and at least one farmer in Namibia reported that the Nama tribe in southern Namibia consume *Lithops* plants fresh (personal communication).

Some of those who study and keep *Lithops* aim to maintain the taxonomic integrity while others have domesticated the genus in the sense of selecting for rare forms, aberrant colour forms (ACFs) and patterns, and some have also produced hybrids (Hammer, 1999; Cole and Cole, 2005). Natural ACFs are very popular among hobby breeders and are frequently reproduced by seed propagation and distributed as cultivars or hybridised with other ACFs to produce even more spectacular colour forms (Hammer, 1999). There are currently many registered cultivars that were produced by dedicated breeders, among whom Y. Shimada from Japan is probably one of the best known.

The cultivation of *Lithops* requires some specialised knowledge of the genus and most beginners lose their plants through over-watering. However, with correct handling, *Lithops* can be successfully grown indoors in most parts of the world, given sufficient light. Getting the plants to flower in cultivation requires a strict watering regime and this can generally be achieved some three years after germination. Much has been written about the cultivation of *Lithops* (e.g. the Mesemb Study Group Bulletin series from 1986 to date; Cole and Cole, 2005; Hammer, 1999). Kurt Dinter was probably the first botanical explorer to grow and export Namibian *Lithops* seeds and live plants to Germany not long after the discovery of *L. pseudotruncatella* in Namibia. Schwantes (1957) reports of the availability of *L. pseudotruncatella* from specialist firms, and since Dinter discovered this species (Cole and Cole, 2005) it is most likely he who distributed seeds of this species from Namibia to Germany. Wilhelm Triebner followed Dinter, also collecting many plants and seeds, and exporting these from Namibia mainly to the USA. Nowadays, many websites describe how to cultivate *Lithops* under a particular set of conditions. Seeds and living plants of every taxon are available online as well as from some South African botanic gardens and succulent nurseries from across the globe and still there is a demand for seeds and living plants from wild populations.

## 1.6 Conservation

### 1.6.1 Threats

Succulent plants appeal to collectors, gardeners, growers, landscapers, nurserymen and tourists. Both historically and presently, illegal collecting of live plants and seeds from wild populations is probably still the most acute threat to the survival of *Lithops* populations. Before national legislation was passed to protect plants, collectors took hundreds of specimens from several populations at a time, either for sale or for personal use, especially after the discovery of a new taxon, already causing concern to the environmentalists of the day (Cole, 1988; Hammer, 1999). Jenkins and Oldfield (1992) noted that collection of succulents from the wild is a significant threat to the survival of wild populations. Generally, plant collectors fall into two categories; those who are happy to buy reliable, verifiable material from a legitimate source and those who are only satisfied with material that is collected directly from wild populations, regardless of whether their actions could cause a population or even a species to go extinct.

Apart from their horticultural appeal, *Lithops* have several other characteristics that render them especially vulnerable to threats such as over-harvesting and habitat destruction or disturbance: (1) they occur in specialized habitats and very often in relatively small patches, with some species such as *L. gesinae* in particularly isolated patches and very small populations; (2) they are relatively slow-growing and take approximately two to three years to reach maturity (Cole and Cole, 2005); (3) their cryptic nature makes them vulnerable to construction projects because they may be overlooked in environmental impact assessment (EIA) processes. More accessible populations depend on remaining undetected to survive unscrupulous collectors, or by having some other kind of protection. The sudden and permanent destruction of approximately 50% of a *L. pseudotruncatella* population in 2015 took place as a secondary effect of a road construction project (Loots, personal observation).

This could possibly have been mitigated if the importance of this site had been more efficiently communicated to local authorities and land-owners.

Initial interest in *Lithops* was paid by European collectors, but was later followed by those from the Far East and the USA. In more than a hundred years, interest in *Lithops* has not waned nor does it seem likely to. On the contrary, a number of collectors, who have compromised their own collections through breeding and hybridisation, have indicated an interest to collect authentic material from wild populations to start new collections. There is currently a growing concern in Namibia as Asian markets are demanding dwarf succulents from Asian businessmen residing in Namibia, but the impact of this demand on wild populations has not yet been determined. However, there is a similar demand for rhinoceros horns from the same part of the world and the devastating impact is plain to see, with 191 rhinos lost from Namibia in just three years: 2015–2017.

Other major threats to wild *Lithops* populations are habitat destruction (Loots, 2011, 2017; Jainta, 2017), followed by predation by insects, rodents, large birds and mammals (Cole, 2005; Loots, 2011).

Preliminary monitoring results suggest that *Lithops* may also be susceptible to long-term droughts. The future effects of modeled climate change scenarios on dwarf succulents in Namibia seem to be unresolved (Musil *et al*., 2005) but secondary effects of changing weather patterns should be included in these models, such as increasing predation on dwarf succulents as a result of prolonged droughts, irrespective of whether the plants themselves may be able to endure prolonged dry periods (Papers II and IV).

A shortage of human and financial resources is a towering threat to the conservation of *Lithops* and biodiversity as a whole because the collection of basic demographic data is of paramount importance to monitor changes in populations over space and time. The extent of threats and other population variables in *Lithops* populations must be verified on the ground. In situ observations as well as meaningful experimental data are needed for formulation of effective and scientifically reliable conservation plans (Schmiedel *et al*., 2012). The aftermath of Namibia’s current economic depression (from around 2016) is likely to have a negative impact on plant conservation over the next few years, and this may leave many populations exposed to a variety of threats.

### 1.6.2 Red List classification and current conservation status of *Lithops* in Namibia

Currently, the IUCN Red List classification system (IUCN, 2001) of the International Union for the Conservation of Nature is the most widely used system in the world to assign species to a category of threat. The system is designed purely to assess the risk of extinction for a particular taxon and has been used to assign some of Namibia's plant species to a conservation status for more than three decades: Hall (1980), Hilton-Taylor (1996, 1997), Oldfield *et al*. (1998), Walter and Gillet (1998), Craven and Loots (2001), Loots (2005) and the status given in Klaassen and Kwembeya (2013).

Hilton-Taylor (1996, 1997) assessed 16 Namibian *Lithops* taxa, most of which he classified as Rare (R), a category which the IUCN no longer uses, but which was subsequently re-introduced in South Africa (Raimondo *et al*., 2009) and adopted also by Namibia for national use (Craven and Loots, 2001; Loots, 2005). Craven and Loots (2001) assessed 24 *Lithops* species and subspecies using the 1994 categories and criteria (IUCN, 1994) while Loots (2005) reassessed the same taxa in order to include them in the first Red Data Book for plants in Namibia and because the IUCN had adopted a new set of criteria (IUCN, 2001).

Correct assignment of plant species to the different categories of threat is reliant on ample information on the type of threats and their impact, the size of the distribution range, number of populations, number of mature plants, condition of the habitat and whether there have been declines in the habitat quality and size or number of plants. Unfortunately, most of this information is rarely available. Most of the assessments of Loots (2005) were based on information from herbarium specimens and literature, which allows one to work out the distribution range and possibly the number of populations. Changes in status assigned to taxa since then, are mainly due to availability of additional data and not because the situation of the taxon has improved or deteriorated. *Lithops* is a high-profile group of plants and because of the recent attention paid to the genus, new contacts made and field work conducted, a considerable body of new data has become available for six taxa, including the recently described *L. amicorum* (Table 2). The 2001 categories and criteria are used in conjunction with the guidelines for using the categories and criteria (IUCN, 2017).

Table 2. *Conservation status of each Namibian Lithops taxon in 2005 (Loots, 2005) and currently (Red List database, 2018). Categories and criteria: DD = Data Deficient; EN = Endangered, VU = Vulnerable (EN and VU are two of the threatened categories); LC = Least Concern; NT = Near Threatened; R = Rare; AOO = Area of Occupancy; EOO = Extent Of Occurrence; D1 = <1000 mature individuals; D2 = AOO < 20 km² or number of locations ≤ 5; A3cd = population reduction suspected to be met in the future based on a decline in the AOO, EOO and/or habitat quality, C2a(i) = number of individuals <10000 and an inferred continuing decline and the number of mature individuals in each subpopulation ≤1000.*

| Species | National status (2005) | National status (2018) | Reasons for change or no change |
|---|---|---|---|
| *L. amicorum* | Not Evaluated (NE) | R | Recently discovered and described; very small AOO, known from <5 locations; believed to be stable |
| *L. dinteri* subsp. *dinteri* | LC | R | Very small AOO, less than 10,000 individuals, habitat specialist; believed to be stable |
| *L. dinteri* subsp. *multipunctata* | VU D2 | VU D2 | Very small AOO, known from ≤5 locations with a plausible threat of illegal collecting |
| *L. fulviceps* | LC | LC | Stable but information is lacking for most populations |
| *L. francisci* | VU D2 | VU D2 | Very small AOO, known from < 5 locations with a plausible threat of illegal collecting; status could rapidly change, should be closely monitored |
| *L. gesinae* | LC | R | Very small AOO, less than 10,000 individuals, very small populations, habitat specialist; not threatened but should be monitored. |
| *L. gracilidelineata* subsp. *brandbergensis* | NT | NT | Assessment needs revision but information is lacking |
| *L. gracilidelineata* subsp. *gracilidelineata* | LC | LC | Believed to be stable |
| *L. hermetica* | VU D2 | VU D2 | Small EOO and AOO; Tsau //Khaeb Natonal Park is opening up to tourism |
| *L. herrei* | LC | VU D1 + D2 | Single, very small population now potentially threatened by activities related to mining |
| *L. julii* subsp. *julii* | NT | NT | Problems with illegal collecting but species may not be threatened as a whole |
| *L. karasmontana* subsp. *bella* | LC | LC | Assessment needs updating but information is lacking for most populations |
| *L. karasmontana* subsp. *eberlanzii* | LC | LC | Believed to be stable but information is lacking for most populations |

| Species | National status (2005) | National status (2018) | Reasons for change or no change |
|---|---|---|---|
| *L. karasmontana* subsp. *karasmontana* | LC | LC | Believed to be stable |
| *L. optica* | NT | NT | Assessment needs updating but information is lacking for many populations |
| *L. pseudotruncatella* subsp. *archerae* | VU D2 | VU D1 + D2 | Single, very small population, Small AOO and EOO |
| *L. pseudotruncatella* subsp. *dentritica* | NT | NT | Status may need to be revised but additional information is needed |
| *L. pseudotruncatella* subsp. *groendrayensis* | VU D2 | VU D2 | Small AOO; vulnerable to illegal collecting and habitat destruction; current listing is appropriate |
| *L. pseudotruncatella* subsp. *pseudotruncatella* | LC | VU A3cd; C2a(i) | Decline in number of populations and mature individuals and continuing decline in AOO due to habitat destruction and erratic weather patterns |
| *L. pseudotruncatella* subsp. *volkii* | VU D2 | EN D | Single population, very small AOO, declining number of mature individuals; threatened by illegal collecting |
| *L. ruschiorum* | LC | LC | Several populations severely disturbed or destroyed, small continuous decline in mature individuals; not threatened with extinction but should be continuously monitored |
| *L. schwantesii* subsp. *gebseri* | VU D2 | DD | Single population with small AOO but exact location unknown; not observed for at least 20 years, may be extinct |
| *L. schwantesii* subsp. *schwantesii* | LC | LC | Believed to be stable although some populations may be exposed to illegal collecting. |
| *L. vallis-mariae* | LC | VU C2a(i) | Continuous decline in number of mature individuals; habitat destruction in some populations, all plants recently removed from a monitoring square. |
| *L. werneri* | VU D2 | VU D2 | Single population, extremely small AOO; illegal collecting could drive this species to extinction in a very short time. |

According to Table 2, 10 Namibian taxa are currently threatened with extinction of which five have remained unchanged (VU D2), three taxa have become threatened with extinction of which one has changed to a higher status of threat (*L. pseudotruncatella* subsp. *volkii*) and one taxon only had its criteria changed (*L. pseudotruncatella* subsp. *archerae* changed from D1 to D1+D2). Four taxa were classified as NT and should therefore be closely monitored. Three taxa were classified as R, indicating that they have a very limited distribution and/or small population size but are currently stable. One taxon is listed as DD and cannot be assigned to an IUCN category. The rest of the taxa are listed as LC, indicating that there is no evidence at present to show that they are facing a risk of extinction. In South Africa, an additional 10 *Lithops* taxa are listed under one of the threatened categories (Raimondo *et al*., 2009).

Imminent risk of extinction is only one of the factors that have to be considered when conservation decisions are made. Pfab and Scholes (2004) have shown that collection of a single live plant per year from a population of less than 1000 mature individuals of *Aloe peglerae*, leads to a dramatic decline in population size and thus a high risk of eventual extinction. These results are relevant also for *Lithops* since most populations in Namibia have fewer than 1000 plants, and they have more or less the same life span range as *Aloe peglerae*, which lives up to 60 years [up to 95 years for *L. optica* according to Schwantes (1957)]. Other factors that should be considered for conservation action are endemism, wild crop relatives, useful species, ecosystem services, keystone species, fodder species or any species that are deemed to be important.

### 1.6.3 Conservation actions for *Lithops* in Namibia

#### ***International conventions and national legislation, policies and strategies***

Namibia has ratified the Convention on Biological Diversity (CBD) and therefore has an obligation to achieve the goals of the Global Strategy for Plant Conservation (2011–2020), the Aichi Targets and the National Biodiversity Strategy and Action Plan II (NBSAP II), which runs from 2013 to 2022. The latter programme has however failed to obtain the necessary buy-in from some of the crucial role-players due to insufficient consultation, and was vastly under-budgeted, resulting in many of the targets being under-achieved or unachieved by the end of the target period. In spite of this, some of the targets are well on track and have been achieved partially or can still be achieved partially, for example Target 4: “By 2022, the rate of loss and degradation of natural habitats outside protected areas serving as ecological corridors or containing key biodiversity or providing important ecosystem services is minimized through integrated land use planning.” The 6th national report for the CBD recognizes that Namibia has areas with high levels of species endemism and diversity, and that they are threatened to various extents by economic, demographic, and social pressures. Target 4 states: “The underlying causes of the loss of such habitats must be addressed through an integrated approach to development which includes integrated land use planning, the mapping and protection of key biodiversity areas and expert working groups on sensitive biodiversity areas.” This, however, is a long way from happening for indigenous plants. The use of environmental impact assessments (EIAs) and strategic environmental assessments to guide development decision making is also cited as a tool to prevent the loss of high biodiversity value habitats, but this process needs a major overhaul before it will work to the advantage of sensitive biodiversity areas. Most EIAs are currently conducted without the benefit of any field work, and if field work is involved, it is usually conducted in the dry season when it is difficult to ascertain what occurs in the target area.

Still, the conservation of dwarf succulents such as *Lithops* can benefit from Target 4 if specific areas are identified and actively protected such as the preliminary Important Plant Areas (IPAs) that were identified already in 2004. These should be further developed and refined. A single *Lithops* species could possibly gain importance if it is combined with other types of biodiversity such as birds, reptiles and mammals or with ecosystem services such as the aesthetic value of the “area”, e.g., the very attractive scenery where *L. werneri* grows.

Vision 2030 is a policy framework for long term national development and addresses biodiversity and the sustainable use thereof. National Development Plans are 5-year plans under Vision 2030 that are budgeted for by the government and destined to reduce poverty and unemployment by ensuring a national, sustainable plan for all economic sectors. While Vision 2030 aims to alleviate poverty by, e.g., sustainable use of natural resources, many obstacles along the way make it unlikely that the development goals are achieved by 2030, unless conservation goals can be aligned with development goals.

The genus is not listed on any CITES appendices, largely owing to a paucity of international trade data.

The Nature Conservation Ordinance (NCO) 4 of 1975 is currently the only national legislation that protects *Lithops* by prohibiting the illegal collecting and sale of live plants and seeds. Unfortunately this legislation is extremely outdated and the enforcement of this law is very poor as far as plants are concerned.

The new “Protected Areas and Wildlife Management Bill” residing under the Ministry of Environment and Tourism promises to address more gaps in the protection of indigenous plants in their natural habitats. As part of this bill, a list of Specially Protected Plants and a list of Protected Plants were drawn up to replace the NCO 4 of 1975. All *Lithops* taxa appear on either one of the two lists. However, the actual protection of populations in situ would still depend on law enforcement, which is a challenge that cannot be met by the staff of the Ministry of Environment and Tourism alone, given the vast tracts of land to be patrolled.

Combined efforts were made by two ministries in the past to make custom officials aware of illegal transport of small succulents by designing posters with pictures of succulents that may not be exported without permits. The success of this method is questionable at best since many of these succulents are very small and their seeds minute, and they are therefore easily concealed.

### *In situ conservation*

Current efforts to conserve *Lithops* populations in their natural habitat in Namibia can be divided into passive and active conservation. Passive conservation refers to plant populations that occur naturally in national parks that were designed and designated to protect animal diversity. Populations of *L. ruschiorum*, *L. gracilidelineata, L. pseudotruncatella* subsp. *archerae, L. optica*, *L. francisci*, *L. hermetica*, *L. herrei* and L. *karasmontana* subsp. *eberlanzii* are protected in this way. A more active approach to their conservation would include the mapping of the locations of all *Lithops* populations within national parks and ensuring that park staff is aware of these locations. This procedure would aid in planning of construction or mining projects within park boundaries.

At present, actively conserved populations of *Lithops* are those that occur on private farmland of conservation-minded farmers. These farm owners strictly monitor access to the populations of *L. amicorum* and *L. werneri* and to some populations of *L. dinteri*, *L. fulviceps*, *L. gesinae*, *L. julii*, *L. karasmontana*, *L. pseudotruncatella*, *L. schwantesii* and *L. vallis-mariae*. The potential problem with this strategy is that when the farm changes hands, the new owner may not place the same conservation value on *Lithops*. This may be the reason that *L. schwantesii* subsp. *gebseri* can no longer be located.

The monitoring plots of *L. ruschiorum* within the license area of Rössing Uranium Limited (RUL) as well as the demarcated “no-go” area (Loots, 2011) were also actively conserved until the end of 2018, but are now facing an uncertain future since the China National Uranium Corporation has become the majority owner of Namibia’s Uranium mine industry. Populations of *L. gracilidelineata* within the license areas of two other uranium mines have an equally uncertain future, and the new majority stakeholders will have to be approached in order to ensure their continued protection.

### *Ex situ conservation*

The National Plant Genetic Resources Centre (Genebank) in Namibia currently holds a total of 8 accessions from 5 *Lithops* species. At least one accession from each species should be targeted for long-term storage in the genebank but this will take several years to achieve.

*Lithops* that grow in road reserves on national roads are vulnerable to illegal collecting, off-road driving and habitat destruction. *Lithops pseudotruncatella* subsp. *pseudotruncatella*, *L. pseudotruncatella* subsp. *groendrayensis* and *L. pseudotruncatella* subsp. *dendritica* are some examples of this scenario. These populations should be among the first to be targeted for seed collecting for ex situ conservation.

Namibia's policies on natural resources focus on "sustainable utilization". A natural resource such as *Lithops* should be utilized in a manner that ensures maximum benefit for the present generation but at the same time does not deprive the next generation of the possibility to use the same resource to the same extent. This automatically necessitates the continued existence of all the relevant taxa in their natural habitats. Presently, there is a need for making indigenous plants more widely available for sale in local Namibian nurseries. Despite the conservation concerns, seed can be harvested sustainably from the most secure populations in all the *Lithops* species, provided that this is carried out by the National Botanical Research Institute, which is the national plant authority and is qualified to collect seed without jeopardizing populations by taking too many seed capsules. The seed can then be distributed to interested, registered growers with an indigenous nursery permit.

Sustainable utilization may contribute to alleviating poverty in communities with few economic opportunities. *Lithops* can be raised from seed with very little input and there is considerable demand from local and international growers. An organised attempt to make authenticated (pure and of known origin) *Lithops* plants commercially available to the public may help to take pressure off wild populations and at the same time provide an income to marginalized rural communities. These communities could benefit by either growing stock for nurseries or by selling directly to the public.

A small number of commercial nurseries are already growing authentic *Lithops* for the succulent market, but anecdotal evidence indicates that soon they will not be able to keep up with the demand of new succulent traders who recently entered the country and are aiming to export to large Asian markets.

### *Long-term monitoring of populations*

Government and private conservationists are monitoring a number of *Lithops* populations to keep track of changes in the population parameters over time.

This information can then be used as an early warning system to prevent detrimental effects of poaching and certain other threats. Remote and less accessible populations are safer from being eroded by poaching. A more hands-on approach to long-term monitoring was taken in 2006 with the request from a local uranium mine (RUL) to conduct a survey to determine the strength of the *L. ruschiorum* population within the license area of the mine. This lead to a national survey of all *L. ruschiorum* populations that could be located as well as the setting up of long term monitoring plots and a monitoring plan (Loots, 2011). This kind of collaborative approach seems to be an effective way of monitoring *Lithops* in their natural habitat. Subsequently, monitoring plots were established for seven populations from four additional species. Monitoring all of these squares on a regular basis remains problematic due to a lack of resources, but collaboration was started with a privately funded citizen science conservation project, which focuses on cultivation and conservation of *Lithops*. The *Lithops* Research and Conservation Foundation is a private conservation organization that has been involved in the cultivation, conservation and monitoring efforts of *Lithops* for several years and has had some success in re-introducing plants in some of the dwindling populations.

### *Collaborative partners*

Monitoring of threatened populations is to an increasing extent carried out by volunteers. South Africa has a very successful programme coordinated by the South African National Biodiversity Institute (SANBI) to collect data on threatened plants populations. Results of their data collecting trips are published in the popular magazine CREW (Custodians of Rare and Endangered Wildflowers). Given the lack of financial and human resources often experienced in government organisations, using such volunteer groups or individuals to collect data on threatened species populations, also called citizen science, can be very valuable for gaining bio-geographic information for conservation (Devictor *et al.*, 2010), provided that the correct training is given. It would be to Namibia’s advantage to seek a similar solution to in situ data capturing and monitoring challenges for the Threatened Plants Programme in general and more specifically for *Lithops* populations. Citizen science is also becoming increasingly digitized with online applications such as the one developed by the Environmental Information System (EIS, www.the–eis.com/) in Namibia that allows naturalists to record biodiversity on their mobile phones by just clicking on drop-down menues. No applications have, however, as yet been developed for monitoring *Lithops* or other dwarf succulents, except for the specifically designed application for certain tagged populations including two populations of *L. optica* in the Tsau //Khaeb national park, as part of the new monitoring framework of this park.

Collaboration with non-governmental research organisations (NGOs) in order to gain long-term monitoring data and perform conservation projects, is a potential resource that has not been tapped into. The Namibia Chamber of the Environment (NCE) is an umbrella organization that acts as a protagonist and a forum for the greater environment sector, performs lobbying and raises funds, and therefore can act as a valuable conservation partner. The Gobabeb Training and Research Centre is an important research and conservation partner, which has conducted comprehensive research in the Namib Desert for over five decades and is currently involved in a project on *L. gracilidelineata*. The Namibia Nature Foundation has been a source of information on new *Lithops* localities while conducting their community work on *Commiphora*. Ideally students from higher education institutions should also be involved, especially in collection and analysis of data on *Lithops* populations. However, providing subsistence and travel budgets for them has been an impediment in making use of this valuable resource.

### 1.6.4 Conclusion

Namibia is making some progress towards protecting its succulent diversity, but this may not be sufficient to save enough *Lithops* populations for the next generation of Namibians. Stronger efforts are urgently needed to ensure that species remain intact in their natural habitat.

# 2 Objectives

## 2.1 Problem statement

The genus *Lithops* (Aizoaceae) is a subject of considerable conservation concern and needs to be protected from the most perilous threats. Delimitation of *Lithops* populations and estimation of plant abundance using standard ecological methods is challenging because of the spatial arrangement and cryptic nature of the plants. Limited information about population parameters and the weight of various habitat variables makes it difficult to assign an accurate conservation status for many of the taxa. Since *Lithops* taxonomy is based on only a few morphological features that may be connected to microhabitat variation, the genus could be over-classified. Clear delimitation of species as well as intra-specific taxa, is essential for assigning the limited conservation resources to preservation of taxa that are perceived to be under threat.

## 2.2 Hypotheses

1. The spatial arrangement of plants in populations of *Lithops* is severely clumped.
2. Habitat parameters related to the altitude, aspect, slope, soil type and substrate, are associated with plant abundance.

3 Long term changes in plant abundance are associated with some or all of the above-mentioned habitat variables.
4 Levels of heterozygosity and total number of alleles (as estimated with DNA markers) are higher in larger/more continuous populations compared to in small and/or severely isolated populations.
5 Genetic distances (as estimated with DNA markers) between populations are correlated with geographic distances as expected for outcrossing species.
6 DNA markers can reflect phylogenetic relationships in the genus, and identify taxa that could be merged in keeping with already noted morphological similarities.

## 2.3 Main objectives

1 To determine the nature of spatial arrangement in wild *Lithops* populations.
2 To determine the best method for estimating plant abundance in *Lithops* populations.
3 To determine habitat profiles in *L. pseudotruncatella* and *L. ruschiorum.*
4 To reveal possible associations between habitat variables and plant abundance in *L. pseudotruncatella* and *L. ruschiorum.*
5 To analyse level of genetic variation within and among populations of *L. ruschiorum,* and possible associations between genetic and geographic distances.
6 To investigate level of genetic variation within and among the different taxa of *Lithops* in Namibia.
7 To improve the taxonomic classification of *Lithops* in Namibia.
8 To improve conservation assessments of *Lithops* taxa in Namibia

### 2.3.1 Paper I Towards better risk assessment for conservation of flowering stones: plant density, spatial pattern and habitat preference of *Lithops pseudotruncatella* in Namibia.

***Specific objectives***

1 To establish the spatial pattern for plants in a *L. pseudotruncatella* population.
2 To identify the optimal method for estimating plant abundance in *Lithops.*
3 To determine the habitat profile of *L. pseudotruncatella* and investigate associations between habitat variables and plant abundance.

### 2.3.2 Paper II Distribution, habitat profile and genetic variability of Namibian succulent *Lithops ruschiorum.*

***Specific objectives***

1. To determine geographic distribution and plant abundance for *L. ruschiorum.*
2. To determine the habitat profile of *L. ruschiorum* and investigate associations between habitat variables and plant abundance.
3. To investigate a possible isolation-by-distance effect among populations of *L. ruschiorum.*

### 2.3.3 Paper III Genetic variation among and within *Lithops* species in Namibia.

***Specific objectives***

1. To estimate AFLP -based variability between and among 15 Namibian *Lithops* species.
2. To determine phylogenetic relationships among the AFLP -investigated *Lithops* taxa.
3. To perform the required taxonomic changes in the genus.

### 2.3.4 Paper IV Changes in plant abundance for the endemic succulent *Lithops ruschiorum* (Aizoaceae).

***Specific objectives***

1. To establish a network of monitoring plots across the distribution area of *L. ruschiorum.*
2. To determine changes over time in plant abundance of *L. ruschiorum.*
3. To assess possible associations between habitat variables and long-term changes in plant abundance in *L. ruschiorum.*

# 3 Material and Methods

## 3.1 Plant populations

### 3.1.1 A single population of *L. pseudotruncatella*

A single population of *L. pseudotruncatella*, situated on a plateau approximately 45 km southeast of Windhoek, was chosen for an in-depth analysis of plant abundance and habitat preferences (Paper I). In total, this population covers about 2.5 ha and it occurs on an east-facing slope with a very gentle gradient and a mean altitude of 1693 m. Within the area, there are gentle quartz rises. Situated within the savanna biome, this population receives approximately 300–360 mm rain annually, mainly in summer. There is no formal grazing management regime but small and large livestock are continuously present.

### 3.1.2 Ten populations of *L. ruschiorum*

The relatively widely distributed *L. ruschiorum* occurs along approximately 600 km of the Atlantic coast in Namibia, and reaches a maximum of 75 km inland. Twenty-one populations were identified based on the National Herbarium (WIND) specimen database, key literature (Cole, 1988a,b) and local experts (Papers II and IV). Field trips in 2006–2008 enabled location of nine of these populations to be used in field surveys of plant abundance and habitat preferences, establishment of permanent monitoring plots (8 populations) and sampling of seed capsules for DNA analyses in 2011 (6 populations), while yet another population was found and sampled for DNA analysis in 2012, bringing the total to 7 (Fig.19).

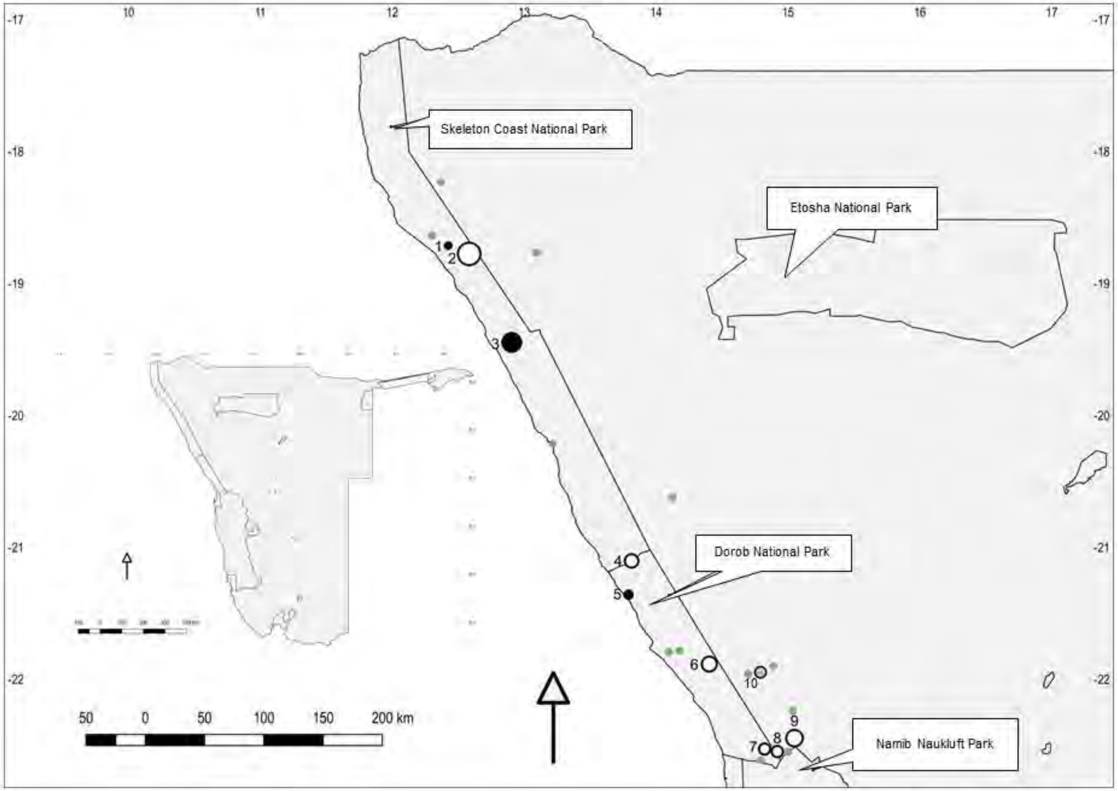


*Figure 19.* Distribution of surveyed *L. ruschiorum* populations. 1. View Point, 2. Khumib River, 3. Hoanib River, 4. Ugab River, 5. Ugab Salt Works, 6. Henties Bay–Uis Road, 7. Rössing Mountain, 8. Feldspar Ridge, 9. Rössing Uranium Limited license area (RUL), 10. Henties Bay–Usakos Road. Circle size 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 distribution area made known from various distribution records, is shown (green dots).

### 3.1.3 Fifteen species of *Lithops*

Information on previously recorded *Lithops* populations in Namibia was obtained from the WIND specimen database, key literature (Cole, 1988b) and local experts (Paper III). Field trips were undertaken from April 2011 to November 2012 to locate suitable populations and sample material for DNA analyses. A total of 41 *Lithops* localities (including the seven *L. ruschiorum* populations mentioned above) were successfully sampled in the field, representing 14 species and 6 additional subspecies according to morphology-based determinations in the field (Fig. 20). Where possible, the perimeter of each population was determined, and seed capsules were collected from at least 10 randomly selected plants across the geographic range of the population. A photograph was taken of each sampled plant for further identification and reference purposes. In populations with few seeds, a single fresh ½ leaf was collected from 10 multi-headed plants and stored directly in silica gel for DNA extraction. In the case of *L. werneri,* four capsules were collected from between the gravel in the single known population (representing <1% of the total number of plants in the population). Locality 10 was divided into two sites (populations 10a and 10b) since *L. karasmontana* subsp. *karasmontana* var. *immaculata* (type locality) and var. *karasmontana* were sampled on the same farm with just a few km between the two varieties. Seeds from the two varieties were collected and kept separately and this brought the number of populations sampled up to 42. For *L. fulviceps,* no seed could be found in the two populations that were visited. However, the same two populations were visited the previous year and herbarium collections were deposited in the WIND herbarium. A small number of seeds were therefore collected from one capsule of each voucher specimen for DNA analysis. This brought the number of species sampled to 15, and populations sampled to 44. Only one *L. gesinae* population could be visited and this was too small to be sampled and therefore left out of the analysis. A total of 28 voucher specimens were collected in populations where voucher specimens had not been collected in the past and these were deposited in the WIND herbarium.

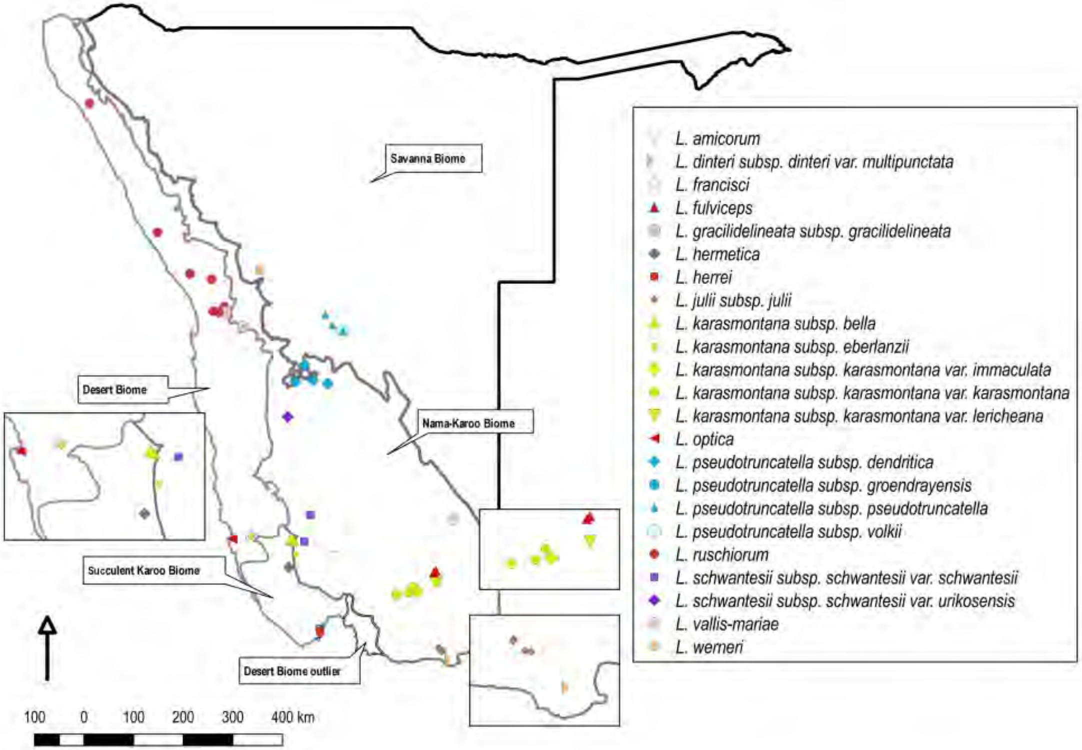


*Figure 20.* Geographic locations for the 44 analysed *Lithops* populations mapped on the biomes of Namibia, adapted from Irish (1994). Mapping done with QGIS version 2.18.25

## 3.2 Estimation of plant abundance and spatial pattern

### 3.2.1 The *L. pseudotruncatella* population

A square of 100×100 m (1 ha) was laid out with corners and 50 m intervals identified with a GPS. Iron droppers were used to demarcate each 50 m point in the hectare to facilitate field work. All *Lithops* plants were marked temporarily with numbered plastic markers (Mannheimer and Loots, 2012) in the dry season of June–July 2012, and counted to provide a census of the whole study area. These plants were then used as a basis for studying plant density, spatial distribution pattern and habitat characteristics. Seven different methods for estimating plant density were applied using the census-detected plants; (1) Nearest Neighbor (Cottam and Curtis, 1956), (2) Closest Individual (Cottam *et al.,* 1953), (3) Kendall-Moran (Kendall and Moran, 1963), (4) Ordered Distance Third Closest Individual (Morisita, 1957), (5) Variable Quadrant Plot (VQP) (Coetzee and Gertenbach, 1977), (6) Belt Transect (Elzinga *et al*., 1998) and (7) Adaptive Cluster Sampling (Philippi, 2005). Finally, a second census of all *Lithops* plants was carried out in the rainy season of February 2013. This time, the 1 ha study area was divided into 100 test plots of 10×10 m each. The locations of all detected *Lithops* plants were again marked temporarily with plastic markers and the number of *Lithops* in each test plot was counted.

In order to detect a possibly clumped plant distribution, a goodness of fit test was carried out to determine if the observed distribution of plants in the 100 test plots differed from the expected distribution of a population with randomly occurring individuals.

### 3.2.2 The *L. ruschiorum* populations

Due to the perceived clumped plant distribution of this species, population boundaries are difficult to define. The smaller area on which a group of plants occurred together on the same topographic feature such as a ridge, outcrop, slope or a gravel plain, was referred to as a “site”. A population was defined as consisting of a group of sites, often separated by unsuitable habitat, occurring at the same geographic location. Populations were separated by significant distances (minimum 10 km), unsuitable habitat and/or geographic barriers.

The most intensely studied population, RUL (Rössing Uranium Limited), occurs in the license area of a uranium mine. Here, a total of 68 sampling points were defined, 1 km apart in grid square format. Seven additional sampling points were placed in areas not covered by the grid but containing dense clusters of *Lithops*. Each sampling point was identified with a set of GPS coordinates. All *Lithops* plants observed in the vicinity of a sampling point, were temporarily marked with a coloured marker. When more than one site could be identified after marking all the plants found within a 500 m radius, a set of GPS coordinates were recorded in the centre of each site. 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 of 51 sites in total. The area of each site was measured using the track log function of the GPS, recording one set of coordinates every second. This area was then used to calculate the density of each site (number of plants/m$^2$). In each of the other eight successfully located populations, all *Lithops* plants were similarly marked using coloured markers, and the boundary for each of 43 sites demarcated with GPS. Plants were counted and density estimated as above. Spearman rank correlation analyses were performed to study associations among occupied area, plant number and plant density.

A total of 36 10×10 m (100 m$^2$) long-term monitoring plots were established in 2007 in each of 21 different sites at RUL and in 15 sites in 7 of the other populations. The four corners of each plot were permanently marked with an iron pole and the GPS coordinates were recorded in the centre. Plants inside the monitoring plots were marked with temporary markers and recorded as mature, damaged or juvenile. The plots at RUL were monitored again in 2008, 2010 and 2016, and plants (mature, damaged and juveniles) were counted and recorded. For the remaining seven populations, six plots (two at Rössing Mountain and four at Henties Bay–Uis Road) were monitored again in 2012, while all plots were monitored in 2016 except three (two at Khumib River and one at Hoanib River). A partial Spearman rank correlation test was performed to detect significant changes in plant number in the 33 re-surveyed monitoring plots.

## 3.3 Determination of habitat preferences

### 3.3.1 The *L. pseudotruncatella* population

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 (the compass direction that a slope faces) was determined with a compass; (3) gradient of the slope was measured with a clinometer; and (4) surface substrate was categorized as: 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 'substrate cover' was subjectively estimated as the percentage of the total area in the test plot that was covered by each of the six substrate types. In addition 'available habitat' was subjectively estimated as the percentage habitat in each test plot that was available to growth of *Lithops* plants. Twelve 1 L soil samples were collected from the uppermost 4–5 cm of the soil layer in the study area, and a 1000 μm sieve was used to separate the soil from stones so that percentage of stone particles in each sample could be determined.

A principal component analysis (PCA) was performed to determine the relationship between the number of plants per 10×10 m plot in the *L. pseudotruncatella* population, and the percentage cover of the different substrates in these plots as well as aspect, gradient and topography. Associations between the percentage cover of the different substrates and plant number was also investigated with the Spearman's rank correlation coefficient. For the 12 test plots where soil samples were taken, the Spearman's rank correlation coefficient was used to compare plant number with percentage stone particles in the soil.

### 3.3.2 The *L. ruschiorum* populations

For each of the 94 sites as well as for the 36 monitoring plots, the following habitat variables were recorded: altitude, aspect, slope (=gradient), soil texture and rock substrate (lithology). For the 43 sites in other populations than RUL, distance between the population and the coast was also recoded. A total of 30 soil samples (22 at RUL and one each in the other populations) were taken in different sites, and pH was determined using a Hanna microprocessor pH meter. All plant and habitat parameters were tested for normality using the Anderson Darling test and transformed into natural logarithms as needed.

Statistical analysis of plant and habitat parameters were carried out in three sets: (a) the 51 sites in the RUL population, (b) the 43 sites in the other 8 populations and (3) the 36 monitoring plots. Spearman rank correlation analyses were performed to study associations between plant number and plant density on the one hand, and the numerical habitat parameters altitude, slope and coastal distance (only for the 8 populations) on the other hand. Analyses of variance (ANOVA) were performed to study the impact of category habitat parameters (aspect, substrate and soil texture) on plant number and plant density, followed by Tukey pairwise comparisons. PCAs were performed to explore the relationship between plant number and most of the habitat parameters, using indicator variables for aspect, substrate and soil texture. 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. 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.

## 3.4 Analysis of genetic variation

### 3.4.1 Plant material and AFLP analysis

Up to 50 seeds per sampled plant were sown in pots in a greenhouse in Alnarp in Sweden, with 14 hours of light per day, and temperatures of 22–25 °C. When available, between 8 and 10 seedlings per population, each from a different mother plant, were sampled for DNA extraction after approximately one year of growth.

DNA was extracted from fresh or frozen leaf material with the DNeasy Qiagen DNA Plant Mini Kit. The samples were then submitted to standard AFLP procedure (Vos *et al.*, 1995) using four primer pair combinations. Polymerase chain reactions (PCR) were performed followed by automated detection of AFLP fragments. AFLP profiles were scored as presence (1) and absence (0) of fragments with a size of 80–300 bp. Bands which were detected in <75% of repetitions of the positive controls were deleted.

### 3.4.2 Data evaluations

The seven populations of *L. ruschiorum* were analysed for genetic variance within and among populations by analysis of molecular variance (AMOVA). Genetic diversity within populations was estimated as percentage of polymorphic loci and expected heterozygosity. Genetic variation within and among populations was evaluated with Principal Coordinate Analysis (PCoA) using Sørensen distances. Finally, an association between genetic and geographic distances among samples was investigated with a Mantel test.

For the 15 species, genetic diversity among and within species, infraspecific taxa and populations were displayed as percentage of polymorphic loci and expected heterozygosity. AMOVAs were applied to estimate the partitioning of genetic variance between taxa at different levels (among and within species, and among and within subspecies and varieties within species), and PCoAs were applied to reveal genetic similarities among and within these taxa. Genetic structure was assessed by Bayesian clustering with 2, 3 and 7 clusters, and a discriminant analysis of principal components (DAPC) with six clusters. Additionally, a neighbour-joining tree based on Nei-Li distances was calculated. Mantel tests were performed to analyse correlations between genetic and geographic distances among all taxa, and among taxa within species represented by several subspecies or varieties.

# 4 Results and Discussion

## 4.1 Plant abundance and spatial pattern

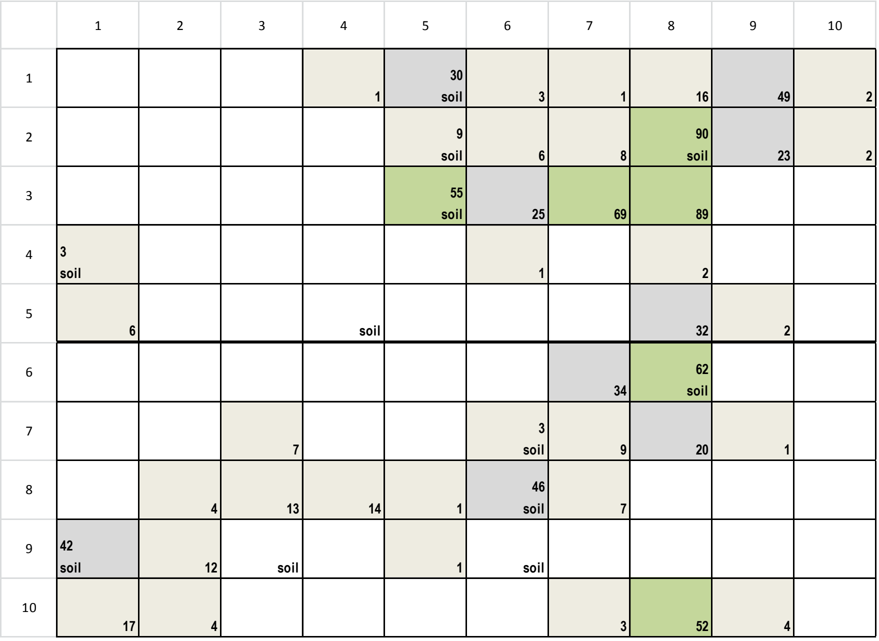
### 4.1.1 The *L. pseudotruncatella* population

The two censuses, conducted in the whole 1 ha study area of a *L. pseudotruncatella* population, detected 448 and 860 plants, respectively (Table 3; Paper I). The 48% increase in plant number from 2012 to 2013 is likely in part due to the division of the study area into more easily surveyed 10×10 m plots, and in part to plants being easier to detect 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, it becomes more difficult in desert populations, where rainfall is erratic.

Table 3. *Results of 2 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 |

The 2013 census revealed an extremely clumped distribution; almost 92% of the total number of plants in the study area occurred in just 20% of the test plots (Fig. 21). A clumped distribution was also confirmed by the goodness of fit test for a Poisson distribution: $\chi^2 = 1959$, df = 10, $P < 0.001$. All of the methods to estimate plant density proved very time consuming and often widely inaccurate, except for the belt transects method. The results obtained with this method (540 plants) and with the somewhat more cumbersome ACS method (557 plants) are the closest to the number of plants obtained in the two censuses. The gross over-estimation obtained with the nearest neighbour method, and the gross under-estimation obtained with the closest individual method and its variants, also confirm a clumped pattern. Accuracy may be further improved by application of other variants of Belt Transects, like placing the transects in opposite directions, e.g. N–S and E–W.



| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | 1 | 30<br>soil | 3 | 1 | 16 | 49 | 2 |
| 2 | | | | | 9<br>soil | 6 | 8 | 90<br>soil | 23 | 2 |
| 3 | | | | | 55<br>soil | 25 | 69 | 89 | | |
| 4 | 3<br>soil | | | | | 1 | | 2 | | |
| 5 | 6 | | | soil | | | | 32 | 2 | |
| 6 | | | | | | | 34 | 62<br>soil | | |
| 7 | | | 7 | | | 3<br>soil | 9 | 20 | 1 | |
| 8 | | 4 | 13 | 14 | 1 | 46<br>soil | 7 | | | |
| 9 | 42<br>soil | 12 | soil | | 1 | soil | | | | |
| 10 | 17 | 4 | | | | | 3 | 52 | 4 | |

*Figure 21*. Number of *Lithops* plants in each 10×10 m plot within the 1 ha study area, as well as location of the 12 soil samples collected. White squares (56 in total) indicate a zero count, light brown indicate ≤ 20 plants, grey indicate 20–50 plants and green indicate > 50 plants.

A clumped distribution has been reported for many other 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) resulting mainly 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.

### 4.1.2 The *L. ruschiorum* populations

During the field work in 2006–2008, only nine *L. ruschiorum* populations were located out of the 21 previously recorded (Papers II and IV), probably because (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.

Since *L. ruschiorum* plants usually appear in clumped patches just like the previously analysed *L. pseudotruncatella* (Paper I), efforts were made to obtain absolute plant counts. A total of 8,456 *L. ruschiorum* plants were recorded at the 94 sites in the nine populations. The Skeleton Coast Park with populations View Point, Khumib River, Hoanib River and Ugab River contained 51% of the total number of recorded plants (Table 4). The largest population was Khumib River with over 2,200 plants, and the second largest was RUL with just over 2,000 plants. The count for View Point was probably quite accurate, since this is a small and very isolated population. In many of the other populations, plant counts are likely to have underestimated true plant number. Especially the Khumib River population is probably much larger than reported 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 found within the license area of RUL, where the species grows, at varying densities, on approximately 52 km².

Table 4. *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 | 8,979 |
| View Point | 1 | 92 | 500 |
| Total | 94 | 8465 | >214,530 |

Each plant was defined as mature, juvenile or damaged. Mean percentage mature plants out of the total number were 90.3%, while 8.6% were damaged and 1.1% were juveniles. Juveniles are exceptionally hard to spot and are likely to be overlooked.

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. The boundaries of a site (i.e. area occupied) were defined by the plants growing on the fringes of the site. As expected, positive correlations were obtained between area occupied and number of plants, both in RUL and in the other eight populations. Area occupied and plant density were instead negatively correlated in both these data sets with the closest association found in RUL. 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. Estimation of plant abundance is not straightforward in species with a patchy or heavily clumped distribution. Plant number and occupied area as used in the Spearman's rank correlation coefficients provide an estimate of the 'size' of a plant site, whereas plant density was more closely associated with most of the habitat variables and may be superior for determination of habitat preferences.

## 4.2 Determination of habitat preferences

### 4.2.1 The *L. pseudotruncatella* population

The PCA shows that plots situated on a southeast- or south-facing rise, with a large percentage of pebbles and gravel, are likely to harbour a high number of *L. pseudotruncatella* plants (Fig. 22; Paper I). By contrast, a high percentage of sand or medium-sized stones are instead negatively associated with plant number as are also slopes, and north- and east-facing aspects. Local adaptation to different edaphic micro-environments has been reported 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).

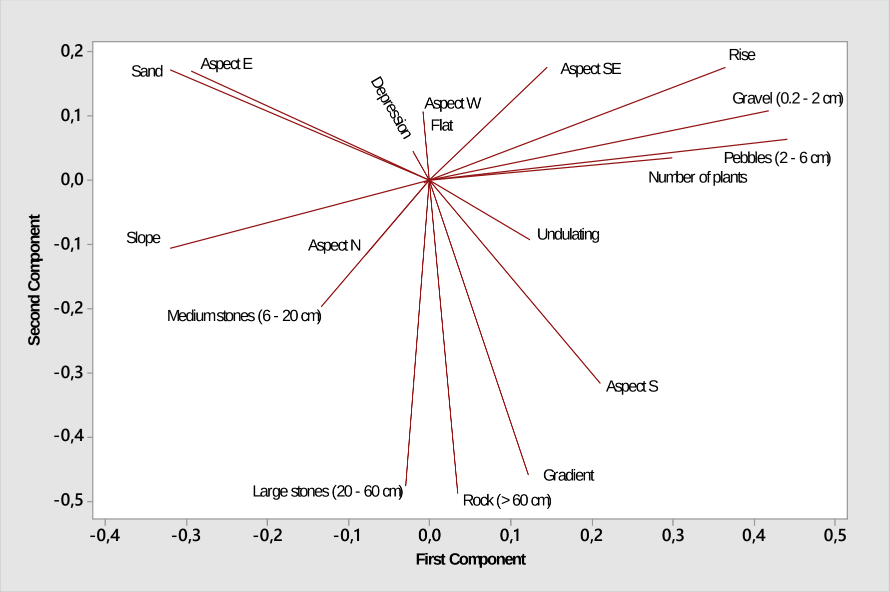


*Figure 22.* Principal Component Analysis showing the relationship between number of *Lithops* plants, substrate cover, slope (gradient), topography and aspect in 100 10×10 m test plots.

*Lithops pseudotruncatella* seems to prefer a habitat with a cover mainly of pebbles and gravel (Fig. 23), providing the plants both stability and protection since the surface substrate remains stable during thunderstorms and probably does not retain excessive amounts of water, especially not on a rise. In the dry season, gravel and pebbles prevents trampling and predation of the plants by livestock. Gravel and pebbles also afford seedlings protection from prolonged exposure to sunlight and help them to become established. By contrast, habitats with larger stones cannot effectively protect *Lithops* plants from being detected by predators, and the loamy soil between the stones may retain too much water which can lead to rotting of the plants.

Due to their cryptic habit (most mature plants reached only 2–8 mm above soil and had an average diameter of 17 mm in this population), the 2 censuses and plant density estimations required about 5 months of field work for 2 persons in total, while the habitat preference study (using pre-marked plants) required an additional month for 2 persons. We believe that the outcome of this study has identified the most accurate methods for determination of plant density and can serve as a model for future research on *Lithops* and other cryptic plant species, and assist in developing a basis for better conservation assessments and protection policies.

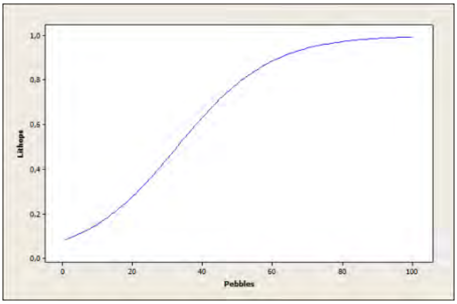


*Figure 23a.* Fitted line plot of number of *Lithops* plants as a function of percentage cover by pebbles in 100 10×10 m test plots

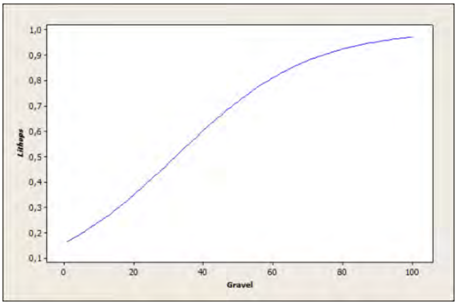


*Figure 23b.* Fitted line plot of number of *Lithops* plants as a function of percentage cover by gravel in 100 10×10 m test plots

### 4.2.2 The *L. ruschiorum* populations

For the qualitative habitat variables, i.e., aspect, soil texture and substrate, ANOVAs estimated impact on plant number and on plant density, respectively, for sites at RUL and for sites in the other eight *L. ruschiorum* populations (Table 5; Paper II). Categories had to be merged in some cases when sample numbers were small and unevenly distributed. Aspect was not significant for number of plants encountered in the different sites at RUL but S+SE+E-facing sites had the highest number followed by SW-facing sites. In the 8 populations data set, the most common aspect was W (8 sites) followed by SW and S+SE+E (7 sites each) and then NE+N+NW (6 sites). Aspect had a significant impact in this data set, with the highest number of plants in sites on SW-facing slopes. Soil texture varied strongly between the two data sets, with only loamy sand (27 sites), silt loam (9 sites) and sand (7 sites) recorded in the eight populations, whereas clay-loam, light clay, loam and silt loam were almost equally common (11–15 sites) at RUL. Soil texture did not affect plant number at RUL but there was significant impact in the 8 populations 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 (6 sites), while quartz+other (17 sites), granite+other (15 sites) and pegmatite+other (4 sites) were most common in the 8 populations data set. 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 at RUL with SW-facing sites harbouring the highest density of plants, as well as in the 8 populations dataset, again with the highest density in sites on SW-facing slopes. Soil texture did not affect plant density in either data set, while substrate had a significant impact for the 8 populations with the highest density on pegmatite+other.

Table 5. *Importance of 3 habitat parameters (aspect, soil texture and substrate) for plant number and plant density determined with* ANOVA *and Tukey pairwise comparisons, for 51 sites at* RUL *and for 43 sites at the other 8 populations.*

*RUL*

| Parameter | df | F | p |
|---|---|---|---|
| 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 8 populations*

| Parameter | df | F | p |
|---|---|---|---|
| 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** |

* 0.05>P>0.01, ** 0.01>P>0.001

Spearman’s rank correlation coefficients were calculated for assessing the impact of the quantitative habitat variables altitude and slope (Table 6). Altitude was not correlated with either plant number or plant density, whereas slope was positively correlated with density in RUL (r = 0.411, p = 0.012) and in the 8 populations (r = 0.504, p = 0.002) as well as with plant number in the 8 populations (r = 0.445, p = 0.006). The latter was confirmed with GLM while no associations were found with plant density. In addition, impact of the distance between the site and the sea coast was investigated for the 8 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 due to the heavily reduced number of sites (26 instead of 43) included in this analysis due to missing values.

Table 6. *Spearman's 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* RUL *population, and at 43 sites in the other 8 populations.*

*RUL*

| Parameter 1 | Parameter 2 | r | N | P |
|---|---|---|---|---|
| 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 8 populations*

| Parameter 1 | Parameter 2 | r | N | P |
|---|---|---|---|---|
| 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* |

In Namibia, the advective fog zone occurs mainly within 15 km from the coast and produces fog precipitation more than 100 days per year, arriving with a south-westerly wind in the afternoon. By contrast, the high fog zone primarily occurs between 20 and 60 km inland from the Atlantic Ocean, and produces fog-derived precipitation 60–120 days per year (Seely and Henschel, 1998; Mendelsohn *et al*., 2002; Robertson *et al*., 2012). In this zone, fog provides up to five times as much precipitation as rain, and is also more predictable, affecting the distribution of many plant species in the Namib Desert (Lancaster *et al.*, 1984; Olivier, 1995; Seely and Henschel, 1998; Hachfeld and Jürgens, 2000; Seely and Pallet, 2008). Although rainfall events trigger germination and initial establishment of *Lithops* plants, fog precipitation could be more important for plant survival and growth in coastal species like *L. ruschiorum*. The Ugab Salt works population is situated less than 1 km from the coast and benefits from advective fog, whereas Hoanib River (19 km from the coast), Khumib River (26.5 km) and Ugab River (26.5 km) possibly benefit from both fog types. At View Point (14 km from the coast), a *Lithops* population grows on a low, exposed hill in a habitat that does not appear to intercept fog effectively. Three further populations occur within the high fog zone, where lower air temperatures and higher humidity allow them to benefit from fog (Seely and Henschel, 1998). The RUL population is instead 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 and Jürgens, 2000) and plants growing here are probably more dependent on rain (Hachfeld, 2000).

## 4.3 Declining plant number in the *L. ruschiorum* monitoring plots

The 21 monitoring plots with *L. ruschiorum* at RUL were observed from 2007 to 2016 (except one), with total number of plants decreasing from 514 (mean 25.7) to 259 (mean 13.0) (Table 7; Paper IV). A partial Spearman's rank correlation analysis indicated a strong decline in plant number with time (Rho = 0.63, $p < 0.001$). Rainfall and fog precipitation data recorded at RUL in 2006–2016 show peaks in 2006, 2008 and 2011 (Fig. 24). A prolific rainfall event every two to three years may be required to sustain this population, which is situated approximately 60 km inland in the outskirts of the high fog zone. Apart from a shortage of water, evidence of animal disturbance was seen in some plots and an unusual number of large herbivores were sighted during the 2016 monitoring session. The prolonged dry period from 2012 to 2016 compelled large herbivores to move into the mining area to find food and water, thus destroying many plants and contributing to the decline.

*Table 7.* Population, plot and number of plants detected in the 36 monitoring plots.

| Population | Plot | 2007 | 2008 | 2010 | 2012 | 2016 |
|---|---|---|---|---|---|---|
| RUL | 1 | 33 | 44 | 45 | * | 41 |
| | 2 | 36 | 36 | 29 | * | 18 |
| | 4 | 8 | 6 | 7 | * | 2 |
| | 5a | 21 | 17 | 14 | * | 18 |
| | 6 | 16 | 10 | 11 | * | 3 |
| | 8 | 31 | 22 | 20 | * | * |
| | 9d | 17 | 16 | 18 | * | 11 |
| | 10 | 10 | 9 | 15 | * | 7 |
| | 13b | 16 | 16 | 15 | * | 2 |
| | 15a | 15 | 12 | 14 | * | 0 |
| | 17a | 38 | 29 | 38 | * | 4 |
| | 18b | 18 | 9 | 7 | * | 0 |
| | 19a | 29 | 11 | 7 | * | 3 |
| | 21 | 10 | 9 | 4 | * | 4 |
| | 46 | 8 | 2 | 3 | * | 3 |
| | 47 | 16 | 7 | 1 | * | 2 |
| | 48a | 29 | 17 | 28 | * | 7 |
| | 48b | 28 | 17 | 18 | * | 5 |
| | 49 | 70 | 55 | 30 | * | 46 |
| | 50 | 15 | 8 | 5 | * | 12 |
| | 68 | 42 | 44 | 63 | * | 70 |
| Rossing Mountain | 51b | 60 | * | * | 75 | 12 |
| | 51c | 89 | * | * | 78 | 39 |
| The Ridge | 52a | 18 | * | * | * | 11 |
| | 52b | 10 | * | * | * | 9 |
| Ugab Salt Works | 53a | 12 | * | * | * | 11 |
| | 53b | 18 | * | * | * | 18 |
| Ugab River | 84a | 36 | * | * | * | 46 |
| | 85b | 24 | * | * | * | 34 |
| Henties Bay-Uis Road | 88a | 24 | * | * | 15 | 22 |
| | 89 | 18 | * | * | 7 | 5 |
| | 90 | 23 | * | * | 6 | 8 |
| | 91 | 73 | * | * | 91 | 96 |

| Population | Plot | 2007 | 2008 | 2010 | 2012 | 2016 |
|---|---|---|---|---|---|---|
| Khumib River | 96a | 63 | * | * | * | * |
| | 96b | 117 | * | * | * | * |
| Hoanib River | 98 | 33 | * | * | * | * |

**no data recorded for this plot in this year*

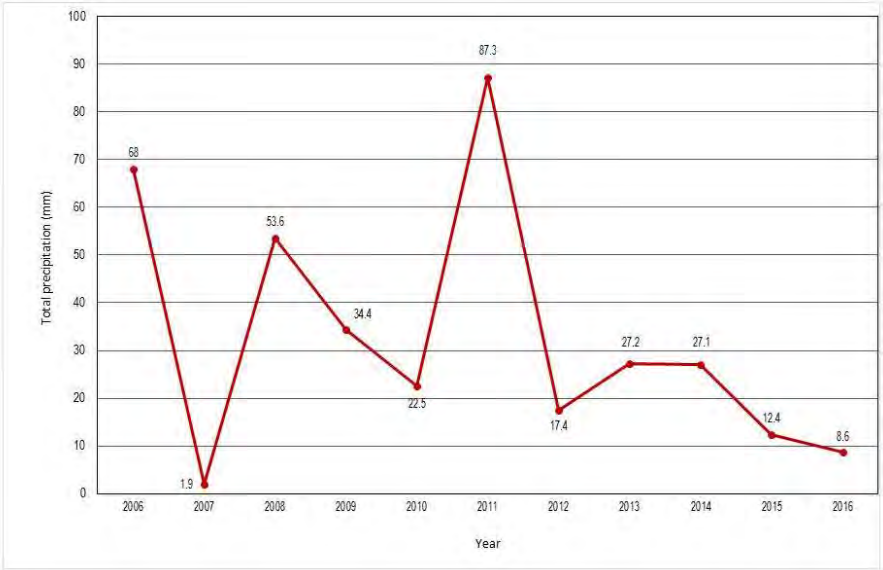


*Figure 24.* Total precipitation recorded for RUL from 2006 to 2016. This includes rain and fog.

Monitoring plots in the other 7 populations, all of which occur closer to the coast and therefore well within the high fog zone or the advective fog zone, showed no statistically significant decline when analysed together but plant number decreased significantly in the Rössing Mountain population. More data is needed to ascertain whether this decline can be ascribed to human activity or changes in weather patterns. Additional surveys should therefore be made, especially for investigating possible effects of the substantial 2018 rains in the central Namib Desert, affecting the southern populations such as RUL and Rössing Mountain.

## 4.4 **AFLP** -based genetic variation in *L. ruschiorum*

### 4.4.1 Within-population variation

AFLP markers have been used to study differentiation at the population level of several species in the sub-family Ruschioideae (Ellis *et al*., 2006; Buys *et al*., 2008) and to perform a phylogenetic study in *Lithops* (Kellner *et al*., 2011). As to our knowledge, the present studies (Papers II and III) are however the first to use DNA markers to study genetic diversity between and within populations in *Lithops*.

Analyses of 52 individuals from seven *L. ruschiorum* populations produced 102 polymorphic AFLP bands (Paper II). Mean percentage polymorphic loci was 66.0 and mean expected heterozygosity was 0.24 (Table 8) which is similar to RAPD-derived estimates for short-lived perennials (0.20), with narrow-range distribution area (0.28), outcrossing breeding system (0.27), water-dispersed seeds (0.27) and growing in early-successional vegetation (0.17; Nybom, 2004). The large Khumib River population had the highest values followed by Rössing Mountain whereas Ugab River had the lowest. Possibly the high diversity in Khumib River is connected with the fact that both varieties of *L. ruschiorum* were found in this population.

Table 8. *AFLP -based estimates of genetic variation, within each of the seven sampled* L. *ruschiorum populations, estimated as Percentage of Polymorphic Loci (PPL) and mean expected heterozygosity ($H_E$), and distribution of molecular variance among and within populations of 7 populations and 6 populations (without Khumib River), with all results highly significant according to permutation tests ($p_{random \geq data} < 0.001$).*

Variation within populations

| 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 |

Distribution of molecular variance among and within populations

| Source of variation | df | Sum of squares | Estimated variance | Percentage of variance |
|---|---|---|---|---|
| *7 populations* | | | | |
| Among populations | 6 | 120.2 | 0.80 | 5 |
| Within populations | 45 | 635.6 | 14.12 | 95 |
| *6 populations (without Khumib River)* | | | | |
| Among populations | 5 | 86.0 | 0.46 | 3 |
| Within populations | 39 | 536.1 | 13.74 | 97 |

### 4.4.2 Between-population differentiation

AMOVA showed that 95% of the variability resided within *L. ruschiorum* populations and 5% between populations (Paper II). Variation between populations declined to less than 3% when repeating the analysis without the Khumib River population (situated approximately 300 km away from the other populations). Only 5% of the genetic variability occurred among populations, indicating a very low level of differentiation. *Lithops* is outcrossing, probably pollinated by a variety of insects (Smith *et al*., 1998; Cole and Cole, 2005) and possibly having prominent gene flow. Higher values, ranging between 25 and 35%, are, however, usually found in outcrossing species sampled from populations within a restricted distribution area (Nybom, 2004). The low differentiation in our study may be indicative of a relatively recent fragmentation of a previously larger population.

A Mantel test indicated a correlation between geographic and genetic distances (Fig 25) when the analysis was performed on all 7 populations ($r = 0.410$, $p < 0.001$). There was, however, no correlation when the test was repeated without Khumib River. Similarly, the PCoA showed some grouping of samples with Khumib River present (Fig. 26) but removal of this population resulted in a loss of this grouping.

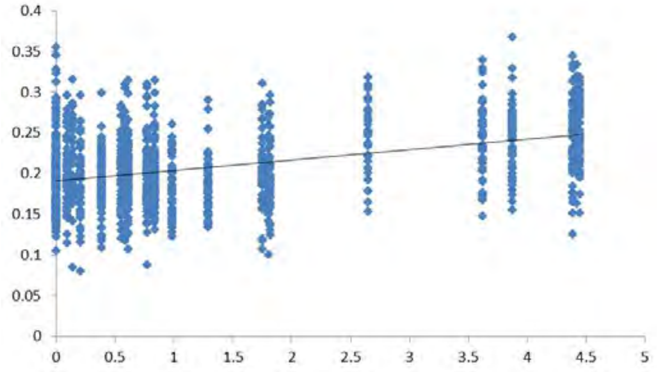


*Figure 25.* Mantel test with all 7 *L. ruschiorum* populations, r = 0.410.

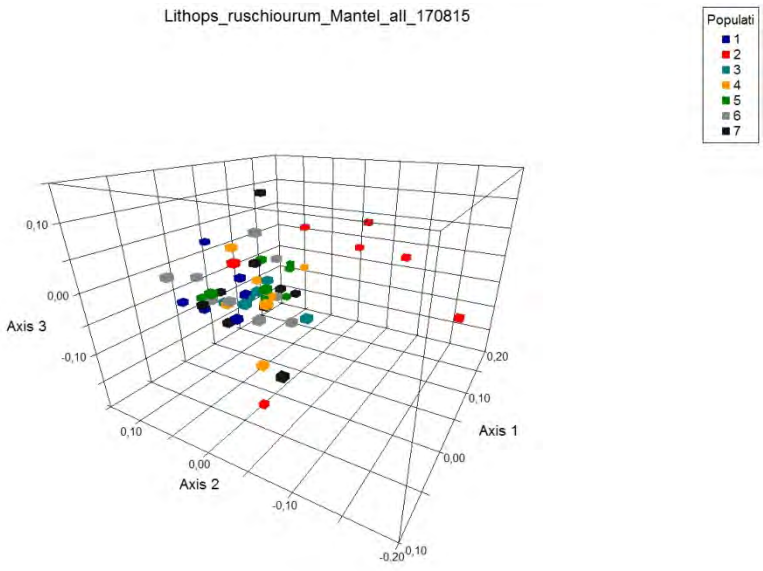


*Figure 26.* PCoA showing between population differentiation for all 7 *L. ruschiorum* populations. Population1. RUL, Population 2. Khumib River, Population 3. Ugab River, Population 4. Feldspar ridge, Population 5. Rössing Mountain, Population 6. Henties Bay–Uis Road, Population 7. Henties Bay–Usakos Road.

## 4.5 AFLP-based genetic variation among and within 15 *Lithops* species

### 4.5.1 Genetic diversity within species and populations

Four AFLP primer pairs produced 92 polymorphic bands in a set of 223 samples representing 15 species and 23 different taxa (Paper III). For each taxon, diversity was estimated as expected heterozygosity, with the lowest values found in *L. karasmontana* var. *lericheana* (0.086), *L. optica* (0.095) and *L. hermetica* (0.113) and the highest values in *L. pseudotruncatella* subsp. *pseudotruncatella* var. *pseudotruncatella* (0.450), *L. werneri* (0.342) and *L. ruschiorum* (0.331). Sample numbers were low and results must be treated with caution. For taxa with only a single population (≥ 6 samples), values varied between 0.189 (*L. amicorum*) and 0.342 (*L. werneri)* providing a crude estimate of within-population diversity. These values are overall similar to previously reported estimates of within-population diversity in perennials with a narrow-range distribution area, outcrossing breeding system, water-dispersed seeds and a preference for early-successional vegetation habitats (Nybom, 2004). *Lithops werneri* has a very restricted distribution in the western-central part of the country and it is presently not possible to explain why this particular taxon is more variable than the others.

AMOVA revealed 23% molecular variance among species, and 24% among different taxa (species, subspecies and varieties). These low levels of differentiation suggest a recently diversified species complex. Seven species were represented by 3–8 populations each, and AMOVAs indicated little differentiation between populations within each species; *L. francisci* 18%, *L. gracilidelineata* 7%, *L. julii* 5%, *L. karasmontana* 12%, *L. pseudotruncatella* 9%, *L. ruschiorum* 2% and *L. schwantesii* 7%. By comparison, variation among populations had an overall mean of 34% in a large metastudy (Nybom, 2004). Factors such as a perennial life form, outcrossing breeding system, insect pollination and small distribution area act to restrict differentiation between populations (Nybom and Bartish, 2000; Nybom, 2004).

A moderate but significant association between genetic and geographic distances was found when all 223 samples were subjected to a Mantel test ($r = 0.329$, $p < 0.001$).

### 4.5.2 Intraspecific variability

Three species, *L. karasmontana*, *L. pseudotruncatella* and *L. schwantesii*, were represented by two or more intraspecific taxa, and were therefore analysed for intraspecific variation. AMOVA showed 13% variance at the subspecies level of *L. karasmontana*, while variance among the three varieties of subsp. *karasmontana* was only 1%. A PCoA similarly indicated some variation between the three subspecies but not between the varieties (Fig. 27). For *L. pseudotruncatella*, AMOVA revealed that only 5% of the variance occurred among the four subspecies, while the PCoA indicated that only subsp. *dendritica* differed from the remainder.

Finally, an AMOVA indicated 7% variance between the two varieties of *L. schwantesii*, but they could not be separated with a PCoA.

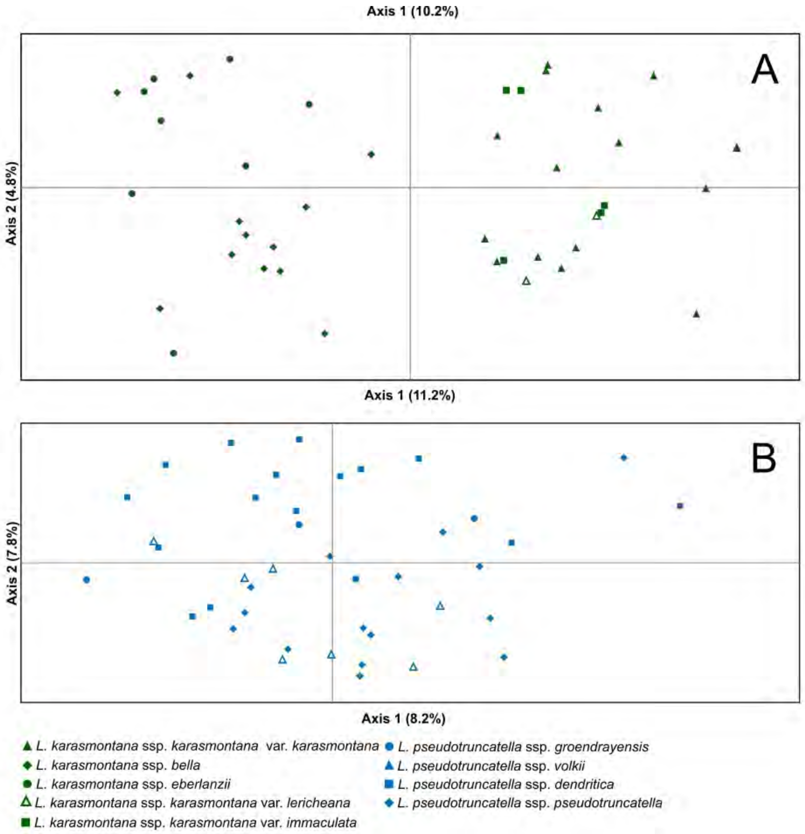


*Figure 27.* PCoA analyses based on AFLP data for 2 *Lithops* species. **A**. *L. karasmontana* including the 3 subspecies *bella*, *eberlanzii* and *karasmontana* with 3 varieties of the latter; var. *immaculata*, var. *karasmontana* and var. *lericheana*. Samples are plotted on the first 2 coordinates which together explain 20% of the variability. **B.** *L. pseudotruncatella* including the 4 subspecies *dendritica*, *groendrayensis*, *pseudotruncatella* and *volkii*. Samples are plotted on the first 2 coordinates which together explain 16% of the variability.

### 4.5.3 Interspecific variability and phylogenetics

Genetic differentiation within and among all 223 samples (Paper III) was evaluated with PCoA; samples of *L. ruschiorum* were clustered in the lower left-hand corner of the plot, while *L. amicorum*, *L. julii* and *L. karasmontana* instead occurred mainly in the lower right-hand corner (Fig. 28). The remaining species overlapped without clear-cut differentiation in the central and upper part of the plot.

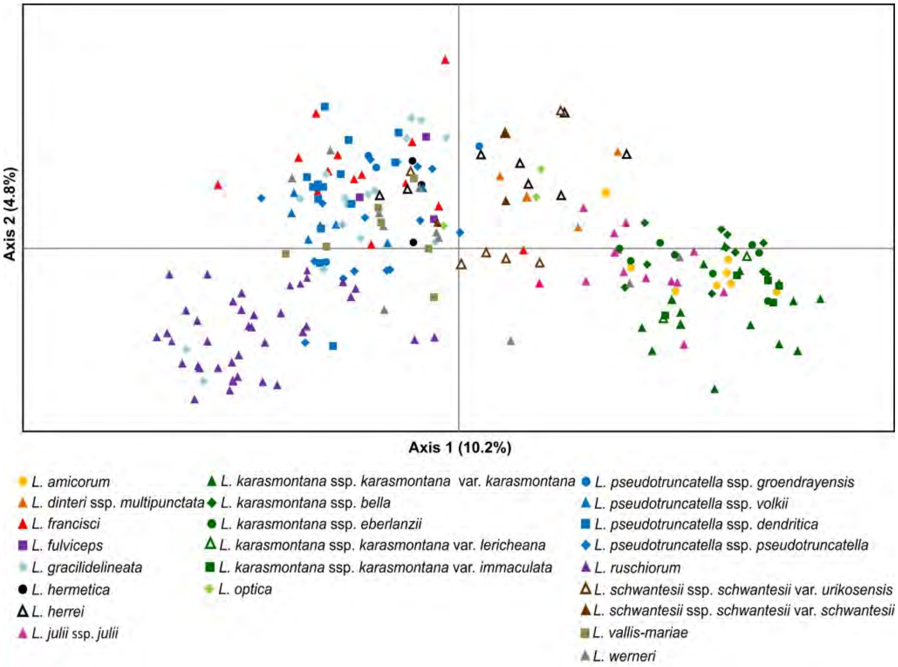


*Figure 28.* PCoA analysis based on AFLP data for 23 *Lithops* taxa. Samples are plotted on the first 2 coordinates which together explain 15% of the variability.

Relationships among taxa were evaluated by an unrooted neighbour-joining phylogenetic analysis; one rather diverse clade consisted of *L. amicorum* and *L. karasmontana*, and 3 smaller clades consisted of *L. dinteri* and *L. julii*, *L. herrei* and *L. optica*, and *L. francisci* and *L. hermetica*, respectively (Fig. 29). Another clade contained all samples of *L. schwantesii* and one sample each of *L. fulviceps* and *L. gracilidelineata*. The remaining species, *L. fulviceps*, *L. gracilidelineata*, *L. pseudotruncatella*, *L. vallis-mariae* and *L. werneri*, were intermingled in 4 clades.

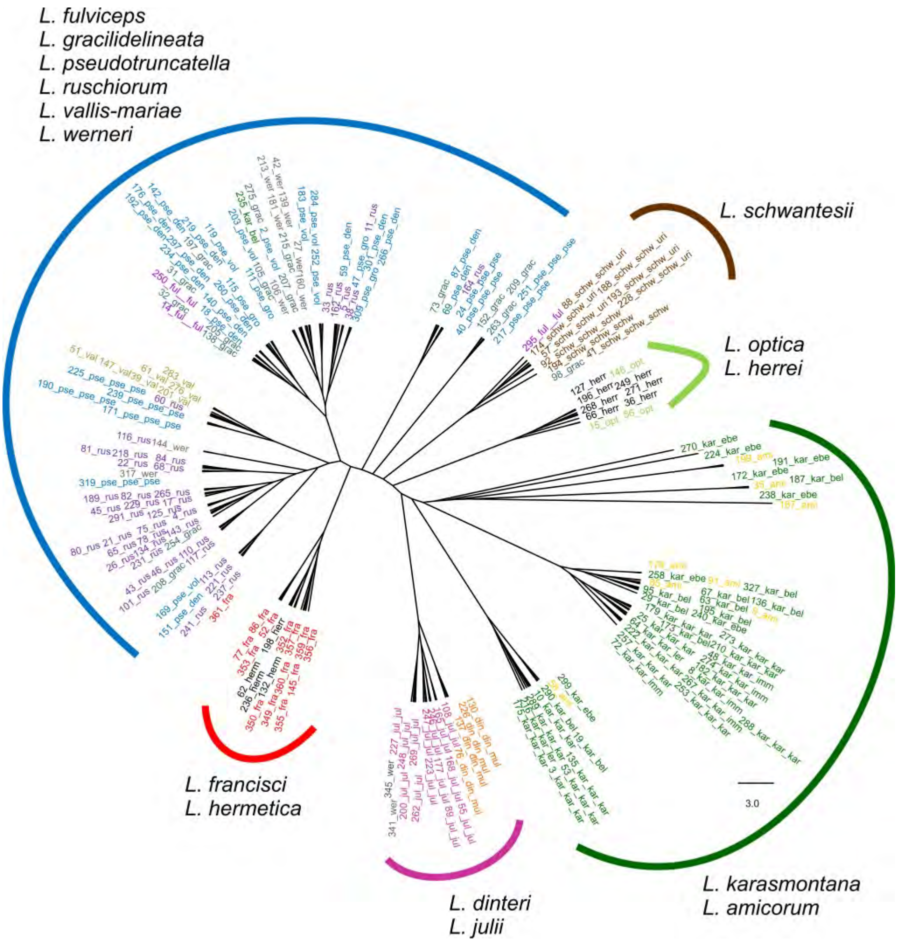
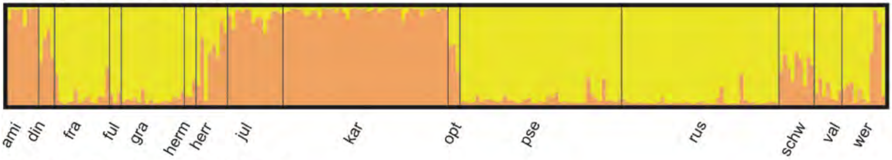


*Figure 29.* Unrooted AFLP-based neighbour-joining tree of 23 *Lithops* taxa.

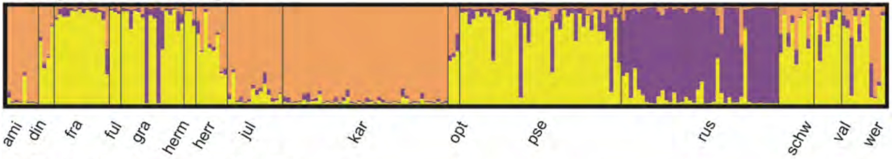
Bayesian clustering (Structure analysis) with two clusters indicated a major division between *L. amicorum, L. julii* and *L. karasmontana* on the one side, and most of the other species on the other side (Fig. 30). With 3 clusters, *L. ruschiorum* occurred in a group of its own. Using 7 clusters produced results similar to the phylogenetic analyses: *L. amicorum* and *L. karasmontana* formed one cluster, *L. herrei* and *L. optica* formed another, and *L. francisci* and *L. hermetica* yet another. *Lithops ruschiorum* occurred in a single-species cluster while most samples of *L. gracilidelineata*, *L. pseudotruncatella* and *L. werneri* formed a large cluster. In this analysis *L. julii* formed a separate cluster, whereas accessions of *L. dinteri* could not be unambiguously assigned to any cluster. In contrast to the other analyses, Bayesian clustering also grouped *L. schwantesii* and *L. vallis-mariae*.

Finally, a discriminant analysis of PCAs (DAPC) assigned each sample to one of 6 clusters, which were almost identical to Bayesian clustering for $K = 7$ except that *L. francisci* and *L. hermetica* no longer formed a cluster of their own.

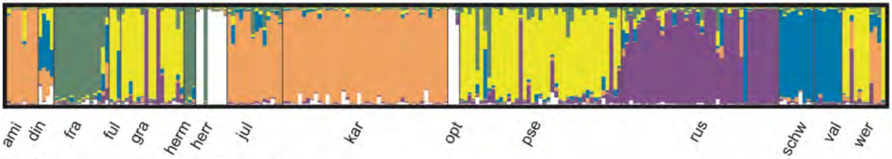
A. Bayesian clustering K = 2



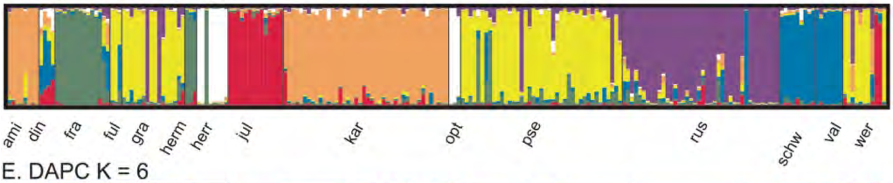
B. Bayesian clustering K = 3



C. Bayesian clustering K = 6*



D. Bayesian clustering K = 7



E. DAPC K = 6

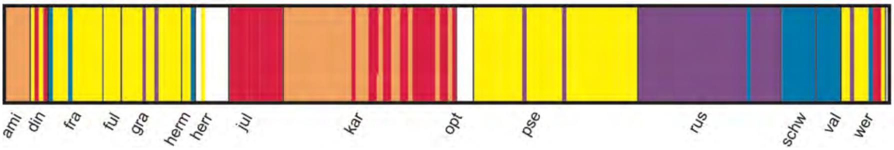


*Figure 30.* Clustering based on AFLP data for 15 *Lithops* species. Each plant is represented by a single vertical bar, which is partitioned into coloured segments representing different clusters. Length of the coloured segment corresponds to the probability (max 1.0) to belong to a certain cluster. A-C. Results from Bayesian clustering for models consisting of 2, 3 or 7 clusters. D. Result from DAPC analysis based on 6 clusters.

Previous phylogenetic analyses using DNA sequencing indicate that overall variation is very low both in the genus *Lithops* (Kellner *et al*., 2011) and in the subfamily Ruschioideae (Klak *et al*., 2003, 2004). The main reason is probably a rapid and recent diversification of succulents after the global expansion of arid environments in the late Miocene (Arakaki *et al*., 2011). Using allozyme data, Wallace (1990) was able to identify 2 major groups (with 2 subgroups each) of *Lithops* taxa and 1 outlier. Based on cultivated accessions for each of 49 taxa (species, subspecies and varieties) from Namibia and South Africa, Kellner *et al*. (2011) identified 9 different clades in *Lithops*, several of which overlapped with the groupings described by Wallace (1990).

In the present study, results from phylogenetic analyses (NJ and Bayes dendrograms), Bayesian clustering analysis and a discriminant analysis, indicate some grouping of species; *Lithops ruschiorum*, *L. fulviceps* + *L. gracilidelineata* + *L. pseudotruncatella* + *L. werneri* + *L. vallis-mariae*, *L. schwantesii* all have yellow flowers but are still morphologically quite distinct. The most divergent species in this large group, *L. ruschiorum*, occurs further west than any of the other species, except for *L. gracilidelineata* with which it has a partially overlapping distribution, and the single *L. werneri* population which is situated within the range of *L. gracilidelineata*. The *L. pseudotruncatella* complex grows mainly in the central part of Namibia, but some distant populations occur within 100 km from the distribution areas for *L. gracilidelineata*, *L. schwantesii* and *L. vallis-mariae*. *Lithops schwantesii* forms an almost discrete group in the NJ dendrogram but is close to *L. vallis-mariae* according to the Bayesian structure and DAPC analyses (Fig. 30), and there is less than 100 km between some of the populations of these 2 species. By contrast, *L. fulviceps* is geographically well separated from the more western species. Kellner *et al*. (2011) reported that *L. ruschiorum* clustered together with *L. pseudotruncatella*, while *L. gracilidelineata* clustered with *L. wernerii*. In contrast to our data, *L. schwantesii* however clustered with *L. fulviceps*, whereas *L. vallis-mariae* occurred close to some outgroup taxa.

*Lithops francisci* + *L. hermetica* occur in the southwestern part of Namibia, but *L. francisci* is much closer to the coast where it can benefit from fog, whereas *L. hermetica* grows further inland (about 80 km) where fog does not reach. Both species have yellow flowers and numerous dusky dots on the leaf surface but differ in several other traits. Jainta (2017) reports that *L. francisci*, *L. hermetica* and *L. gesinae* (the latter not included in our study) are sufficiently similar in morphology to be merged into one species, namely *L. francisci*. Kellner *et al.* (2011) reported that *L. francisci* clustered with *L. gracilidelineata* and *L. werneri* in spite of the 550 km distance, including the entire Namib sand sea, which separates *L. francisci* from the other species.

*Lithops optica* + *L. herrei* differ in flower colour (white, and yellow with a white centre, respectively) but still form a close-knit group, well separated from the remaining species. Both species occur within 50 km of the coast in southwestern Namibia, but are still separated by some 80 km. These two species formed a group also according to Wallace (1990) and Kellner *et al*. (2011). Cole and Cole (2005) reported of several pairs of taxa with very similar leaf colour, pattern and markings but with yellow versus white flowers.

*Lithops dinteri* + *L. julii* occur in close proximity in southeastern Namibia but are morphologically very different, including their yellow and white flowers, respectively. In the study by Kellner *et al*. (2011), *L. dinteri* instead clustered with *L. karasmontana*. A certain similarity between these two species and also *L. julii* was indicated in our study. Some populations in the outskirts of the distribution area of *L. karasmontana* complex occur within 100 km from *L. dinteri* and within 60 km of *L. julii*.

*L. karasmontana* + *L. amicorum* form a close-knit group in all of our analyses, which is concordant with both the geographic distribution in southwestern Namibia and morphology. Although *L. amicorum* could be merged with *L. karasmontana*, it does not comfortably fit under any of the already existing subspecies or varieties but is distinctive enough to be ranked as a subspecies of its own. *Lithops amicorum* was not investigated by Kellner *et al*. (2011), while *L. karasmontana* clustered with *L. dinteri* as reported above.

### 4.5.4 Taxonomic treatment

Three nomenclatural changes are made (Paper III), affecting: (1) *L. optica* and *L. herrei*, (2) *L. amicorum* and *L. karasmontana*, and (3) subspecies *bella* and *eberlanzii* of *L. karasmontana*.

# 5 Conclusions and perspectives

## 5.1 Concluding remarks on the present study

Carefully made descriptions of *Lithops* populations and their micro-habitats, could be very useful in pointing, e.g., plant conservationists to promising areas where *Lithops* can be found, and also help to determine what areas should be protected. Unfortunately, the extremely clumped spatial arrangement of *Lithops* plants, as demonstrated in a *L. pseudotruncatella* population, makes it difficult to determine population boundaries and plant abundance but a simple transect method can be used with some degree of success. Dividing a population into 10×10 m plots improves estimation of plant number, but is very time consuming.

Analysis of the habitat profile of *L. pdeudotruncatella* shows that a dense cover of quartz gravel and pebbles is a major factor in the establishment and survival of this species. By contrast, analysis of the habitat profile of *L. ruschiorum* indicates that amount of precipitation is probably the most crucial habitat characteristic for this species. Habitats that can intercept fog are especially important for plant density in populations that are closer to the coast, while rain is more important for populations that are situated further inland. Long-term monitoring of *L. ruschiorum* populations suggest that they are very susceptible to prolonged droughts and secondary effects thereof such as increased grazing pressure by livestock.

AFLP-based estimates of genetic variation within populations were typical for long-lived, perennial and outcrossing species. By contrast, differentiation among populations was very low, implicating considerable gene flow between populations and/or recent population fragmentation.

Proper delimitation of species as well as subspecific taxa, is an important basis for making correct assignments of plant conservation status. AFLP analyses were able to confirm some previous reports on relatedness among the investigated *Lithops* taxa of Namibia. Main reason for the overall low variability in *Lithops* is probably a rapid and recent diversification of succulents after the global expansion of arid environments in the late Miocene. The genus thus appears to be over-classified and three taxonomic changes were effected by merging two pairs of species as well as the subspecies of yet another species. Further changes are expected pending the clarification of relationships between *L. francisci, L. fulviceps, L. gracilidelineata, L. hermetica, L. pseudotruncatella, L. vallis-mariae* and *L. werneri*. Additional samples will have to be collected in the field, and possibly other methods could be applied such as DNA sequencing.

## 5.2 Perspectives for the conservation of *Lithops* in Namibia

Future climate change scenarios suggest that dwarf succulents are already experiencing the temperature threshold that they can endure (Musil *et al.*, 2005), which also suggests that local population extinctions may lead to further fragmentation. Namibia may have fewer fog days by 2070 and *Lithops* will experience a 60% loss of habitat as a result (Guo *et al.*, 2017). These effects will have to be mitigated to ensure the continued existence of *Lithops* and other succulent diversity.

Recruitment probably takes place only periodically in most populations. Adult persistence may be more important for population survival, as reported for *Haworthia koelmaniorum* Oberm. & D.S.Hardy (Witkowski and Liston, 1997) suggesting that conservation of adult plants in their habitat must be prioritised.

The importance of long-term monitoring of plant populations must not be underestimated. As an example, the global status of *L. optica*, with which *L. herrei* is combined (Paper III), has now changed from NT to VU A4cd (IUCN, 2001, 2017) in South Africa, indicating a suspected population reduction of at least 30%. This assessment was based on long-term monitoring data for 10 of the 24 known South-African populations through. Long-term monitoring of two *L. optica* populations on the Namibian side was set up in 2018.

The destruction of 60% of the area of the studied population of *L. pseudotruncatella* subsp. *pseudotruncatella* (the largest known population of this subspecies) in 2015 confirmed the vulnerability of dwarf succulents. If the area had been marked as a research area that should not be disturbed, the catastrophic destruction of close to 1000 plants could have been halted or at least postponed pending discussions with the new land owner. The decrease in this population together with the projected steady decline of other populations as a result of, e.g., illegal collecting, extreme weather conditions and building construction, the status of subsp. *pseudotruncatella* was revised to Vulnerable (VU A3cd; C2a(i)). The conservation status of the other subspecies was also revised: subsp. *archerae* = VU D1+D2; subsp. *dendritica* = NT; subsp. *groendrayensis* = VU D2 and subsp. *volkii* = EN D.

The conservation status of *L. ruschiorum* is currently LC, but many threats such as off-road driving and illegal collecting prevail. Insufficient precipitation can increase the pressure from grazing animals as demonstrated at RUL, leading to higher mortalities in populations already under stress. The present study revealed at least two populations with over 2,000 plants with the largest at Khumib River in Skeleton Coast Park. The second largest, at RUL, should be especially conserved, considering its distance from the Skeleton Coast Park and the fact that the mine has changed ownership.

# References

Bittrich V. & Hartmann H.E.K. (1988). The Aizoaceae - A new approach. *Botanical Journal of the Linnean Society* 97: 239–254.

Burke A. & Mannheimer C.A. (2003). Towards a density measure for assessments of arid plant populations – examples from the southern Namib Desert. *African Journal of Ecology* 41: 254–256.

Buys M.H., Janse van Rensburg L.-L., Mienie C.M.S., Barker N., Burgoyne P.M., Mills L., Van Rensburg L. & Hartmann H.E.K. (2008). Applying AFLPS in Aizoaceae: The *Delosperma herbeum* complex as a case study. *Biochemical Systematics and Ecology* 36: 92–100.

Chesselet P., Smith G.F. & van Wyk A.E. (2002). A new tribal classification of Mesembryanthemaceae. Evidence from floral nectaries. *Taxon* 51: 295–308.

Clark J.Y. (1996). A key to *Lithops* N.E. Brown (Aizoaceae). *Bradleya* 14: 1–9.

Cockburn W. (1974). Crassulacean Acid Metabolism in *Lithops insularis*, a non-halophytic member of the Mesembryanthemaceae. *Planta* (Berlin) 118: 89–90.

Coetzee B.J. & Gertenbach W.P.D. (1977). Technique for describing woody vegetation composition and structure in inventory type classification, ordination and animal habitat surveys. *Koedoe* 20: 67–75.

Cole D.T. (1988a). *Lithops flowering stones*. Acorn Books CC & Russel Friedman Book CC, Johannesburg.

Cole D.T. & Cole N.A. (2014). *Lithops: Lithops locality data C001–C420*. Available at http://www.lithops.info/en/lists/lld.html (2019.03.29)

Cole D.T. (2000). *Lithops hermetica* Cole. *Cactus & Co* 4: 157–161.

Cole D.T. & Cole N.A. (2001). Ruschioideae: *Lithops* in Hartmann H.E.K. (Ed.). *Illustrated Handbook of succulent plants: Aizoaceae, F-Z.* pp 114–135. Springer, Berlin.

Cole D.T. & Cole N.A. (2005). *Lithops* flowering stones. Cactus & Co., Tradate, Italy.

Cole D.T. (2006). *Lithops* - two new taxa. *Cactus & Co* 10: 57–59.

Cole D.T. (2012). *Lithops karasmontana* subsp. *karasmontana* var. *immaculata*. *Cactus & Co.* 16: 8–13.

Cottam G. & Curtis J.T. (1956). The use of distance measures in phytosociological sampling. *Ecology* 37: 451–460.

Cottam G., Curtis J.T. & Wilde H.B. (1953). Some sampling characteristics of a population of randomly dispersed individuals. *Ecology* 34: 741–757.

Craven P. & Loots S. (2001). *Namibia*. In Golding J.S. (Ed.). *Southern African Plant Red Data Lists.* Southern African Botanical Diversity Network Report Series No. 14. SABONET, Pretoria.

Devictor V., Whittaker R.J. & Beltrame C. (2010). Beyond scarcity: citizen science programmes as useful tools for conservation biogeography. *Diversity and Distributions* 16: 354–362.

Egbert K.J., Martin C.E. & Vogelmann T.C. (2008). The influence of epidermal windows on the light environment within the leaves of six succulents. *Journal of Experimental Botany* 59: 1863–1873.

Eller B.M. & Ruess, B. (1982). Water relations of *Lithops* plants embedded into the soil and exposed to free air. *Physiologia Plantarum* 55: 329–334.

Ellis A.G. & Weis A.E. (2006). Co-existence and differentiation of “flowering stones”: the role of local adaptation to soil micro-environment. *Journal of Ecology* 94: 322–335.

Ellis A.G., Weis A.E. & Gaut B.S. (2006). ‘Evolutionary radiation of "stone plants" in the genus *Argyroderma* (Aizoaceae): unravelling the effects of landscape, habitat, and flowering time’. *Evolution* 60: 39–55.

Elzinga C.L., Salzer D.W. & Willoughby J.W. (1998). *Measuring & monitoring plant populations.* US Department of the Interior, Bureau of Land Management. Denver, Colorado. Technical Reference: 1730–1731.

Fearn B. (1968). *An investigation into the taxonomy and phytogeography of the genus Lithops N.E.Br.* Diss. M.Sc.University of Sheffield, Botany, Sheffield.

Field K.J., George R., Fearn B., Quick W.P. & Davey M.P. (2013). Best of both worlds: Simultaneous high light and shade tolerance adaptations within individual leaves of the living stone *Lithops aucampiae*. *PloS One* 8 (10): 1-7

Germishuizen G. & Meyer N.L. (Eds.). (2004). Plants of southern Africa: an annotated checklist. Strelitzia 14. National Botanical Institute, Pretoria.

Hachfeld B. (2000). ‘Rain, fog and species richness in the central Namib Desert in the exceptional rainy season of 1999/2000’. *Dinteria* 26: 113–146.

Hachfeld B. & Jürgens N. (2000). Climate patterns and their impact on the vegetation in a fog driven desert: the central Namib Desert in Namibia. *Phytocoenologia* 30: 567–589.

Hall A.V., de Winter M., de Winter B. & van Oosterhout S.A.M. (1980). Threatened plants of southern Africa. South African National Scientific Programmes Report No. 45. CSIR, Pretoria.

Hammer S.A. (1999). *Lithops treasures of the veld. Observations on the genus Lithops N.E.Br.* British Cactus & Succulent Society, Norwich.

Hanski I.A. & Gilpin M.E. (1997). *Metapopulation Biology: Ecology, Genetics and Evolution.* Academic Press, San Diego.

Hartmann H.E.K. (1988). Fruit types in Mesembryanthema. *Beiträge zur Biologie der Pflanzen* 63: 313–349.

Hartmann H.E.K. (2006). Adaptations and phytogeography in the ice-plant family (Aizoaceae) – the interaction of the genetic equipment and ecological parameters. II. Hide-and-seek: plants sunk in the ground. *Bradleya* 24: 1–38.

Hassan N.S., Thiede J. & Liede-Schumann S. (2005). Phylogenetic analysis of Sesuvioideae (Aizoaceae) inferred from nr DNA internal transcribed spacer (ITS) sequences and morphological data. *Plant Systematics and Evolution* 255: 121–143.

Hilton-Taylor C. (1996). *Red Data List of southern African Plants*. Strelitzia 4. National Botanical Institute, Pretoria.

Hilton-Taylor C. (1997). Red Data List of southern African Plants 2. Corrections and additions. *Bothalia* 27: 195–209.

Ihlenfeldt H.D. (1983). Dispersal of Mesembryanthemaceae in arid habitats. *Sonderbaende des Naturwissenschaftlichen Vereins Hamburg* 7: 381–390.

Ihlenfeldt H.D. (1994). Diversification in an arid world: the Mesembryanthemaceae. *Annual Review of Ecology and Systematics* 25: 521–546.

Irish J. (1994). The biomes of Namibia, as determined by objective categorisation. *Navorsinge van die Nationale Museum Bloemfontein* 10: 550–591.

IUCN (1994). *IUCN Red List Categories*. Prepared by the Species Survival Commission. IUCN. Gland, Switzerland.

IUCN (2001). *IUCN Red List Categories and Criteria: Version 3.1*. IUCN Species Survival Commission, IUCN, Gland and Cambridge.

IUCN Standards and Petitions Subcommittee (2017). *Guidelines for using the IUCN Red List categories and criteria version 13*. Prepared by the Standards and Petitions Subcommittee. Downloadable from http://www.iucnredlist.org/documents/RedListGuidelines.pdf

Jainta H. (2017). *Wild Lithops*. Klaus Hess publisher, Göttingen and Windhoek.

Jenkins M. & Oldfield S. (1992). *Wild plants in trade.* A TRAFFIC Network Report. TRAFFIC International. Cambridge.

Kellner A., Ritz C.M., Schlittenhardt P. & Hellwig F.H. (2011). Genetic differentiation in the genus *Lithops* L. (Ruschioideae, Aizoaceae) reveals a high level of convergent evolution and reflects geographic distribution. *Plant Biology* 13: 368–380.

Kendall M.G. & Moran P.A.P. (1963). *Geometric probability.* Charles Griffin & Co., London.

Klaassen E.S. & Kwembeya E.G. (Eds.) (2013). *A Checklist of Namibian Indigeous and Naturalised Plants.* Occasional Contributions No. 5, National Botanical Research Institute, Windhoek.

Klak C., Khunou A., Reeves G. & Hedderson T. (2003). A phylogenetic hypothesis for the Aizoaceae (Caryophyllales) based on four plastid DNA regions. *American Journal of Botany* 90:1433–1445.

Klak C., Reeves G., Hedderson T. (2004). Unmatched tempo of evolution in Southern African semi-desert ice plants. *Nature* 427: 63–65.

Klak C., Bruyns P.V., Hanáček P. (2013). A phylogenetic hypothesis for the recently diversified Ruschieae (Aizoaceae) in southern Africa. Molecular *Phylogenetics and Evolution* 69: 1005–1020.

Korn R.W. (2011). Window patterns in *Lithops*. *International Journal of Plant Sciences* 172: 1101–1109.

Lancaster J., Lancaster N. & Seely M.K. (1984). Climate of the central Namib Desert. *Madoqua* 14: 5–61.

Landrum J.V. (2001). Wide-band tracheids in leaves of genera in Aizoaceae: the systematic occurrence of a novel cell type and its implications for the monophyly of the subfamily Ruschioideae. *Plant Systematics and Evolution* 227: 49–61.

Loots S. (2005). *Red Data Book of Namibian plants*. Southern African Botanical Diversity Report No. 38. SABONET, Pretoria and Windhoek.

Loots S. (2011). *National conservation assessment and management of two Namibian succulents, with specific reference to the Rössing Uranium Mine*. Report. Rio Tinto - Rössing Uranium Limited, Windhoek.

Loots S. & Nybom H. (2017). 'Towards better risk assessment for conservation of flowering stones: Plant density, spatial pattern and habitat preference of *Lithops pseudotruncatella* in Namibia'. *South-African Journal of Botany* 109: 112–115.

Maggs G.L., Craven P. & Kolberg H. (1998). Plant species richness, endemism and genetic resources in Namibia. *Biodiversity and Conservation* 7: 435–446

Mannheimer C.A. (2006*). A taxonomic revision of the genera of the subtribe Dracophilinae (Aizoaceae: Ruschioideae).* Diss. M.Sc. Rhodes University, Grahamstown. Rhodes University.

Mannheimer C.A. & Loots S. (2012). A simple aid to assessing cryptic succulents in the field. *Dinteria* 32: 78–80.

Mendelsohn J., Jarvis A., Roberts C. & Robertson A. (2002). *Atlas of Namibia*. David Phillip Publishers, Cape Town.

Morisita M. (1957). A new method for the estimation of density by spacing method applicable to non-randomly distributed populations. *Physiology and Ecology* 7: 134 –144.

Musil C.F., Schmiedel U. & Midgley G.F. (2005). Lethal effects of experimental warming approximating a future climate scenario on southern African quartz-field succulents: a pilot study. The New *Phytologist* 165: 539–547.

National Herbarium of Namibia (WIND). Specimen Database (2006). National Botanical Research Institute. Ministry of Agriculture, Water and Forestry, Windhoek, Namibia.

Nel G.C. (1946). *Lithops*. University Press, Stellenbosch.

Nobel P.S. (1989). Shoot temperatures and thermal tolerances for succulent species of *Haworthia* and *Lithops*. *Plant, Cell and Environment* 12: 643–651.

Nybom H. (2004). Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* 13: 1143–1155.

Nybom H. & Bartish I.V. (2004). Effects of Life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspectives in *Plant Ecology, Evolution and Systematics* 3: 93–114.

Olivier J. (1995). Spatial distribution of fog in the Namib. *Journal of Arid Environments* 29: 129–138.

Parolin P. (2006). Ombrohydrochory. Rain operated seed dispersal in plants – With special regard to jet action dispersal in Aizoaceae. *Flora* 201: 511–518.

Philippi T. (2005). Adaptive Cluster Sampling for estimation of abundances within local populations of low-abundance plants. *Ecology* 86: 1091– 1100.

Pfab M.F. & Scholes M.A. (2004). Is the collection of *Aloe peglerae* from the wild sustainable? An evaluation using stochastic population modelling. *Biological Conservation* 118: 695–701.

Plant Red List database of Namibia (2016). National Botanical Research Institute, Ministry of Agriculture, Water and Forestry, Windhoek, Namibia.

R Core Team (2015) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna.

Raimondo D., von Staden L., Foden W., Victor J.E., Helme N.A., Turner R.C., Kamundi D.A. & Manyama P.A. (2009). Red List of South African Plants. Strelitzia 25. South African National Biodiversity Institute, Pretoria.

Robertson A., Jarvis A., Mendelsohn J. & Swart R. (2012). Chapter 2: Weather and water in: *Namibia's coast – ocean riches and desert treasures*. Directorate of Environmental Affairs, Ministry of Environment and Tourism, Windhoek, Namibia.

Schmiedel U. & Jürgens N. (1999). Community structure on unusual habitat islands: quartz fields in the Succulent Karroo, South Africa. *Plant Ecology* 142: 57–69.

Schmiedel U., Dengler J., & Etzold S. (2012). Vegetation dynamics of endemic-rich quartz fields in the Succulent Karoo, South Africa, in response to recent climatic trends. *Journal of Vegetation Science* 23: 292–303.

Schwantes G. (1957). *Flowering Stones and mid-may flowers*. Ernest Benn, London.

Scogin R. (1972). Proteins of the genus *Lithops* (Aizoaceae): developmental and comparative studies. *Journal of South African Botany* 38: 55–61.

Seely M.K. & Pallet J. (2008). *Namib Secrets of a Desert Uncovered*. Venture Publications, Windhoek.

Seely M.K. & Henschel J.R. (1998). *The climatology of Namib fog*. In: Proceedings of the 1st Conference on fog and fog collection, 19–24 July 1998, Vancouver.

Smith G.F. & Crouch N.R. (1999). Mesembs in the muthi market: *Lithops leslei* as an ethnomedicinal plant. *British Cactus and Succulent Journal* 17: 133–137.

Smith G.F., Chesselet P., Van Jaarsveld E.J., Hartmann H.E.K., Hammer S., Van Wyk B.E., Burgoyne P., Klak C. & Kurzweil H. (1998). *Mesembs of the World: Illustrated Guide to a Remarkable Succulent Plant Group.* Briza Publications, Pretoria.

Strohbach B. (2001). Vegetation survey of Namibia. *Namibia Scientific Society Journal* 49: 93–124.

Thiede J. (2004). Phylogenetics, systematics and classification of the Aizoaceae: a reconsideration based on molecular data. Schumannia 4. *Biodiversity and Ecology* 2: 51–54.

Turner J.S. & Picker M.D. (1993). Thermal ecology of an embedded dwarf succulent from southern Africa (*Lithops* spp.: Mesembryanthemaceae). *Journal of Arid Environments* 24: 361–385.

Van Jaarsveld E. (1987). The succulent riches of South Africa and Namibia. *Aloe* 24: 45–92.

Vos P., Hogers R., Bleeker M., Reijans M., Van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M. & Zabeau M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23, 4407–4414.

Von Willert D.J., Eller B.M., Werger M.A., Brinckmann E. & Ihlenfeldt H.-D. (1992). *Life strategies of succulents in deserts – with special reference to the Namib Desert.* Cambridge University Press, Cambridge.

Wallace R.S. (1985). Taxonomic usefulness of leaf idioblast patterns in the genus *Lithops* (Mesembryanthemaceae). *Bulletin New Jersey Academy of Science* 30: 56.

Wallace R.S. & Fairbrothers D.E. (1985). Systematic investigation of the genus *Lithops* N.E.Br., the stone plants of southern Africa (Mesembryanthemaceae). *American Journal of Botany* 72: 974–974.

Wallace R.S. & Fairbrothers D.E. (1986). The value of microcharacters in systematics of the genus *Lithops* N.E.Br. (Mesembryanthemaceae). *American Journal of Botany* 73: 793–793.

Wallace R.S. (1988). Tanniniferous idioblast patterns as taxonomic characters in the genus *Lithops* N.E.Br. (Mesembryanthemaceae). In Goldblatt P. & Lowry P.P. (Eds.). *Monographs in Systematic Botany from the Missouri Botanical Garden* 25: 295–303.

Wallace R.S (1990). Systematic significance of allozyme variation in the genus *Lithops* (Mesembryanthemaceae). *Mitteilungen aus dem Institut fuer Allgemeine Botanik Hamburg* 23: 509–524.

Walter K.S. & Gillet H.J. (Eds.) (1998). *1997 IUCN Red List of Threatened Plants.* IUCN – The International Union for the Conservation of Nature. Gland, Switzerland & Cambridge, UK.

Wiki-travel (2018). *Travel Encyclopedia*. *Free collection of 40+ large maps of African Countries.*Available at https://wiki-travel.com/large-map-of-african-countries-3.html. Visited 24.04.2019.

Witkowski E.T.F. & Liston R.J. (1997). Population structure, habitat profile and regeneration of *Haworthia koelmaniorum*, a vulnerable dwarf succulent, endemic to Mpumalanga, South Africa. *South-African Journal of Botany* 63: 363–370.

# Popular science summary

Many plants in the dry areas of Southern Africa have special features to help them thrive in spite of the restricted access to water. One of the most specialized and characteristic plant groups is the genus *Lithops* with plants that consist of only one pair of leaves that appear to be fat and swollen (succulent leaves) and are used for storing water. Since they often resemble the stones and pebbles in the habitat where they grow, *Lithops* are known as “living stones”. There are 16 species of *Lithops* in Namibia, most of them found in the Namib Desert along the coast or in semi-dry areas further inland. Unfortunately, *Lithops* are vulnerable to, e.g., over-harvesting by plant collectors, various forms of habitat destruction including increased desertification, and predation by both wild animals and livestock. Much-needed legislation for protecting these emblematic plants is dependent on their conservation status in the Red List database as, e.g. ‘endangered’ or ‘vulnerable’. However, in order to assign an accurate conservation status, information is needed about the plant populations, as well as about how they are affected by environmental variables in their habitat.

Estimating plant abundance is very difficult in populations of *Lithops*; the plants are small and blend in with their habitat, and also tend to grow in small clumps here and there over vast areas. Plant number, spatial arrangement of plants and habitat specificity was therefore investigated in a 1 ha study area in a population of *L. pseudotruncatella*. We used seven different methods based on counting only a subset of the plants and then estimating the total number. Only two of these methods produced results that were reasonably similar to the number obtained from counting all plants in the whole area. We also laid out 100 10×10 m test plots. The number of plants in these test plots was positively associated with a high percentage cover of gravel and pebbles in the plots as opposed to sand or stones, and with a gentle rise as opposed to slopes and depressions in the topography.

Plant abundance and habitat variables were also evaluated in nine populations of *L. ruschiorum*. In each population, one to several sites were defined, each site consisting of an area with a continuous presence of *Lithops*. The most meticulously studied population, RUL, was divided into 51 sites, while another 43 sites were recognised in the remaining eight populations. Plant abundance was assessed in each site using two estimators: plant number and plant density (number of plants/size of site area). Statistical evaluations showed that plant number and/or plant density was associated with several variables measured/observed in the sites: aspect (compass direction), degree of slope, soil texture, geological substrate and geographic distance from the coast, but not with the altitude. Moreover, our results suggest that there is a strong impact of fog-based precipitation on plant density in the coastal populations, whereas rain is probably more important at RUL which is situated further inland. A total of 36 10×10 m plots in eight of the populations, were permanently marked out for repeated monitoring. All plants in these plots were counted 3–4 times from the start in 2007 up to 2016. Plant number dropped by 50% in the 21 plots monitored at RUL, most likely due to a prolonged drought in 2012–2016. Plant number declined also in some of the 15 monitoring plots in the other populations, especially between 2012 and 2016. Although *Lithops* are adapted to a very dry habitat, the increased desertification observed in recent years may pose a very serious threat.

DNA markers (Amplified Fragment Length Polymorphism, AFLP) were employed to study 52 individuals from seven populations of *L. ruschiorum*. Analysis of molecular variation demonstrated medium high levels of variability within populations as expected. By contrast, genetic differentiation between populations was very low, with only the spatially most distant population (300 km apart from the remainder) being significantly different. Possibly the present populations are only the remains of previously larger and more continuous populations, that have become fragmented in recent times.

In *Lithops*, different taxa (i.e. species, subspecies and varieties) are mostly defined according to leaf characters that are adapted to plant habitat and probably governed by a few genes only. This may have resulted in an over-emphasis of the genetic differentiation, and with too many taxa being described. AFLP markers were used to study 223 individuals from 44 populations representing 15 *Lithops* species and 23 taxa in total. Analyses of genetic structuring and phylogenetic relationships identified several groups of closely related species, some of which could be merged. Subspecies and varieties of the same taxon often overlapped considerably and could also be merged in some cases. Main reason for the overall low variability in *Lithops* is probably a rapid and recent diversification of succulent species after the global expansion of arid environments in the late Miocene (23 to 5.3 million years ago). Three changes in the taxonomy were made, affecting: (1) *L. optica* and *L. herrei*, (2) *L. amicorum* and *L. karasmontana*, and (3) subspecies *bella* and *eberlanzii* of *L. karasmontana*.

# Populärvetenskaplig sammanfattning

Många växter i Södra Afrikas öknar och halvöknar har speciella egenskaper, som hjälper dem att växa och frodas trots bristen på vatten. En av de mest specialiserade och karaktäristiska växtgrupperna är släktet *Lithops* med växtindivid som består av bara ett bladpar. Dessa blad är uppsvällda (fetbladsväxter = suckulenter) och används för att lagra vatten. Eftersom bladen ofta liknar stenarna i miljön där de växer, kallas *Lithops* ‘levande stenar’. Det finns 16 arter av *Lithops* i Namibia varav flertalet i Namib-öknen längs med kusten eller i halvöknar längre in i landet. Tyvärr är *Lithops* sårbara för rovdrift av illegala växtsamlare och för miljöförstöring inklusive ökenspridning, samt för både vilda och tama betesdjur. Ett välbehövligt juridiskt hållbart skydd för dessa växter, som har stort symbolvärde, är avhängigt deras skyddsstatus i den nationella rödlistan, exempelvis ‘starkt hotad’ eller ‘sårbar’. För att kunna fastställa en korrekt skyddsstatus, krävs dock att man har tillgång till information om växtpopulationerna samt hur dessa påverkas av miljöbetingelserna på sina ståndorter.

Det är svårt att uppskatta växtrikedomen i *Lithops*-populationer; de enskilda växterna är små och smälter in i omgivningen, samt tenderar att återfinnas i små grupper utspridda på mycket stora ytor. Antal växtindivid, deras utbredning och beroende av ståndortsvariationen undersöktes därför på en 1 hektar stor yta i en population av *L. pseudotrunctella*. Vi använde 7 olika metoder som bygger på att man bara räknar en del av växtindividen och sedan uppskattar det totala antalet. Endast två metoder gav resultat som överensstämde någorlunda med siffran vi fått fram genom att istället räkna alla växtindivid i hela området. Vi lade också ut 100 10×10 m försöksrutor. Antal växtindivid i dessa rutor ökade med ett ökat inslag av grus och småsten till skillnad från sand eller större stenar, och med en lättare höjning istället för sluttningar och sänkor i topografin.

Växtrikedom och ståndortsvariation undersöktes även i nio populationer av *L. ruschiorum*. I varje population markerades en eller flera växtytor som i sin tur bestod av en yta med sammanhängande förekomst av *Lithops*. Den mest välstuderade populationen, RUL, delades in i 51 växtytor medan ytterligare 43 växtytor markerades i övriga åtta populationer. Växtrikdomen beräknades på två sätt: antal växtindivid respektive växttäthet (antal individ/växtytans storlek). Statistisk bearbetning visade att antalet växtindivid och/eller växttätheten påverkades av flera omvärldsvariabler som mätts/observerats för växtytorna: väderstreck, lutning, jordmån och bergart samt det geografiska avståndet till kusten men däremot inte av höjden över havet. Dessutom tyder våra resultat på ett stort inflytande av nederbörd som dimma i de kustnära populationerna medan regn troligen är viktigare i RUL som ligger längre in i landet. Sammanlagt 36 10×10 m försöksrutor lades ut i åtta populationer för återkommande observationer. Alla växtindivid i dessa rutor räknade 3–4 gånger från starten 2007 fram till 2016. Antalet växtindivid föll med 50% i de 21 rutorna i RUL, troligen på grund av långvarig torka 2012–2016. Antalet växtindivid minskade även i några av de 15 rutorna i de andra populationerna, speciellt mellan 2012 och 2016. Trots att *Lithops* är anpassade till en mycket torr växtmiljö, kan den tilltagande ökenspridningen utgöra ett allvarligt hot.

DNA markörer (Amplified Fragment Length Polymorphism, AFLP) användes för att studera 52 växtindivid från sju populationer av *L. ruschiorum.* Variansanalys påvisade, som väntat, mellanhög variation inom populationerna. Den genetiska differentieringen mellan populationer var däremot oväntat låg, och endast en poulation (belägen 300 km från övriga) avvek signifikant. Möjligen kan dagens populationer utgöra kvarlevor av tidigare mycket större och mer sammanhängande populationer, vilka fragmenterats i modern tid.

Inom släktet *Lithops* avgränsas olika taxa (dvs arter, underarter och varieteter) i huvudsak efter bladens egenskaper, vilka är anpassade till ståndorten och troligen styrda av endast ett fåtal gener. Detta kan ha medfört en överdriven betoning av genetisk differentiering, och därmed erkännande av alltför många taxa. AFLP markörer användes för att studera 223 växtindivid från 44 populationer, som representerade 15 *Lithops* arter och totalt 23 taxa. Flera grupper av närbesläktade arter kunde påvisas genom analyser av genetisk strukturering och evolutionära samband. Några av dessa arter borde slås samman. Underarter och varieteter av samma art överlappade ofta, och borde också slås samman i vissa fall. Den begränsade variationen hos *Lithops* beror nog främst på en snabb, nutida artbildning inom suckulenter efter en global ökning av torra landområden i slutet av Miocen (23–5,3 millioner år sedan). Tre taxonomiska förändringar har gjorts, vilka påverkar: (1) *L. optica* och *L. herrei,* (2) *L. amicorum* och *L. karasmontana* samt (3) subspecies *bella* och *eberlanzii* av *L. karasmontana.*

# Acknowledgements

A PhD is never completed all by yourself and therefore can never really belong entirely to yourself.

Hilde Nybom is without question the best main supervisor anyone could ask for, a brilliant teacher, kind and generous, she patiently but sternly gets you there. The many fond memories I have of her (and her canines) will stay with me as long as I live. My German supervisor Dr. Christiane Ritz, most kind, most capable and efficient, a wonderful person that I feel honoured and blessed to have worked with. Larisa Gustavsson also competently and willingly provided assistance whenever I needed it and Rodomiro Ortiz enthusiastically and proficiently oversaw the last part of the journey as main supervisor.

The logistical and moral support that Dr. Moneim Fatih provided so many times will not be under-estimated or forgotten, nor the help from his son Samy and the often unexpected and kind assistance from his wife. These memories will be kept safe and revisited many times.

Close friends Mohammed Elsafi, Busi, Tiny, Elnura and Alphonsine could always be relied upon to assist in times of need and they will always be remembered.

Jan Eric Englund deserves special recognition for his assistance with statistical problems.

Jasna Sehic extracted DNA and patiently assisted with scoring data under very difficult circumstances and deserves a very special thank you.

Michaela Schwager taught me the ins and outs of lab work and Veit’s research took me to Prague!

My colleagues at the NBRI provided so much encouragement, support, professional assistance and field work: Esmerialda Strauss, Frances Chase,

Quanita Daniels, Kahimbi Sikute, Elisabeth Lucas, Leevi Nanyeni, Silke Rugheimer, Remmie Hilukwa, Vanessa Steyn, Joceline Dentlinger, David Aiyambo, Marianne Hochobes, Belinda Polster and Steve Carr and former colleague Salome Kruger.

Support from Deputy Director Vincent Louw and Director Joseph Hailwa as well as the ministerial training committee of MAWF and the Executive Director are also recognized.

Colleagues and friends at Alnarp and Balsgård provided assistance with various matters and I would like to thank all of them: Tomas Bryngelsson and Anders Carlsson assisted with various administrative matters, Mulato Gelata helps whenever he can see the desperation on your face, even when you are not his student. Others who helped with various issues that were important to me or proved to be a valuable friend at one time or another were Sergey Hegay, Faraz Muneer, Firuz Odilbekov, Faiza Rasheed, Michael Vagiri, Mbaki Muzilla Ibrahim Tahir, Weibo, Alexandra Nikolic and Karl-Johan Bergstrand, Anja Zborowska, Helena Persson Hovmalm, Abel, Ann-Sofie Fält, Helen Lindgren, as well as those behind the scenes in the finance section, the IT section and all other faculty members who contributed towards my education and of course everyone who provided interesting conversation and cake during teatimes.

Tok Schoeman (may he rest in peace), Hilde and Frikkie Mouton, Roy Earle, Keith Green provided information about localities and assisted in many other ways.

Other friends assisted in different ways like Christina and Åke Karlsson, Lida Loots assisted with field work, Engela Maritz and Lizelle Miller provided moral support.

Coleen Mannheimer and Antje Burke commented on some of the manuscripts and Ben Strohbach gave tips on conducting field experiments.

I would like to thank everyone at Alnarp who have provided assistance to me at some point: Helen Lindgren, Annelie Ahlman, Maria Luisa Prieto-Linde, Li-Hua Zhu, Mariette Andersson, and all who have just been friendly Elaine Ceresino, Åsa Grimberg, Rui Guan, Ida Lager, Pia Ohlsson, Marjan Ghasemkhani, Masoud Ahmadi and Mariette Andersson. Gary and especially Peter at the printing division displayed remarkable patience with this ever-changing thesis.

This work would not have been possible without the kind assistance from farm owners who granted access to their farms, provided information about *Lithops* populations and gave up their time: Mr and Ms G.S. Berg, Ms B. Boehm-Erni,

C. Buhrman, W. Diergaardt, L. Gessert, J and S. Hopkins, B. and L. Eksteen, H. Esterhuizen, W. Itzko, Ms. Koch, A. Louw, J. and J. van Niekerk, Mr. I and Ms. du Plooy (Farm Garub), N. and C. Pretorius, H. and O. Pretorius, A. Rusch, F. Snyman, B.N. and J. Steyn, P. and W. Swiegers, W. Teubner, G.G. Viviers, D. De Wet and R. and R. von Wielich.

Lize von Staden of SANBI and the Threatened Species Programme, Pieter van Wyk from SANParks, and the Custodians of Rare and Endangered Wildlife for data to re-assess *Lithops optica* / *L. herrei* are gratefully acknowledged.

Financial support was received and is gratefully acknowledged from the SADC Plant Genetic Resources Centre (SPGRC) and Sida. We thank the colleagues from the Senckenberg Biodiversitäts- und Klimaforschungszentrum (SBik-F) for technical support and V. Herklotz (Senckenberg Museum of Natural History Görlitz) for help with statistical problems. The Ministry of Environment and Tourism of Namibia granted research and entry permits into National Parks. Rössing Uranium Mine, Husab Uranium mine, Langer Heinrich Mine, Namdeb diamond mine and Scorpion Zinc Mine granted access and assistance in their license areas.

# Appendix 1

South African Journal of Botany 109 (2017) 112–115

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

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

S. Loots [a,b,*], H. Nybom [c]

[a] Swedish University of Agricultural Sciences, Department of Plant Breeding, Box 101, 230 53 Alnarp, Sweden
[b] National Botanical Research Institute, Ministry of Agriculture, Water and Forestry, Private Bag 13184, Windhoek, Namibia
[c] Swedish University of Agricultural Sciences, Department of Plant Breeding, Balsgård, Fjälkestadsvägen 459, 29194 Kristianstad, Sweden

ARTICLE INFO

*Article history:*
Received 18 December 2015
Received in revised form 25 November 2016
Accepted 13 December 2016
Available online xxxx

Edited by T Kraaij

*Keywords:*
*Lithops pseudotruncatella*
Plant density
Spatial arrangement
Habitat preferences
Belt transect

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

---

* Corresponding author at: National Botanical Research Institute, Ministry of Agriculture, Water and Forestry, Private Bag 13184, Windhoek, Namibia.
*E-mail addresses:* sonja.loots@slu.se, LootsS@mawf.gov.na (S. Loots), hilde.nybom@slu.se (H. Nybom).

http://dx.doi.org/10.1016/j.sajb.2016.12.023

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 southeast 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

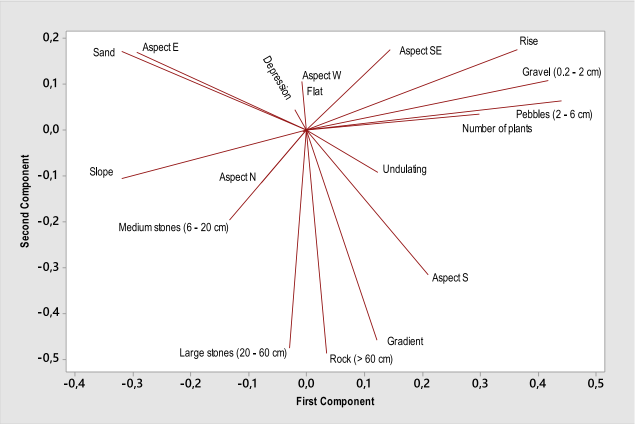


**Fig. 1.** Principal Component Analysis showing the relationship between number of *Lithops* plants, substrate cover, gradient, topography and aspect in 100 10 × 10 m test plots.

**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.

## Acknowledgements

Jan-Eric Englund and Patrick Graz assisted in statistical data analyses, Ben Strohbach provided scientific input, and Antje Burke, Coleen Mannheimer and Esmerialda Strauss commented on the manuscript. Kahimbi Sikute and Elisabeth Lucas of the National Botanical Research Institute of Namibia assisted in the field. Moneim Fatih is gratefully acknowledged for logistical support. Financial support was received from the Nordic-SADC Plant Genetic Resources Centre Network Programme, Lusaka, Zambia and the Ministry of Agriculture, Water and Forestry of Namibia.

## References

Burke, A., Mannheimer, C.A., 2003. Towards a density measure for assessments of arid plant populations — examples from the southern Namib Desert. African Journal of Ecology 41, 254–256.

Coetzee, B.J., Gertenbach, W.P.D., 1977. Technique for describing woody vegetation composition and structure in inventory type classification, ordination and animal habitat surveys. Koedoe 20, 67–75.

Cole, D.T., Cole, N.A., 2005. *Lithops* Flowering Stones. Cactus & Co.

Cottam, G., Curtis, J.T., 1956. The use of distance measures in phytosociological sampling. Ecology 37, 451–460.

Cottam, G., Curtis, J.T., Wilde, H.B., 1953. Some sampling characteristics of a population of randomly dispersed individuals. Ecology 34, 741–757.

De Pauw, E., Coetzee, M.E., Calitz, A.J., Beukes, H., Vits, C., 1998. Production of an agro-ecological zones map of Namibia (first approximation). Part II: results. Agricola 10, 27–43.

De Pauw, E., Coetzee, M.E., Calitz, A.J., Beukes, H., Vits, C., 1999. Addendum to the Agricola 1998/1999: preliminary agro-ecological zones. Agricola 1998/1999, 1–69.

Ellis, A.G., Weis, A.E., 2006. Co-existence and differentiation of “flowering stones”: the role of local adaptation to soil micro-environment. Journal of Ecology 94, 322–335.

Ellis, A.G., Weis, A.E., Gaut, B.S., 2006. Evolutionary radiation of “stone plants” in the genus *Argyroderma* (Aizoaceae): unravelling the effects of landscape, habitat and flowering time. Evolution 60, 39–55.

Elzinga, C.L., Salzer, D.W., Willoughby, J.W., 1998. Measuring & Monitoring Plant Populations. US Department of the Interior, Bureau of Land Management, Technical Reference 1730-1.

Giess, W., 1998. A preliminary vegetation map of Namibia. Third revised edition. Dinteria 4, 5–112.

Hartmann, H.E.K., 2006. Adaptations and phytogeography in the ice-plant family (Aizoaceae) — the interaction of the genetic equipment and ecological parameters. II. Hide-and-seek: plants sunk in the ground. Bradleya 24, 1–38.

Henderson, P.A., Seaby, R.M.H., 1999. Density From Distance (DFD): Version 1.1. Pisces Conservation Ltd., IRC House Penington, Lymington, UK.

Ihlenfeldt, H.D., 1983. Dispersal of Mesembryanthemaceae in arid habitats. Sonderbaende des Naturwissenschaftlichen Vereins Hamburg 7, 381–390.

Ihlenfeldt, H.D., 1994. Diversification in an arid world: the Mesembryanthemaceae. Annual Review of Ecology and Systematics 25, 521–546.

Irish, J., 1994. The biomes of Namibia, as determined by objective categorisation. Navorsinge van Die Nationale Museum Bloemfontein 10, 550–591.

IUCN, 2001. IUCN Red List Categories and Criteria: Version 3.1. IUCN Species Survival Commission. IUCN, Gland, Switserland and Cambridge, UK.

IUCN Standards and Petitions Subcommittee, 2013. Guidelines for Using the IUCN Red List Categories and Criteria Version 10.1. Prepared by the Standards and Petitions Subcommittee.

Kendall, M.G., Moran, P.A.P., 1963. Geometric Probability. Griffin, London.

Krebs, C.J., 1999. Ecological Methodology (Vol. 2nd). Addison-Wesley Educational Publishers, Inc., Menlo Park, California.

Loots, S., 2005. A red data book of Namibian plants. Southern African Botanical Diversity Network Report No. 38. SABONET, Pretoria and Windhoek.

Loots, S., 2011. National conservation assessment and management of two Namibian succulents, with specific reference to the Rössing uranium mine. Report on a Partnership Project Between the National Botanical Research Institute of Namibia, Rössing Uranium Limited, the Rio Tinto Group and the Royal Botanic Gardens, Kew. Windhoek, Namibia.

Mannheimer, C.A., 2006. A Taxonomic Revision of the Genera of the Subtribe Dracophilinae (Aizoaceae:Ruschioideae). (Thesis submitted in fulfilment of the requirements for the degree of Master of Science of Rhodes University). Rhodes University, South Africa.

Mannheimer, C.A., Loots, S., 2012. A simple aid to assessing cryptic succulents in the field. Dinteria 32, 78–80.

Meteorological Service Division, 2012. Tabulation of Climate Statistics for Selected Stations in Namibia. Ministry of Works and Transport, Republic of Namibia.

Miller, R.M., 2008. The Geology of Namibia Volume 1: Archaean to Mesoproterozoic. Ministry of Mines and Energy. Geological Survey, Windhoek, Namibia.

Morisita, M., 1957. A new method for the estimation of density by spacing method applicable to non-randomly distributed populations. Physiology and Ecology 7, 134–144.

Philippi, T., 2005. Adaptive cluster sampling for estimation of abundances within local populations of low-abundance plants. Ecology 86, 1091–1100.

Plant Red List database of Namibia. 2016. National Botanical Research Institute, Windhoek, Namibia.

Raimondo, D., von Staden, L., Foden, W., Victor, J.E., Helme, N.A., Turner, R.C., Kamundi, D.A., Manyama, P.A., 2009. Red List of South African Plants. South African National Biodiversity Institute, Pretoria, South Africa.

Schmiedel, U., Jürgens, N., 1999. Community structure on unusual habitat islands: quartz fields in the succulent Karroo, South Africa. Plant Ecology 142, 57–69.

Schwantes, G., 1957. Flowering Stones and mid-day Flowers. Ernest Benn Limited, London.

Strohbach, B., 2001. Vegetation survey of Namibia. Namibia Scientific Society Journal 49, 93–124.

# Appendix 2

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

Ms. Sonja Loots, Senior forester, [1]Swedish University of Agricultural Sciences, Department of Plant Breeding, Box 101, 230 53 Alnarp, Sweden, +46(0)40415565, sonja.loots@slu.se, sonja.loots.solo@gmail.com, M. Sc., +46(0)737750915.
[2]National Botanical Research Institute, Ministry of Agriculture, Water and Forestry, Private Bag 13184, Windhoek, Namibia, +264612022020, Sonja.Loots@mawf.gov.na

Dr. Christiane M. Ritz, Section leader, Senckenberg Museum für Naturkunde Görlitz, Am Museum 1, D-02826 Görlitz, Germany, +49(0)358147605330, Christiane.ritz@Senckenberg.de, Ph.D.,

Ms. Michaela Schwager, Technical assistant, Senckenberg Museum für Naturkunde Görlitz, Am Museum 1, D-02826 Görlitz, Germany, +49(0)358147605152, Michaela.schwager@Senckenberg.de,

Ms. Jasna Sehic, Laboratory engineer, Swedish University of Agricultural Sciences, Department of Plant Breeding, Balsgård, Fjälkestadsvägen 459, 29194 Kristianstad, Sweden, +46(0)44265802, jasna.sehic@slu.se, Fil. Mag. +46(0)702215324,

Dr. Larisa Garkava-Gustavsson, Reader, Swedish University of Agricultural Sciences, Department of Plant Breeding, Box 101, 23053 Alnarp, Sweden, +46(0)40415163, larisa.gustavsson@slu.se, Ph.D., +46(0)702858114,

Dr. Veit Herklotz, Scientific assistant, Senckenberg Museum für Naturkunde Görlitz, Am Museum 1, D-02826 Görlitz, Germany, +49(0)358147605303, Veit.herklotz@Senckenberg.de, Ph.D.,

Prof. Hilde Nybom, Professor emerita, Swedish University of Agricultural Sciences, Department of Plant Breeding, Balsgård, Fjälkestadsvägen 459, 29194 Kristianstad, Sweden, Tel. +46(0)44265802, hilde.nybom@slu.se, Ph.D., +46(0)703444369

## Abstract

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 since detailed field data and assessments of genetic diversity are lacking.

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

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 was recorded as well as altitude, aspect, slope, soil texture and substrate. AFLP markers were employed to study 52 individuals from seven populations.

In total 8,465 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.

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.

## Introduction

Southern Africa harbours many endemic leaf succulents because of the environmental conditions with aridity and variable geology (Young & Desmet 2016), but few of these taxa have been thoroughly studied in spite of imminent threats from 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 & Cole 2005; 2006) that are often referred to as "stone plants" or "flowering stones" due to their ability to blend in with their substrates on gravelly plains, rocky outcrops and slopes, making 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 at 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 five cm of the soil layer and must compete with other species with roots 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 since 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.

Genetic variability and processes such as isolation-by-distance and genetic drift, can be studied by DNA-based markers, and appear to be closely associated with life form, breeding system, mode of seed dispersal and successional status (Nybom 2004). 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 variables 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.

## Materials and Methods

### *Study area and habitat parameters*

*Lithops ruschiorum* grows in the northern and central Desert biome of Namibia (Irish 1994), and has a distribution that stretches for more than 600 km along the coast and approximately 75 km inland (Cole & Cole 2005). Most of the localities are found within Skeleton Coast National Park (encompassing almost the entire northern Namib Desert, north of the Ugab River) and Dorob National Park (the major part of the central Namib Desert). The central Namib Desert is characterised by mean monthly minimum and maximum temperatures of +1 to 41 °C. Average annual rainfall is very sparse and unpredictable, ranging from 15 mm at the coast to 87 mm further inland (approx. 100 km) in the central Namib (Lancaster et al. 1984), and from 11 to 42 mm in the northern Namib (Irish 1994). However, much more precipitation is instead received as fog, caused by moist air flowing over the cold Benguela current along the Namibian coastline, and ranging from 183 mm per year near the coast to 3 mm 100 km inland in the central Namib (Lancaster et al. 1984). More detailed climate data for the Namib Desert is not accessible. 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.

*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 license area in the central Namib Desert, to Khumib River in the northern Namib (Figure 2). In 2011, 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 cross-pollination between populations 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 (Rössing Uranium Limited), received special attention due to its size and to previous plant and habitat analyses. This population is situated in an area with a variety of habitat types approximately 60 km inland while all the other populations are found well within the fog zone, either closer to the coast (seven populations) and/or on much lower and flatter land (one population). Burke (2005) divided the RUL population into 20 biotopes based on the dominant plant species and number of endemic and Red Listed species in each biotope. Twelve of these contained *L. ruschiorum* and/or *Adenia pechuelii* (Engl.) Harms, another endemic succulent plant species. In the present study, a total of 68 sampling points, one km apart in grid square format, was 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 GPS coordinates (in WGS84 format) and saved as waypoints in a GPS to enable 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 were 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 (Suppl. Table 1a). 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/m$^2$). A total of 22 soil samples were taken in different sites, and pH was determined for each sample using a Hanna microprocessor pH meter.

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 (Suppl. Table 1b). 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 intra-specific 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 analysis*

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 ½ leaf was collected from 10 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 hours 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. DNA 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 rpm, and in the last step the DNA was eluted in only 2 × 50 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 was 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); 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). PCR reactions 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. AFLP profiles were scored as presence (1) and absence (0) of fragments ranging from 80 to 300 bp, using the software Genographer 2.1.4 (Banks & Benham 2008). Bands which were detected in <75% of repetitions of the positive controls were deleted.

### *Statistical analysis*

The numerical plant and habitat parameters (altitude, slope, distance from coast, occupied area, plant number, 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: (a) sites in the RUL population and (b) sites in the other eight populations since RUL was represented by more data than all the other populations taken together and would otherwise result in a heavily skewed data set. First 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, due to 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. One-way ANOVAs 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. Due to 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, which were log transformed and tested with Shapiro-Wilk for normality. 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 et al. 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.

## 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 8,465 *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 2,200 plants, and the second largest was RUL in the southern part of the distribution area, with just over 2,000 plants. Due to the considerable challenges in detecting *Lithops* plants in their natural habitat, plant numbers reported here are likely lower than reality, but relative differences between populations and sites should still be accurate.

Percentage mature plants out of the total number was 90.3%, while 8.6% were damaged and 1.1% were juveniles (Supplementary Tables 1a and 1b). Juveniles are exceptionally hard to spot and many are likely to have been overlooked. The Khumib River population had the highest percentage juvenile plants (4.8%) while RUL had only 0.6% and View Point and Ugab Salt Works none at all. The highest percentage 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 two plants to 161, the eight sites at Henties Bay–Uis Road from five 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 m² to 12,397 m², the five Rössing Mountain sites ranged from below 5,000 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*

Since *Lithops* have a very patchy distribution also on apparently suitable habitats, and since 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 sites instead of entire populations. Information about plant number and occupied area for each site is presented in Supplementary Tables 1a and 1b.

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 ten m² to 12,000 m², with a mean of 1,101 m² (not measured for fewer than three plants), while plant number per site ranged from one 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 and a median value of 0.029 plants per m².

In the other eight populations, *Lithops* plants occurred in one to 12 sites per population, and a total of 43 sites were analysed. Occupied area for these sites varied from 200 m² to 22,684 m² with an average of 3,885 m², and plant number from three 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 and a median value of 0.039 plants per m².

The boundaries of a site (i.e. occupied area) were determined by 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. 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 datasets have a high percentage of sites with large occupied areas that have low densities (39% for RUL; 50% for the other eight), but RUL has the highest percentage small sites with high densities (33% for RUL; 10% for the other eight) 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). 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 Supplementary Tables 1a and 1b. 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 since these varied too much within-site. PCA analyses were performed on all the habitat variables together with occupied area and number of plants for RUL (Figure 2a) and the eight populations (Figure 2b). 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 to 704 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 over-representation of the unusual substrates felspar 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 (Suppl. Table 2). Level sites had the most plants followed by SW-facing sites. Aspect was not associated with plant number according to ANOVA (Table 3) or GLM (Table 4). In the eight populations data set, the most common aspect was W (8 sites) followed by SW and S+SE+E (7 sites each) and NE+N+NW (six sites). According to ANOVA, aspect had a significant impact (F = 3.64, p = 0.016) with the highest number of plants in sites on SW-facing slopes whereas GLM did not detect any association.

Soil texture differed considerably between the two data sets, with only loamy sand (27 sites), silt loam (9 sites) and sand (7 sites) recorded in the eight populations, whereas clay-loam, light clay, loam and silt loam were almost equally common (11–15 sites) at RUL (Suppl. Table 2). 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 (6 sites), while quartz+other (17 sites), granite+other (15 sites) and pegmatite+other (4 sites) were most common in the eight populations data set (Suppl. Table 2). 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). GLM 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 (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 ($r = 0.445$, $p = 0.006$) but these associations were not found according to GLM. 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 due to the heavily reduced number of sites (26 instead of 43) 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 AFLP bands. Mean percentage 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 6). 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 Khumib River population.

The PCoA is a multi-variate 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 3a and 3b). 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 (Figures 3c and 3d).

## 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 due mainly to 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.

Due to 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). Since *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 Rössing Uranium Limited License Area (RUL where the species grows, at varying densities, on approximately 52 km$^2$.

### *Plant number, occupied area and plant density*

A clumped distribution has been described for many species in the family Aizoaceae (Ihlenfeldt 1983; Burke & Mannheimer 2003), 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 while median density was similar. 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 meter.

### *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 (Lancaster, Lancaster & Seely 1984; Olivier 1995;

Seely & Henschel 1998; Hachfeld & Jürgens 2000; 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 percentage contribution was greatest 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 been described also 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 2b) 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 and plant number, and between slope and plant density. These associations are most likely due to 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. Since 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 due to the fact that 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 hold 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 under-estimated (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. In contrast, the large Khumib River population is further inland but situated on slopes in fog-trapping valleys.

South-facing slopes should be especially beneficial since they are less exposed to sunshine and the plants therefore suffer less from desiccation. As expected, SW- and S to E-facing slopes harboured sites with the highest plant density in both data sets. Our data suggest that there is, however, also an effect of the type of precipitation received in the different populations. Plant number was the highest on SW-facing slopes in the eight populations. 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 instead found on S to E-facing slopes. In Namibia, rain usually arrives with eastern winds, and the rain-dependent *L. pseudotruncatella* had its highest plant density in SE- or S-facing plots (Loots & Nybom 2016).

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 is 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 characterized by dark mottled and banded gneiss of the Khan formation. Here, the higher sunlight adsorption resulting from the dark colour, is perhaps outweighed by positive factors like the short distance from the sea, and beneficial elevation and aspect.

### *Genetic structure*

AFLP markers have been used to successfully investigate differentiation at population level of species in the sub-family Ruschioideae (Ellis, Weis & Gaut 2006; Buys et al. 2008), 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 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 (Smith et al. 1998; Cole & Cole 2005) 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 (i.e., some centuries ago) 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 L. ruschiorum*

In the assessment for the Red Data Book (Loots 2005), the largest population of *L. ruschiorum* was inferred to contain no more than 1,100 mature plants. The present study shows that there are at least two populations with over 2,000 plants with the largest at Khumib River in Skeleton Coast Park. The second largest, at RUL, should also be conserved, especially considering its distance from the Skeleton Coast Park, the fact that the mine has changed ownership and the long-term monitoring programme based there for *L. ruschiorum*.

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

The current status of LC 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 thirty 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. Recent anecdotal evidence suggests that poaching of *Lithops* is on the increase. 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, particularly on plant density in populations closer to the coast, whereas rain is probably more important at RUL (Rössing Uranium Limited) 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.

## Acknowledgements

J.-E. Englund is acknowledged for statistical help, M. Fatih for logistical support in Sweden and the staff of the Senckenberg BIK-F laboratory (Frankfurt am Main, Germany) for technical support in AFLP analyses. Financial support was received from Rio Tinto, RBG Kew, Rössing Uranium Limited, MAWF and Sida (Swedish International Development Cooperation Agency) as part of

Nordic support to SADC Plant Genetic Resources Project. Staff of the NBRI assisted with field work and logistical support.

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

## References

Banks, T.W. & Benham, J.J., 2008, *Genographer version 2.1.4*. Montana State University.

Bates, D., Maechler, M., Bolker, B. & Walker, S. 2015, 'Fitting linear mixed-effects models using lme4', *Journal of Statistical Software* 67, 1–48, doi:10.18637/jss.v067.i01.

Burke, A. & Mannheimer, C.A., 2003, 'Towards a density measure for assessments of arid plant populations – examples from the southern Namib Desert', *African Journal of Ecology* 41, 254–256.

Burke, A., 2005, '*Rössing's Biodiversity strategy: Biotope mapping, reconstruction of the pre-mining situation and assessment of biodiversity value'*. Phase 2 report.

Buys, M.H., Janse van Rensburg, L.-L., Mienie, C.M.S., Barker, N., Burgoyne, P.M., Mills, L., Van Rensburg, L. & Hartmann, H., 2008, 'Applying AFLPs in Aizoaceae: The *Delosperma herbeum* complex as a case study', *Biochemical Systematics and Ecology* 36, 92–100.

Cole, D.T., 1988a, '*Lithops* Flowering Stones', Acorn Books CC & Russel Friedman Book CC, Johannesburg.

Cole, D.T., 1988b, '*Lithops* locality data. Numerical index C001-C392, and alphabetical species index', 3rd ed., Johannesburg.

Cole, D.T. & Cole, N.A., 2001, 'Ruschioideae: *Lithops*' in H.E.K. Hartmann (ed.), *Illustrated Handbook of succulent plants: Aizoaceae, F-Z.* pp 114–135, Springer, Berlin.

Cole, D.T. & Cole, N.A., 2005, '*Lithops* Flowering Stones'. Cactus & Co., Tradate, Italy.

Cole, D.T. 2006, '*Lithops* – two new taxa', *Cactus and Co*. X: 57-63.

Ellis, A.G., Weis, A.E. & Gaut, B.S., 2006, 'Evolutionary radiation of "stone plants" in the genus *Argyroderma* (Aizoaceae): unravelling the effects of landscape, habitat, and flowering time', *Evolution* 60, 39–55.

Guo, D., Young, A.J., Desmet, P.G. & Midgley, G.F., 2017, ‘Climate change impacts on dwarf succulents in Namibia as a result of changes in fog and relative humidity’, *Journal of Water Resource and Hydraulic Engineering* 6, 57–63.

Hachfeld, B., 2000, ‘Rain, fog and species richness in the central Namib Desert in the exceptional rainy season of 1999/2000’, *Dinteria* 26, 113–146.

Hachfeld, B. & Jürgens, N., 2000, ‘Climate patterns and their impact on the vegetation in a fog driven desert: the central Namib Desert in Namibia’, *Phytocoenologia* 30, 567–589.

Ihlenfeldt, H.D., 1983, ‘Dispersal of Mesembryanthemaceae in arid habitats’, *Sonderbaende Des Naturwissenschaftlichen Vereins Hamburg* 7, 381–390.

Irish, J., 1994, ‘The biomes of Namibia, as determined by objective categorisation’, *Navorsinge van die Nationale Museum Bloemfontein* 10, 550–591.

IUCN, 2001, *‘IUCN Red List Categories and Criteria’:* Version 3.1, IUCN Species Survival Commission, IUCN, Gland and Cambridge.

Jainta, H., 2017, ‘Wild *Lithops*’, Klaus Hess publisher, Göttingen and Windhoek.

Kellner, A., Ritz, C.M., Schlittenhardt, P. & Hellwig, F.H., 2011, ‘Genetic differentiation in the genus *Lithops* L. (Ruschioideae, Aizoaceae) reveals a high level of convergent evolution and reflects geographic distribution’, *Plant Biology* 13, 368–380.

Klaassen, E.S. & Kwembeya, E.G., (Eds), 2013, ‘A Checklist of Namibian Indigenous and Naturalised Plants’, *Occasional Contributions* No. 5, National Botanical Research Institute, Windhoek.

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B., 2017, ‘lmerTest Package: Tests in Linear Mixed Effects Models’, *Journal of Statistical Software* 82, 1–26, doi: 10.18637/jss.v082.i13

Lancaster, J., Lancaster, N. & Seely, M.K., 1984, ‘Climate of the central Namib Desert’, *Madoqua*, 14, 5–61.

Loots, S., 2005, ‘Red Data Book of Namibian plants’, *Southern African Botanical Diversity Report* No. 38. SABONET, Pretoria and Windhoek, 124 pp.

Loots, S., 2011, ‘*National conservation assessment and management of two Namibian succulents, with specific reference to the Rössing Uranium Mine’.* Report on a partnership project between the National Botanical Research Institute of Namibia, Rössing Uranium Limited, the Rio Tinto Group and the Royal Botanic Gardens, Kew, Unpublished.

Loots, S. & Nybom, H., 2017, ‘Towards better risk assessment for conservation of flowering stones: Plant density, spatial pattern and habitat preference of *Lithops pseudotruncatella* in Namibia’, *South-African Journal of Botany* 109, 112–115.

Mannheimer, C.A. & Loots, S., 2012, ‘A simple aid to assessing cryptic succulents in the field’, *Dinteria* 32, 78–80.

Mendelsohn, J., Jarvis, A., Roberts, C. & Robertson, A., 2002, ‘*Atlas of Namibia’*, David Phillip Publishers, Cape Town.

National Herbarium of Namibia (WIND), 2006, ‘*SPMNDB Database’, National Herbarium of Namibia (WIND),* National Botanical Research Institute, MAWF, Windhoek.

Nei, M., 1978, ‘Estimation of average heterozygosity and genetic distance from a small number of individuals’, *Genetics* 89, 583–590.

Nybom, H., 2004, ‘Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants’, *Molecular Ecology* 13, 1143–1155.

Olivier, J., 1995, 'Spatial distribution of fog in the Namib', *Journal of Arid Environments* 29, 129–138.

Peakall, R. & Smouse, P., 2012, 'GENALEX 6.5: Genetic analysis in Excel. Population genetic software for teaching and research - an update', *Bioinformatics* 28, 2537–2539.

R Core Team, 2018, '*R: A language and environment for statistical computing'.* R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Robertson, A., Jarvis, A., Mendelsohn, J. & Swart, R., 2012, 'Chapter 2: Weather and Water' in: *Namibia's Coast: Ocean Riches and Desert Treasures*, Directorate of Environmental Affairs, Ministry of Environment and Tourism, Namibia, pp 20–45.

Schuelke, M., 2000, 'An economic method for the fluorescent labelling of PCR fragments', *Nature Biotechnology* 18, 233–234.

Schwantes, G., 1957, '*Flowering Stones and Mid-Day Flowers'*, Ernest Benn, London.

Seely, M.K. & Pallet, J., 2008, '*Namib Secrets of a Desert Uncovered'*, Venture Publications, Windhoek.

Seely, M.K. & Henschel, J.R., 1998, '*The climatology of Namib fog'*, in Proceedings of the 1st Conference on fog and fog collection, 19-24 July 1998, Vancouver.

Smith, G.F., Chesselet, P., Van Jaarsveld, E.J., Hartmann, H., Hammer, S., Van Wyk, B.E., Burgoyne, P., Klak, C. & Kurzweil, H., 1998, '*Mesembs of the World: Illustrated Guide to a Remarkable Succulent Plant Group'*, Briza Publications, Pretoria.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. & Zabeau, M., 1995, 'AFLP: a new technique for DNA fingerprinting', *Nucleic Acids Research* 23, 4407–4414.

Witkowski, E.T.F. & Liston, R.J., 1997, 'Population structure, habitat profile and regeneration of *Haworthia koelmaniorum*, a vulnerable dwarf succulent, endemic to Mpumalanga, South Africa', *South-African Journal of Botany* 63, 363–370.

Young, A.J. & Desmet, P.G., 2016, 'The distribution of the dwarf succulent genus *Conophytum* N.E.Br. (Aizoaceae) in southern Africa', *Bothalia* 46(1), a2019. http://dx.doi.org/10.4102/abc.v46i1.2019

Table 1. *Investigated population 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 | 8,979 |
| View Point | 1 | 92 | 500 |
| *Total* | *94* | *8465* | *>214,530* |

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 RUL population, and at 43 sites in the other eight populations.*

*RUL*

| Parameter 1 | Parameter 2 | r | N | P |
|---|---|---|---|---|
| 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 8 populations.*

| Parameter 1 | Parameter 2 | r | N | P |
|---|---|---|---|---|
| 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* |

* 0.05>P>0.01, ** 0.01>P>0.001, *** P<0.001

Table 3. *Importance of three habitat parameters (aspect, soil texture and substrate) for plant number and plant density determined with* ANOVA *and Tukey pairwise comparisons, for 51 sites at* RUL *and for 43 sites at the other eight populations. For an extended version see Supplementary Table.*

*RUL*

| Parameter | df | F | p |
|---|---|---|---|
| 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 8 populations*

| Parameter | df | F | p |
|---|---|---|---|
| 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** |

* 0.05>P>0.01, ** 0.01>P>0.001

Table 4. *Factors and covariables selected by a GLM model to explain variation in plant density and total plant number for 29 sites at* RUL.

| Plant density | | | | | |
|---|---|---|---|---|---|
| Source of variation | Sum of squares | Mean squares | df | F-value | Pr(>F) |
| Aspect | 4.5694 | 1.1424 | 4 | 0.851 | 0.515 |
| Soil type | 6.4139 | 2.138 | 3 | 1.593 | 0.233 |
| Substrate | 2.9853 | 0.9951 | 3 | 0.741 | 0.544 |
| Soil type:Substrate | 8.5289 | 2.843 | 3 | 2.118 | 0.141 |
| Total plant number | | | | | |
| Source of variation | Sum of squares | Mean squares | df | F-value | Pr(>F) |
| Altitude | 0.006 | 0.00603 | 1 | 0.005 | 0.945 |
| Slope | 0.2198 | 0.21982 | 1 | 0.187 | 0.677 |
| Aspect | 3.8622 | 0.96556 | 4 | 0.822 | 0.546 |
| Soil type | 0.0869 | 0.02897 | 3 | 0.025 | 0.994 |
| Substrate | 0.7989 | 0.26629 | 3 | 0.227 | 0.875 |
| Altitude:Slope | 0.2396 | 0.23956 | 1 | 0.204 | 0.664 |
| Altitude:Aspect | 0.7563 | 0.25211 | 3 | 0.215 | 0.884 |
| Slope:Aspect | 2.7229 | 0.90764 | 3 | 0.773 | 0.541 |
| Altitude:Slope:Aspect | 1.1132 | 1.11315 | 1 | 0.948 | 0.359 |

Table 5. *Factors and covariables selected by a GLM to explain variation in plant density and total plant number for 26 sites at the other eight populations.*

Plant density

| Source of variation | Sum of squares | Mean squares | df | F-value | Pr(>F) | |
|---|---|---|---|---|---|---|
| Altitude | 0.0452 | 0.0452 | 1 | 29.181 | 1.0000 | |
| Coastal distance | 0.0413 | 0.0413 | 1 | 26.662 | 1.0000 | |
| Soil texture | 2.2046 | 1.1023 | 2 | 711.924 | 0.0014 | ** |
| Substrate | 2.8772 | 0.7193 | 4 | 464.555 | 0.0021 | ** |
| Aspect | 6.3412 | 1.5853 | 4 | 1023.846 | 0.0010 | *** |
| Slope | 0.4381 | 0.4381 | 1 | 282.930 | 1.0000 | |
| Soil texture:Substrate | 3.0655 | 1.5328 | 2 | 989.923 | 0.0010 | *** |
| Soil texture:Aspect | 0.4117 | 0.1372 | 3 | 88.620 | 0.0112 | * |
| Substrate:Aspect | 2.0906 | 0.6969 | 3 | 450.065 | 0.0022 | ** |
| Soil texture:Slope | 0.0000 | 0.0000 | 1 | 0.015 | 1.0000 | |
| Substrate:Slope | 2.2547 | 2.2547 | 1 | 1456.146 | 1.0000 | |
| Aspect:Slope | 1.0152 | 1.0152 | 1 | 655.636 | 1.0000 | |

Total plant number

| Source of variation | Sum of squares | Mean squares | df | F-value | Pr(>F) | |
|---|---|---|---|---|---|---|
| Altitude | 0.4868 | 0.4868 | 1 | 0.360 | 1.0000 | |
| Coastal distance | 0.14786 | 0.14786 | 1 | 0.109 | 1.0000 | |
| Soil texture | 0.19031 | 0.09515 | 2 | 0.070 | 0.9343 | . |
| Substrate | 2.48332 | 0.62083 | 4 | 0.460 | 0.7709 | |
| Aspect | 0.27828 | 0.06957 | 4 | 0.051 | 0.9913 | |
| Slope | 0.00852 | 0.00852 | 1 | 0.006 | 1.0000 | |
| Soil texture:Substrate | 2.84732 | 1.42366 | 2 | 1.053 | 0.4872 | |
| Soil texture:Aspect | 0.74887 | 0.24962 | 3 | 0.185 | 0.8990 | |
| Substrate:Aspect | 1.64287 | 0.54762 | 3 | 0.405 | 0.7677 | |
| Soil texture:Slope | 0.05525 | 0.05525 | 1 | 0.041 | 1.0000 | |
| Substrate:Slope | 0.00358 | 0.00358 | 1 | 0.003 | 1.0000 | |
| Aspect:Slope | 0.3391 | 0.3391 | 1 | 0.251 | 1.0000 | |

Table 6. *AFLP-based estimates of genetic variation, within each of the seven sampled L. ruschiorum populations, estimated as Percentage of Polymorphic Loci (PPL) and mean expected heterozygosity ($H_E$), and distribution of molecular variance among and within populations of seven populations or six populations (without Khumib River) based on AFLP data, with all results highly significant according to permutation tests ($p_{random \geq data} < 0.001$).*

*Variation within populations*

| 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 |

*Distribution of molecular variance among and within populations*

| 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 |

*Figure 1A* *Figure 1B*

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

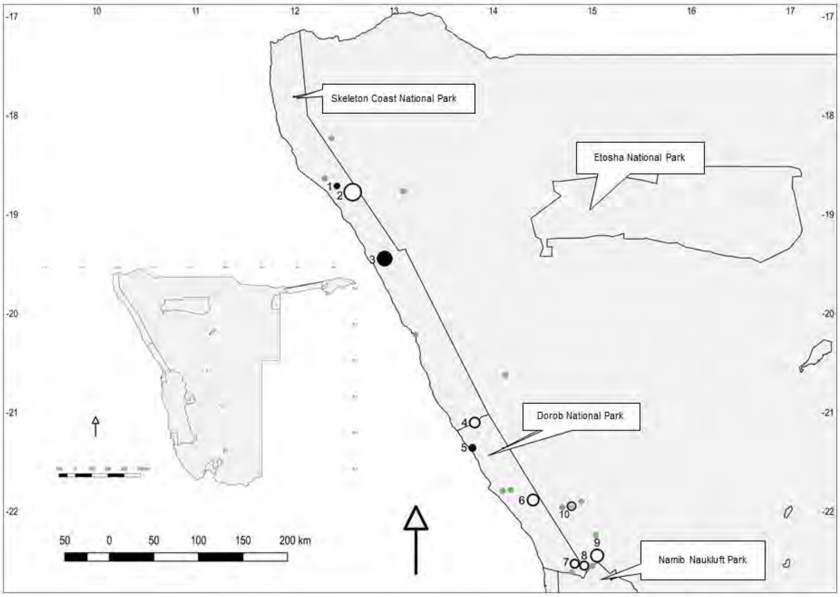


*Figure 2.* Distribution of surveyed *L. ruschiorum* populations. 1. View Point, 2. Khumib River, 3. Hoanib River, 4. Ugab River, 5. Ugab Salt Works, 6. Henties Bay–Uis Road, 7. Rössing Mountain, 8. Feldspar Ridge, 9. Rössing Uranium Mine License Area (RUL), 10. Henties Bay–Usakos Road. Circle size is 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 (green dots).

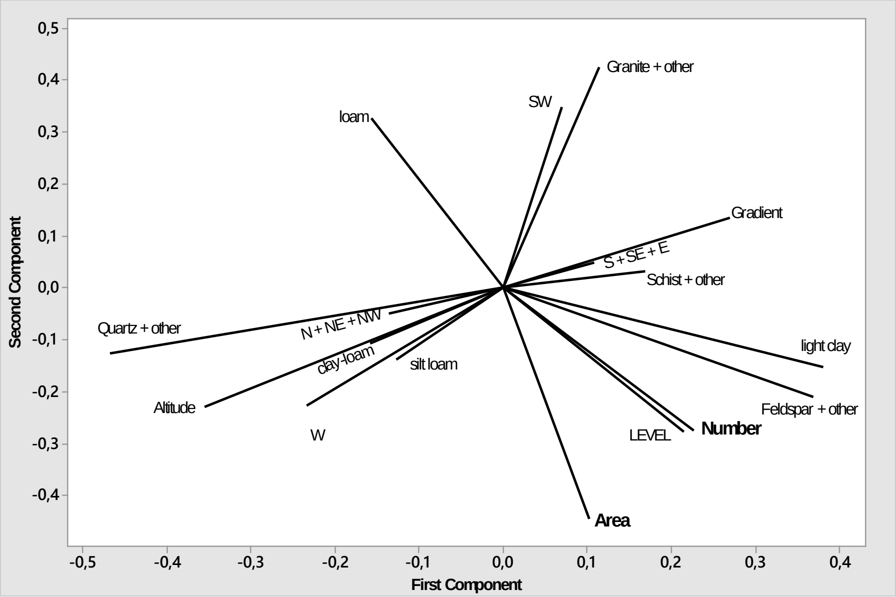


*Figure 3a.* Principal Component Analysis (PCA) showing the relationship between number of plants (Number), occupied area (Area) and habitat parameters: altitude, aspect (N+NE+NW, SW, S+SE+E, LEVEL), gradient (degree of slope), soil texture (loam, light clay, silt loam, clay-loam) and substrate (Quartz+other, Granite+other, Schist+other, Feldspar+other) in the 51 sites at RUL.

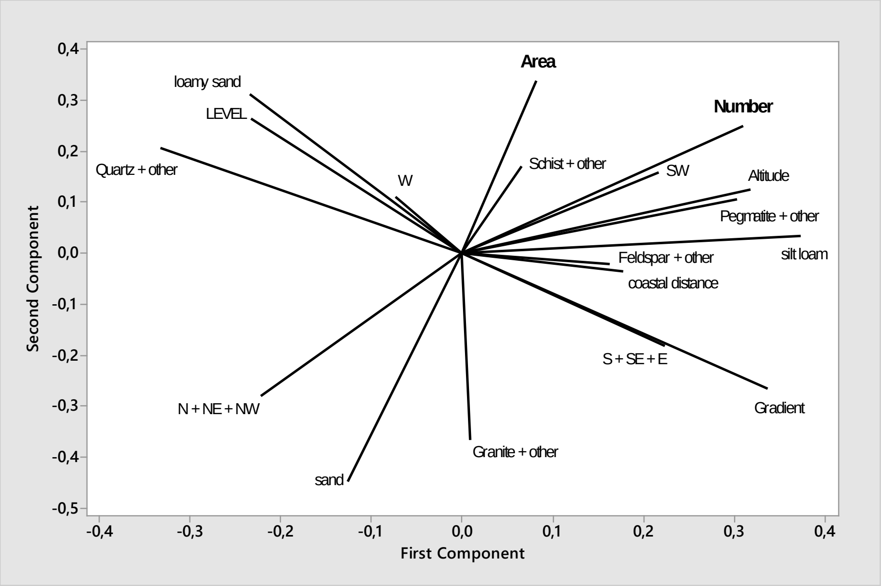


*Figure 3b.* Principal Component Analysis (PCA) showing the relationship between number of plants (Number), occupied area (Area) and habitat parameters: altitude, aspect (LEVEL, W, SW, S+SE+E, N+NE+NW), coastal distance, gradient (degree of slope), soil texture (loamy sand, silt loam, sand) and substrate (Quartz+other, Schist+other, Pegmatite+other, Feldspar+other, Granite+other) in the 43 sites of the other eight populations.

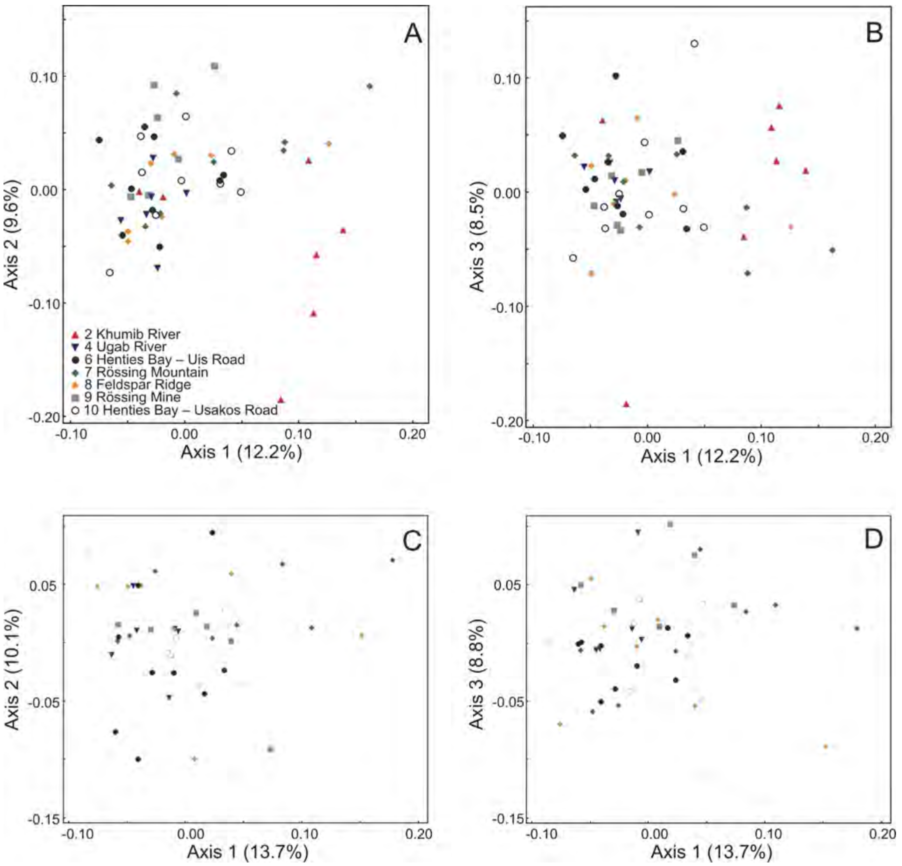


*Figure 4.* Principal Coordinate Analysis (PCoA) of AFLP data for 52 plants derived from seven populations of *L. ruschiorum.* 4A and 4C show dimensions 1 and 2, while 4B and 4D show dimensions 1 and 3. 4A and 4B include all populations, whereas 4C and 4D exclude the somewhat deviating population Khumib River.

Supplementary Table 1a. *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 (m²) | 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 |

| Site | Altitude (masl) | Soil texture | Substrate | Aspect | Slope | Mature plants | Damaged plants | Juveniles | Total number of plants | Occupied area (m²) | Density (number of plants/ occupied area) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 |

| Site | Altitude (masl) | Soil texture | Substrate | Aspect | Slope | Mature plants | Damaged plants | Juveniles | Total number of plants | Occupied area (m²) | Density (number of plants/ occupied area) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 |
| 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 |

| Site | Altitude (masl) | Soil texture | Substrate | Aspect | Slope | Mature plants | Damaged plants | Juveniles | Total number of plants | Occupied area (m²) | Density (number of plants/ occupied area) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 |

| Site | Altitude (masl) | Soil texture | Substrate | Aspect | Slope | Mature plants | Damaged plants | Juveniles | Total number of plants | Occupied area (m²) | Density (number of plants/ occupied area) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | | |

Supplementary Table 1b. *Field data, including habitat parameters, plant number, occupied area and plant density, collected for 43 Lithops ruschiorum sites in the other 8 surveyed populations. Sites 51 a–e are in Rössing Mountain (for geographic location see Fig. 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 tex-ture | Substrate | Aspect | Slope | Mature plants | Damaged plants | Juveni les | Total number of plants | Occu-pied 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 |

| Site | Altitude (masl) | Coastal distance (km) | Soil texture | Substrate | Aspect | Slope | Mature plants | Damaged plants | Juveniles | Total number of plants | Occu-pied area (m²) | Density (number of plants / occupied area) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 |

| Site | Altitude (masl) | Coastal distance (km) | Soil tex-ture | Substrate | Aspect | Slope | Mature plants | Damaged plants | Juveniles | Total number of plants | Occu-pied area (m²) | Density (number of plants / occupied area) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 |
| 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 |

| Site | Altitude (masl) | Coastal distance (km) | Soil texture | Substrate | Aspect | Slope | Mature plants | Damaged plants | Juveniles | Total number of plants | Occu-pied area (m²) | Density (number of plants / occupied area) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 |

| Site | Altitude (masl) | Coastal distance (km) | Soil tex-ture | Substrate | Aspect | Slope | Mature plants | Damaged plants | Juveniles | Total number of plants | Occu-pied area (m²) | Density (number of plants / occupied area) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | | |

Supplementary Table 2. *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 |
| | N+NE+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 |
| | N+NE+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 |
| | N+NE+NW | 6 | 1.4 | | | | A |

| Parameter | Categories | N | Mean | DF | F | P | Ttg |
|---|---|---|---|---|---|---|---|
| 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 |
| | N+NE+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 |

# Appendix 3

# Genetic variation among and within *Lithops* species in Namibia

Sonja Loots
[1]University of Agricultural Sciences, Department of Plant Breeding, Box 101, SE-23053 Alnarp, Sweden. Tel. +4640415565. Email: sonja.loots.solo@gmail.com. ORCID: 0000-0001-5769-9009.
[2]National Botanical Research Institute, Ministry of Agriculture, Water and Forestry, Private Bag 13184, Windhoek, Namibia. Tel. +264612022020. Email: Sonja.Loots@mawf.gov.na

Hilde Nybom
Swedish University of Agricultural Sciences, Department of Plant Breeding, Balsgård, Fjälkestadsvägen 459, SE-29194 Kristianstad, Sweden, ORCID: 0000-0002-4355-8106.

Michaela Schwager
Senckenberg Museum of Natural History Görlitz, Am Museum 1, D-02826 Görlitz, Germany, ORCID: 0000-0002-8147-8596.

Jasna Sehic
Swedish University of Agricultural Sciences, Department of Plant Breeding, Balsgård, Fjälkestadsvägen 459, SE-29194 Kristianstad, Sweden ORCID: 0000-0002-4406-4431.

Christiane M. Ritz
Senckenberg Museum of Natural History Görlitz, Am Museum 1, D-02826 Görlitz, Germany, ORCID: 0000-0002-7246-1004.

## Abstract

The dwarf succulent genus *Lithops* is endemic to Southern Africa. Species delimitation is often problematic and based mainly on leaf morphology, which is strongly associated with plant habitat.

Relationships between taxa and populations in Namibia were studied with Amplified Fragment Length Polymorphisms (AFLP) using 44 wild *Lithops* populations representing 15 species and 23 taxa in total. Four primer pairs produced 92 polymorphic bands in the 223 samples. Expected heterozygosity ($H_e$) within taxa ranged from 0.086 to 0.450.

Genetic and geographic distances were correlated according to a Mantel test. Analysis of molecular variance (AMOVA) showed only 23% variation among the 15 investigated species. Genetic differentiation and structuring was investigated with a principal coordinate analysis (PCoA), a neighbour-joining cluster analysis, a Bayesian Structure analysis and a discriminant analysis of principal components (DAPC).

In all four analyses, *L. optica* (Marloth) N.E.Br. and *L. herrei* L.Bolus, which differ only in flower colour, clustered closely together and are here combined under *L. optica*. The morphologically similar species *L. amicorum* D.T.Cole and *L. karasmontana* (Dinter & Schwantes) N.E.Br. clustered together, and *L. amicorum* is here reduced to the subspecific level: *L. karasmontana* subsp. *amicorum* (D.T.Cole) Loots & Ritz. Subspecific taxa overlapped to a large extent except for in *L. karasmontana* where 13% of the variability resided among subspecies; the nominal subspecies differed from the other two, subsp. *bella* (N.E.Br.) D.T.Cole and subsp. *eberlanzii* (Dinter & Schwantes) D.T.Cole, which are here combined under *L. karasmontana* subsp. *bella* (N.E.Br.) D.T.Cole.

**Key words**: Aizoaceae, Molecular marker, Phylogenetics, Succulent, Taxonomy

## Introduction

The well-known succulent genus *Lithops* N.E.Br. ('living stones') belongs to the family Aizoaceae, also known as the ice plant family, and can be found in almost any botanical garden. Aizoaceae is the largest succulent plant family in the world (Van Jaarsveld 1987) with an important centre of evolution in the arid parts of Southern Africa (Klak et al. 2003). Of the four proposed subfamilies, Ruschioideae, to which *Lithops* belongs, is the largest with 112 genera and some 1600 species (Klak et al. 2013).

*Lithops* plants store water in the reduced, highly succulent leaves which constitute their most prominent morphological feature. One plant often consists of a single leaf pair, fused at the base and cone-shaped in appearance, but can occasionally have up to 20 heads or more, depending on the species (Cole and Cole 2005). A fissure divides the top of the leaf pair into two, more or less equal halves. The name *Lithops* is derived from the Greek "*lithos*", which means stone and "*-ops*", which means face, and was first published by N.E. Brown in 1922 (Cole and Cole 2005).

The first *Lithops* species was described already in 1811 by Haworth as *Mesembryanthemum turbiniforme* Haw. based on material discovered by William John Burchell in South Africa (Nel 1946). Almost 100 years later, the second *Lithops* species was collected by K. Dinter in 1897 and published by N.E. Brown in 1908 as *Lithops pseudotruncatella* (Berg.) N.E.Br., from the vicinity of Windhoek, Namibia. During the following decades, dozens of species were published, especially in connection with expeditions headed by European plant collectors and the subsequent commercialisation of this material. A total of 93 *Lithops* taxa, including species, subspecies and varieties are currently recognised (Cole and Cole in Hartmann 2001; Cole and Cole 2005; Cole 2006, 2012), with 40 taxa occurring in Namibia. Some of these taxa overlap in morphology to such an extent that they are not easily identifiable without information about geographic origin, thus indicating an over-classification. For Namibia, 26 taxa, including 16 subspecies, are currently accepted by the National Herbarium of Namibia (WIND 2006–2011; Klaassen and Kwembeya 2013) but the classification has never been critically evaluated. By contrast, the South African National Biodiversity Institute (SANBI) National Herbarium recognizes only 25 species and 9 subspecies as occurring in South Africa, including the 4 species shared between South Africa and Namibia (Chesselet et al., 2003; Cole and Cole 2005) although one of these, *L. dinteri* subsp. *dinteri,* is not recognized by SANBI.

The combination of high public appeal and need of a very specific habitat to ensure plant survival and episodic reproduction, has made *Lithops* a group of great conservation concern. Presently, serious threats stem from both climate change and from direct human activities such as illegal collecting of seeds and live plants, and indirectly through habitat destruction resulting from prospecting, mining and off-road driving and recently also motion pictures filming projects, in addition to grazing by domestic livestock and wild animals (Cole and Cole 2005; Loots 2005, 2011; Loots et al. manuscript submitted; Jainta 2017).

Based mainly on literature, the rather limited herbarium material and field inventories, a national conservation status has been assigned to all of the Namibian *Lithops* species (IUCN 2001; Loots 2005; Klaassen and Kwembeya 2013; Plant Red List Database of Namibia 2016), and habitat preferences have been investigated in-depth for two of these species (Loots and Nybom 2017; Loots et al. manuscript submitted). More information is, however, needed in order to determine an accurate status for each individual taxon. An important aspect is the taxonomic classification within the genus, including the understanding of how factors such as adaptation to specific microhabitats have affected speciation. In addition, estimates of genetic variability within and among populations are necessary.

Within *Lithops* two subgenera; *Leucolithops* Schwantes (white flowers) and *Xantholithops* Schwantes (yellow flowers) have been described (Schwantes 1957). Other reproductive characters, like number of seed locules (5–7) and seed surface (tuberculate or rugose), have also been considered in *Lithops* taxonomy. Major emphasis has, however, been placed on leaf morphology including the occurrence of windows (i.e. special areas on the upper leaf surface that allow light transmittal to the inner parts of the leaves) thus prompting a division into sections *Fenestratae* Nel and *Afenestratae* Nel (Nel 1946). Leaf shape, colour and markings vary widely in the genus, apparently as an evolutionary adaptation to different substrates and soil types, and have been the basis of all *Lithops* taxonomic treatments (Nel 1946; Schwantes 1957; Hammer 1999), and a comprehensive book (Cole 1988a) and identification keys (Clark 1996; Cole and Cole in Hartmann 2001). These leaf traits could, however, have evolved as local adaptations to the phenominal variety of small ecological niches typical of Southern Africa, and their taxonomic value remains to be established. Cole and Cole (2005) produced an updated edition of their book with some new taxa and an updated key to the species. An annotated photographic book including maps was recently produced with *Lithops* in habitat (Jainta 2017). Similarly, a stone plant in South Africa, the genus *Argyroderma* N.E.Br., has apparently undergone diversification through a process of adaptive speciation in response to habitat selection (Ellis et al. 2006).

Using allozyme analysis, Wallace (1990) found considerable genetic similarity within *Lithops* and suggested that the genus must have evolved recently since its rapid morphological diversification is not accompanied by genetic divergence of a corresponding magnitude. This was later supported by Klak et al. (2003, 2004), who concluded that the core Ruschioideae, which include *Lithops*, must have radiated recently since there is extremely limited variation in nucleotide sequences. This is in concordance with the recent radiation of several unrelated succulent plant groups, that diversified during a period of global aridification in the late Miocene (Arakaki et al. 2011).

The only molecular phylogenetic study conducted specifically for the genus *Lithops* was published by Kellner et al. (2011), who analysed nuclear ribosomal DNA and plastid sequences from plant nursery-derived material of more than half of the currently recognised taxa as well as approximately 20 species in related genera. Polymorphism was again very low and samples of different genera often grouped together. However, when applying Amplified Fragment Length Polymorphism (AFLP) to 11 outgroup taxa and 49 *Lithops* taxa (species and subspecies), higher levels of polymorphism were encountered and nine clades could be identified within the genus *Lithops* (Kellner et al., 2011). Some of these nine clades do not fit the current morphology-based taxonomy, suggesting the need for a thorough taxonomical revision. Recently, the use of AFLPs in a study on intraspecific genetic variation in 7 Namibian populations of *L. ruschiorum* yielded medium to high genetic diversitiy within populations whereas genetic differentiation between populations was very low (Loots et al., Bothalia ABC, in press).

The aim of the present study was to produce information that can assist in making plant conservation decisions for *Lithops* in Namibia. More specifically, an evaluation of present-day taxonomy was undertaken by investigating genetic relatedness among the Namibian *Lithops* taxa using AFLP data, and by determination of the amount and partitioning of genetic variation between and within taxa and populations. The study was based on a large collection effort with freshly sampled material from 44 natural *Lithops* populations in Namibia, potentially representing 15 species and 23 taxa including subspecies and varieties.

## Materials and methods

### *Plant material*

Sixteen *Lithops* species are reported for Namibia according to Cole and Cole (2005) and Cole (2006, 2012). Most of these species grow mainly in the Nama Karoo Biome (Fig. 1) which occupies the largest portion of the interior of southern Namibia and is characterised by dwarf shrubland and grasses, and an annual mean rainfall of 50–230 mm (Irish 1994). The Succulent Karoo Biome, with low winter rainfall (40–90 mm) and extreme summer aridity, also contains a number of *Lithops* species. By contrast, only four species grow in the true Desert Biome with very harsh environmental conditions including a mean annual rainfall from 11 mm along the west coast to 22 mm in areas further inland: *L. ruschiorum* and *L. gracilidelineata* in the central and northern Namib Desert, and *L. francisci* in the southern Namib while *L. optica* occurs in the Desert Biome and the Succulent Karoo Biome. *Lithops pseudotruncatella* occurs in the Nama Karoo Biome and the Savanna Biome, characterised by a grassy ground layer and a distinct upper layer of woody plants, and an average summer rainfall ranging from 170 to 700 mm. Some *L. gracilidelineata* populations are also found in the Savanna Biome while the single known population of *L. werneri* occurs on the border between this biome and the Nama Karoo Biome.

Information on previously recorded *Lithops* populations in Namibia was obtained from the National Herbarium of Namibia (WIND, 2006-2011), key literature (Cole 1988a, 1988b) and some local experts. Field trips were undertaken from April 2011 to November 2012 to locate populations and sample material for DNA analyses. A total of 44 *Lithops* populations, representing 15 species and several subspecies and varieties according to morphology-based determinations in the field, were located and surveyed to determine the perimeter of each population (Table 1, Fig. 1). Where possible, seed capsules from at least 10 plants were sampled in a transect across the geographical range of the population. A photograph was taken of each sampled plant for further identification and reference purposes. Herbarium vouchers were collected and deposited in WIND if the population was large enough and if vouchers had not been collected previously (Table 2). In populations where seed was scarce but the plants produced many heads, a single fresh ½ leaf was collected from 10 multi-headed plants and stored directly in silica gel for DNA extraction. In the case of *L. werneri*, four seed capsules were picked from between the gravel in the single known population.

Up to 50 seeds per sampled plant were sown in pots in a greenhouse in Alnarp in Sweden, with 14 hours 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 approximately one year of cultivation.

### *Molecular analysis*

DNA extraction was performed on fresh, frozen or silica-dried leaf material using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions, with two modifications: all centrifugations were run at 14,000 rpm, and in the last step the DNA was eluted in only 2×50 µL to prevent excessive dilution in samples with low DNA concentration. DNA quality was checked by agarose gel-electrophoresis and photometry using Nanodrop 2000/2000c (Thermo Scientific, Wilmington, USA).

The DNA samples were submitted to standard AFLP procedure (Vos et al. 1995) except that restriction and ligation was 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); E46/M51 (ATT/CCA). In addition, E33/ M47 (AAG/CAA) was selected since it had proved useful in a previous study (Loots et al. manuscript submitted).

To avoid direct labelling of specific primers, the “poor man’s approach” according to Schuelke (2000) was used. PCR reactions were performed using an Eppendorf Mastercycler ep gradient S (Eppendorf AG, Hamburg, Germany). Automated detection of AFLP fragments was carried out by the Senckenberg Biodiversitäts- und Klimaforschungszentrum (SBik-F; Frankfurt am Main, Germany), using an ABI 3730 sequencing machine (ABI Life Technologies, Darmstadt, Germany) and the size standard LIZ-600 (ABI Life Technologies). To ensure unambiguous scoring of AFLP fragments, ten positive controls were repeated in each run consisting of 96 samples, thus each of the positive controls was run three to five times in total.

### *Data analysis*

Genetic diversity displayed as Percentage of Polymorphic Loci (PPL) and expected Heterozygosity ($H_e$), Analyses of Molecular Variance (AMOVA) and Principal Coordinate Analyses (PCoA) were computed with GenAlEx v. 6.5 (Peakall and Smouse 2012).

Genetic structure in the data was further assessed by Bayesian clustering using the program Structure 2.3.4 (Pritchard et al. 2000; Falush et al. 2007) and applying the admixture model with correlated allele frequencies. We analysed 10 replicates of models ranging from 1 to 15 clusters ($K = 15$) based on 1,000,000 generations after a burn-in of 500,000 generations. The rate of change in log probability across clusters was estimated according to the method of Evanno et al. (2005) and revealed peaks at 2, 3 and 7 clusters (Online supplement Fig. S1). Results of these models were graphically displayed using Distruct v. 1.1 (Rosenberg 2004). We performed a Discriminant Analysis of Principal Components (DAPC) using the package adegenet 2.0.0 (Jombart et al. 2010) in R environment (R Core Team 2015). Based on the Bayesian Information Criterion (BIC) we chose the model consisting of six clusters (Online supplement Fig. S1). Additionally, we calculated a Neighbour-joining tree based on Nei-Li distances and tested robustness by bootstrapping (1000 replicates) with Paup v. 4b10 (Swofford 2002). A Bayesian phylogeny was computed with MrBayes v. 3.2.6 (Huelsenbeck and Ronquist 2001) based on the restriction site model with coding bias “noabsencesites” and the Dirichlet distribution prior on state frequencies (3:1) estimated from the data set. Two runs each for 2,000,000 generations were performed with four independent chains, a sample frequency of 100 and a burn-in of 2500.

To analyse correlations between genetic (Bray-Curtis) distances and geographic distances (WGS84 coordinates transformed into Euclidean distances), we performed Mantel tests based on 9999 replications computed with the ADE-4 package (Dray et al. 2007) in R.

## Results

### *Genetic diversity within species and populations*

The four primer pairs produced 92 polymorphic bands for the final set of 223 samples. AMOVA showed 23% molecular variance among the 15 investigated species, and 24% when the material was instead divided into 23 different taxa (species, subspecies and varieties) (Table 3). Seven species were represented by 3–8 populations each. An AMOVA was performed to estimate percentage of molecular variance between populations within each species; *L. francisci* 18%, *L. gracilidelineata* 7%, *L. julii* 5%, *L. karasmontana* 12%, *L. pseudotruncatella* 9%, *L. ruschiorum* 2% and *L. schwantesii* 7% (Table 3).

For each taxon, diversity was estimated as expected heterozygosity (Table 1), with the lowest values found in *L. karasmontana* var. *lericheana* (0.086), *L. optica* (0.095) and *L. hermetica* (0.113) and the highest values in *L. pseudotruncatella* subsp. *pseudotruncatella* var. *pseudotruncatella* (0.450), *L. werneri* (0.342) and *L. ruschiorum* (0.331). Sample number was however restricted for many taxa, especially for those with low values of expected heterozygosity, and results should therefore be treated with caution. For taxa with only a single population sampled (with at least 6 samples each), values varied between 0.189 (*L. amicorum*) and 0.342 (*L. werneri)* thus providing a crude estimate of within-population diversity.

A moderate but significant association between genetic and geographic distances was found when all 223 samples were subjected to a Mantel test, $r = 0.329$, $p < 0.001$.

### *Intraspecific taxonomy*

Three of the sampled species, *L. karasmontana*, *L. pseudotruncatella* and *L. schwantesii*, were represented by two or more intraspecific taxa and were therefore analysed for variation at the intraspecific level. Plant material of *L. karasmontana* represented three subspecies: subsp. *bella*, subsp. *eberlanzii* and subsp. *karasmontana*. Of the latter subspecies, sampling had been undertaken of the nominal variety as well as of the varieties *immaculata* and *lericheana*. AMOVA showed 13% variance at the subspecies level, while variance among the three varieties of subsp. *karasmontana* was only 1% (Table 3). A PCoA was conducted for the total of 42 samples of *L. karasmontana* (Fig. 2A). Subspecies *karasmontana* (22 samples) was significantly separated from the other two subspecies (12 and 8 samples, respectively) which instead overlapped to a high degree. By contrast, there was no pattern of differentiation among the three varieties of subsp. *karasmontana* (15, 5 and 2 samples, respectively). A significant positive association was found between genetic and geographic distances when all 42 samples of *L. karasmontana* were subjected to a Mantel test, $r = 0.422$, $p < 0.001$.

For *L. pseudotruncatella*, 41 samples were available in total: subsp. *dendritica* (15 samples), subsp. *groendrayensis* (4 samples), subsp. *pseudotruncatella* (15 samples) and subsp. *volkii* (7 samples). An AMOVA showed that only 5% of the variance occurred among subspecies. In the PCoA, only subsp. *dendritica* showed some differentiation from the remainder (Fig. 2B). A weak but significant association was found between genetic and geographic distances when all 41 samples were subjected to a Mantel test, $r = 0.165$, $p < 0.001$.

Two varieties had been collected from *L. schwantesii*, the nominal variety and var. *urikosensis*, with 3 and 6 samples each. An AMOVA indicated 7% variance between these varieties but they could not be separated with a PCoA (not shown).

### *Interspecific variation*

Genetic differentiation within and among all 223 samples was evaluated with PCoA (Fig. 3). In spite of considerable overlapping, some differentiation among species or groups of species could be discerned. Samples of *L. ruschiorum* were clustered in the lower left-hand corner of the plot, while *L. amicorum*, *L. julii* and *L. karasmontana* instead occurred mainly in the lower right-hand corner. In the central and upper part of the plot, the remaining species overlapped without a clear-cut differentiation.

Relationships among taxa were evaluated by an unrooted neighbour-joining phylogenetic analysis (Fig. 4). Unfortunately, clades did not receive any support by bootstrap values but several of these clades nevertheless correspond to results of the other analyses (see below) and some appear to reflect present-day taxonomy. We detected one clade consisting of *L. amicorum* and *L. karasmontana* but without any differentiation between the two species, or between the infraspecific taxa of *L. karasmontana*. Three clades consisted of two species each: *L. dinteri* and *L. julii*, *L. herrei* and *L. optica*, and *L. francisci* and *L. hermetica*, respectively. Another clade contained all samples of *L. schwantesii* and one sample each of *L. fulviceps* and *L. gracilidelineata*. The remaining species, *L. fulviceps, L. gracilidelineata, L. pseudotruncatella, L. vallis-mariae* and *L. werneri,* were intermingled in four clades. Among these, only *L. vallis-mariae* (7 samples in total) formed a close-knit unit. The Bayesian phylogeny (Online supplement Fig. S2) yielded very similar topology but it was also not supported by posterior probabilities. In contrast to the neighbor-joining tree, most samples of *L. ruschiorum* formed a separate clade.

The models of Bayesian clustering (Structure analysis) with optimal numbers of clusters (2, 3 and 7 clusters; Evanno et al. 2005; Online supplement Fig. S1) are presented in Fig. 5. The model with 2 clusters (Fig. 5A) indicated a major division between *L amicorum, L. julii* and *L. karasmontana* on the one side and most of the other species on the other side. Admixture was, however, indicated for *L. dinteri, L. herrei, L. schwantesii, L. optica* and *L. werneri*. When the model $K = 3$ (Fig. 5B) was employed, *L. ruschiorum* appeared to constitute a cluster of its own. Application of the model $K = 7$ (Fig. 5C) resulted in a similar basic pattern compared to the phylogenetic analyses: *L. amicorum* and *L. karasmontana* formed one cluster (orange), *L. herrei* and *L. optica* formed another (white), and *L. francisci* and *L. hermetica* yet another (green), while *L. ruschiorum* occurred in a single-species cluster (violet) and most samples of *L. gracilidelineata, L. pseudotruncatella* and *L. werneri* were contained in the yellow cluster. In this analysis *L. julii* formed a separate cluster (red), whereas accessions of *L. dinteri* could not be unambiguously assigned to any cluster. In contrast to the other analyses, Bayesian clustering also grouped *L. schwantesii* and *L. vallis-mariae* (blue cluster).

Finally, a discriminant analysis of PCAs (DAPC) assigned each sample to one of six clusters (Fig. 5D). The obtained clusters were almost identical to Bayesian clustering for $K = 7$ except that *L. francisci* and *L. hermetica* no longer formed a cluster of their own. For some species, samples were placed in different clusters. Thus, almost half of the samples of *L. dinteri, L. karasmontana* and *L. werneri* were more similar to *L. julii* than to other samples of their own species.

## Discussion

### *Genetic variation in Lithops*

Application of AMOVA (Table 3) showed that 23% of the genetic variance occurred among the 15 studied species of *Lithops,* and 24% among the 23 taxa (also subspecies and varieties). These low levels of differentiation suggest that the *Lithops* taxa are closely related and represent a recently diversified species complex. Data on ongoing gene flow among species are scarce and contradictory: based on allozyme analysis, Wallace (1990) suggested a high degree of inter-fertility among yellow-flowered species and a similarly high degree of inter-fertility among white-flowered species. This view was not supported by Cole and Cole (2005), who found only about 12 possibly hybridogenic *Lithops* colonies out of 450 investigated.

Variance attributed to differences among populations of the same species varied from 2 to 18% (Table 3). This is considerably lower than RAPD- and AFLP-based parameters reported in a meta study by Nybom (2004) in which variation among populations had an overall mean of 34%. Our results can probably be explained by the perennial life form, outcrossing breeding system, insect pollination and small distribution area of most of the species, all of which act to restrict the differentiation between populations (Nybom and Bartish 2000; Nybom 2004).

Clearly, sample number affects the amount of within-population diversity ($H_e$). For populations with a minimum of 6 samples, $H_e$ varied between 0.19 and 0.24 except for the surprisingly high value encountered in *L. werneri* (0.34; Table 1). Previously reported analysis of the same 7 *L. ruschiorum* populations as in the present study (but a slightly larger set of samples) yielded a mean within-population diversity of 0.24 (Paper II) which is very similar to previously reported molecular marker-derived estimates of within-population diversity in perennials with a narrow-range distribution area, outcrossing breeding system, water-dispersed seeds and a preference for early-successional vegetation habitats (Nybom 2004). *Lithops werneri* has a very restricted distribution in the western-central part of the country and it is presently not possible to explain why this particular taxon would be more variable than the others.

### *Relatedness among taxa*

Previous attempts to utilize sequencing information for investigating phylogenetic relationships in *Lithops* have not been very informative since overall variation appears to be very restricted both in the genus (Kellner et al. 2011) and in the subfamily Ruschioideae (Klak et al. 2003, 2004). The main reason is probably a rapid and recent diversification of succulent plants from different families, brought about by the global expansion of arid environments in the late Miocene (Arakaki et al. 2011). Nevertheless, several studies have shown that AFLP markers provide meaningful information in recently diversified genera in Aizoaceae; e.g. in *Lithops* (Kellner et al. 2011), *Argyroderma* (Ellis et al. 2006) and *Delosperma* N.E.Br. (Buys et al. 2008) as well as in other plant genera for example in *Aylostera* Speg. (Cactaceae; Ritz et al. 2016) and *Rosa* L. (Koopman et al. 2008).

Performing a cluster analysis on allozyme data, Wallace (1990) was able to identify two major groups (with two subgroups each) of *Lithops* taxa and one outlier. Using three cultivated accessions for each of 49 taxa (species, subspecies and varieties) from Namibia and South Africa, Kellner et al. (2011) identified 9 different clades in *Lithops*, several of which overlapped with the groupings described by Wallace (1990).

Subjecting our AFLP data, obtained from sampling wild populations in Namibia, to phylogenetic analyses (NJ and Bayes dendrograms), Bayesian clustering analysis and a discriminant analysis, produced relatively concordant results. Taken together, these analyses suggest an almost linear continuum from [1] *L. ruschiorum* to [2] *L. fulviceps* + *L. gracilidelineata* + *L. pseudotruncatella* + *L. werneri* + *L. vallis-mariae* to [3] *L. francisci* + *L. hermetica* to [4] *L. schwantesii* + *L. vallis-mariae* to [5] *L. herrei* + *L. optica* to [6] *L. dinteri* to [7] *L. julii* to [8] *L. karasmontana* + *L. amicorum*. *Lithops vallis-mariae* yielded contradictory results and thus appears in two groups. It should also be noted that samples of *L. werneri* were quite heterogeneous rendering the position of this species very uncertain. Below, we discuss the proposed groups in more detail.

**[1+2+4]** ***L. ruschiorum, L. fulviceps + L. gracilidelineata + L. pseudotruncatella + L. werneri + L. vallis-mariae, L. schwantesii.*** This large group contains only yellow-flowering taxa, most of which are morphologically very distinct but in some cases geographically close in the western parts of the country. *Lithops ruschiorum* occurs further west than any of the other species, at least 170 km from *L. pseudotruncatella* and even further from the other species except for *L. gracilidelineata*, with which it has a partially overlapping distribution, and the single *L. werneri* population, which is situated within the range of *L. gracilidelineata*. *Lithops ruschiorum* is separated from the remainder in most analyses (Figs 3 and 5, PCoA, Bayesian structure, DAPC) but there is some overlap according to the NJ dendrogram (Fig. 4).

The *L. pseudotruncatella* complex is found mainly in the central part of Namibia, but some distant populations occur within 100 km from the distribution areas for *L. gracilidelineata*, *L. schwantesii* and *L. vallis-mariae*. *Lithops schwantesii* forms an almost discrete group in the NJ dendrogram (Fig. 4) but is close to *L. vallis-mariae* according to the Bayesian structure and DAPC analyses (Fig. 5), and there is less than 100 km between some of the populations of these two species. By contrast, *L. fulviceps* is geographically well separated by distance as well as by the Great Karas Mountains which appear to form a barrier between *L. fulviceps* and the more western species.

*Lithops pseudotruncatella* with its truncate profile, extremely variable size and many different leaf colours and patterns is morphologically very dissimilar to *L. ruschiorum* with its distinctly cordate profile and opaque, greyish to pale or creamy white face with few or no facial markings, but these two species have supposedly hybridized in cultivation to produce *L. steineckeana* Tisch. (Cole and Cole 2005). Whether this can be taken as an indication of a close genetic relationship is uncertain, and there is as yet no evidence of spontaneous natural hybridisation between these taxa. Also the remaining taxa in this group are, for the most part, quite distinctive in their morphology. Nervertheless, Kellner et al. (2011) reported that *L. ruschiorum* clustered together with *L. pseudotruncatella*, while *L. gracilidelineata* clustered with *L. wernerii*. In contrast to our data, *L. schwantesii* however clustered with *L. fulviceps*, whereas *L. vallis-mariae* occurred close to some outgroup taxa (Kellner et al. 2011).

**[3] *L. francisci* + *L. hermetica.*** A separate group for the two species *L. francisci* and *L. hermetica* was indicated by the NJ dendrogram and the Bayesian structure analysis (Figs 4 and 5). Both species grow in the southwestern part of Namibia, in the succulent Karroo, about 110 km apart although *L. francisci* occurs much closer to the coast where it can benefit from fog, whereas *L. hermetica* occurs further inland (about 80 km) where fog does not reach. Both species have yellow flowers and numerous dusky dots on the leaf surface but vary in several other traits. *Lithops francisci* has 15–20 mm wide flowers and forms more elevated and often multi-headed plants with a distinctly cordate-truncate profile and more or less occluded or sometimes absent windows, while *L. hermetica* has larger flowers (18–23 mm) but is otherwise smaller with fewer heads and distinctive dark grey windows on the leaves that blend in with the grey limestone habitat (Cole and Cole 2005). Jainta (2017) considers *L. francisci*, *L. hermetica* and *L. gesinae* de Boer (the latter not included in our study) to be sufficiently similar in morphology to be merged into one species, namely *L. francisci*.

Considerable genetic similarity between *L. francisci* and *L. hermetica*, and the large group described above, was indicated by the PCoA, the DAPC, and the Bayesian structure analyses with K = 2 and K = 3 (Figs 3 and 5). In the study of Kellner et al. (2011), *L. hermetica* was not analysed but *L. francisci* clustered with *L. gracilidelineata* and *L. werneri* in spite of the 550 km distance, including the entire Namib sand sea, that separates *L. francisci* from the other species. *Lithops francisci* is also morphologically very different from *L. gracilidelineata* and *L. werneri*, with the latter being one of the smallest species (leaves 20×15 mm) with very distinctive branched markings on the leaf face that are completely different from any other species. The much larger plants (leaves 30×25 mm) of *L. gracilidelineata* in the southern range of the distribution, where they look somewhat different from the northern populations, have equal-sized, mostly very pale leaf lobes, making the plants appear round or elliptic. This is fairly uncommon in *Lithops* where most species have unequal lobes. In addition, the high degree of rugosity with few markings on the face also gives these plants a unique appearance.

**[5] *L. optica* + *L. herrei*.** All our analyses suggested that *L. optica* (white flowers, leaves 20×15 mm) and *L. herrei* (yellow flowers with a white centre, leaves 23×17 mm) form a close-knit group, which is well separated from the remaining species. These two species formed a group also according to Wallace (1990) and Kellner et al. (2011), in the latter study together with the South-African species *L. geyeri* (yellow flowers, leaves 20×15 mm; not sampled here). Jainta (2017) proposed to combine *L. geyeri* with *L. herrei* under the name *L. herrei* L.Bolus, because of similarities in their body shape and facial patterns. In addition, *L. geyeri* was already reduced once before to varietal status under *L. herrei* by de Boer and Boom (1961).

Both *L. optica* and *L. herrei* occur within 50 km of the coast in southwestern Namibia, but the two species seem to be geographically separated by some 80 km. Most of the *L. herrei* populations grow in close proximity of the Orange River, where they probably benefit from fog coming up from the river valley. South of this river, *L. geyeri* populations occur within 100 km of *L. herrei*. In all three species, leaves are cordate-truncate and usually distinctly convex but populations closer to the coast have open windows and those further inland have maculate windows (Cole and Cole 2005). In the more coastally distributed *L. optica*, one half of the face is often lower than the other and the windows more often open than maculate, while in *L. geyeri* windows in most populations are opaque and less often open, possibly as an adaptation to the high amount of sunlight. Leaf colour of the three species is similar, except for the ’Rubra’ form in *L. optica*, which was once abundant but now is extremely rare due to over-collecting; one reason that this species is of conservation concern.

Cole and Cole (2005) reported several pairs of taxa with very similar leaf colour, pattern and markings but with yellow versus white flowers. They concluded that more analyses are needed to determine whether these similarities are due to convergent evolution or mis-classification. The genetic similarity detected in our study suggest that *L. optica* and *L. herrei* are very closely related, possibly with only a recent divergence in petal colour, and we propose to treat the two taxa under *L. optica*.

**[6+7] *L. dinteri* + *L. julii*.** *Lithops dinteri* subsp. *multipunctata* (yellow flowers, 25–30 mm) and *L. julii* subsp. *julii* (white flowers, 25–35 mm) cluster together in the NJ dendrogram (Fig. 4), while two samples of *L. dinteri* overlap with *L. julii* in the DAPC (Fig. 5). *Lithops julii* instead appears to constitute a group of its own in the Bayesian structure analysis (Fig. 5). *Lithops dinteri* and *L. julii* grow in close proximity in southeastern Namibia but are morphologically very different. *Lithops dinteri* subsp. *multipunctata* forms 2–5 headed plants with truncate, flat to slightly convex (20×15 mm) leaves, that have extensive linear markings in a very obscure window or an opaque face (windows often absent). Margins on the face are not clearly distinguishable, and the face itself is marked with numerous bold red lines, dots, hooks and/or dashes that are referred to as rubrications. *Lithops julii* subsp. *julii* forms 2–4 headed plants with similar but somewhat larger leaves (25×20 mm), and a slightly reniform face. Windows are usually occluded but there are channels in a network of narrow, slightly impressed grooves with linear rubrications, that are sometimes displayed only at the inner margins on the inside of the leaf lobe opposite the fissure (Cole and Cole 2005). The colours on the faces of these two species are also very different.

In the study by Kellner et al. (2011), *L. dinteri* clustered with *L. karasmontana* (white flowers, 20–35 mm). A certain similarity between these two species and *L. julii* was indicated also in our study, especially in the PCoA and the DAPC (Figs 3 and 5). Some populations in the outskirts of the distribution area of *Lithops karasmontana* complex occur within 100 km from *L. dinteri* and within 60 km of *L. julii*, but the species are morphologically quite different. In *L. karasmontana* subsp. *karasmontana* the leaves are truncate to slightly convex or even slightly concave; windows are usually absent or occluded and the rubrications are much less pronounced than in either *L. dinteri* or *L. julii*.

**[8] *L. karasmontana* + *L. amicorum*.** *Lithops karasmontana* and *L. amicorum* form a close-knit group in all of our analyses, which is concordant with both the geographic distribution in southwestern Namibia and morphology. *Lithops amicorum* grows in close proximity with subsp. *eberlanzii* of *L. karasmontana*, which also has white flowers and often grey–white leaves and facial features with rubrications similar to those of *L. amicorum* (Cole and Cole 2005; Cole 2006). However, all the intraspecific taxa of *L. karasmontana* have an elliptic-reniform face, whereas *L. amicorum* leaves have a mostly elliptic face. In addition, plants of *L. karasmontana* subsp. *eberlanzii* are medium-sized (leaves 25×20 mm, flowers 25–30 mm), whereas *L. amicorum* is the smallest *Lithops* taxon in Namibia (leaves 15×10 mm, flowers 20–25 mm). Although *L. amicorum* could be merged with *L. karasmontana*, it does not comfortably fit under any of the already existing subspecies or varieties but is distinctive enough to be ranked as a subspecies of its own. *Lithops amicorum* was not investigated by Kellner et al. (2011), while *L. karasmontana* clustered with *L. dinteri* as reported above.

### *Intraspecific classification*

The three investigated subspecies of *L. karasmontana* differ according to the AMOVA (13%, Table 3). The PCoA (Fig. 2) suggests that this is caused by the separation of subsp. *karasmontana* from subsp. *bella* and subsp. *eberlanzii*, which grow in closer proximity than the other subspecies and are not always easily distinguishable, although subsp. *bella* has much bolder, translucent windows. By contrast, populations of subsp. *karasmontana* and subsp. *bella* are relatively well separated geographically and are easy to distinguish morphologically; subsp. *bella* has a cordate-truncate profile with distinctly convex leaves, whereas subsp. *karasmontana* leaves usually are truncate and flat to only slightly convex. The channels on the face of subsp. *bella* are very broad, distinct and impressed, irregular and large, usually without rubrications, whereas channels in varieties of subsp. *karasmontana* are narrow, much reduced or absent altogether except for var. *lericheana*, which has broad, distinct but irregular channels with some rubrications. In subspecies *eberlanzii* the channels are present but not quite as bold as in subsp. *bella* and windows are usually occluded; subsp. *eberlanzii* is also extremely variable in colour, and known for its occasional but striking light green avocado-shaded specimens.

By contrast, the three varieties of subsp. *karasmontana* could not be separated by either analysis. These varieties are not geographically well separated and morphologically they are extremely variable, even within populations, which can make them hard to distinguish.

The four subspecies of *L. pseudotruncatella*, subsp. *dendritica*, subsp. *groendrayensis*, subsp. *pseudotruncatella* and subsp. *volkii*, showed much less differentiation (5% in the AMOVA) than anticipated, whereas grouping the material by population showed a higher differentiation (9%, Table 3). Although not well-separated geographically, these subspecies are morphologically very distinct. Subspecies *pseudotruncatella* is extremely variable in size (leaves 25–40×20–30 mm; flowers 25–40 mm), colour and patterns, the reniform leaf lobes are usually very unequal in size and have numerous dusky dots. By contrast, subsp. *dendritica* has a distinct round-elliptic leaf face, with more or less equal lobes and often reddish in colour. Subspecies *groendrayensis* is also very distinct with its large size (leaves 35×30 mm, flowers 30–35 mm), monocephalous habit, flat and truncate top, round-elliptic leaf face, equal lobes and pale grey-white colour. Finally, subsp. *volkii* has a slightly reniform leaf face, unequal lobes and pale bluish-white colour (Cole and Cole 2005).

## Conclusions

Partitioning the molecular marker variability into 15 species or into 23 taxa yielded similar and comparatively low values of genetic differentiation, indicating that the genus is over-classified to some extent.

*Lithops optica* and *L. herrei* clustered together in all our analyses as well as in two previous molecular studies and are here combined into one species as *L. optica* with two flower morphs (white and yellow). Similarly, *L. amicorum* and *L. karasmontana* grouped together in all our analyses and are here combined under *L. karasmontana,* with *L. amicorum* as a subspecies.

Further work is required to resolve the relationships between *L. francisci*, *L. fulviceps*, *L. gracilidelineata*, *L. hermetica*, *L. pseudotruncatella*, *L. vallis-mariae* and *L. werneri*. Two additional species, *L. ruschiorum* and *L. schwantesii*, grouped with these species in some analyses but not in others and appear to be relatively well-differentiated from the remainder.

Conservation of *Lithops* diversity in Namibia can benefit from the acknowledgement of intraspecific taxa, provided that these can be easily distinguished. The two extra-nominal subspecies described in *L. karasmontana,* subsp. *eberlanzii* and subsp. *bella,* are howeveer very similar and are here summarized under the name *L. karasmontana* subsp. *bella.* The presently acknowledged varieties of *L. karasmontana* subsp. *karasmontana* show no differentiation according to DNA analyses and are morphologically extremely variable and difficult to identify. Rejection of these varieties by SANBI and WIND is therefore justified.

### Taxonomic treatment

***Lithops optica*** (Marloth) N.E.Br. ≡ *Mesembryanthemum opticum* Marloth, Transactions of the Royal Society of South Africa 1: 405–406, plate 27 (1908) [1910]. – TYPE: Namibia, Luderitz Bay, Prince of Wales Bay, March 1924, *E. Stöber*, Marloth 4675, 2615CA (PRE0389969-0) = *Lithops herrei* L.Bolus **syn. nov.** – TYPE: South Africa, Malmesbury Division, Swartwater, *s. d., H. Herre, SUG9176* (BOL133432, holo).

***Lithops karasmontana*** N.E.Br. **subsp. *amicorum*** (D.T.Cole) Loots & Ritz, **comb. nov.** *Lithops amicorum* D.T.Cole, Cactus & Co. 10(1): 59 (57–58; photos) (2006). – TYPE: Namibia, SE of Aus, 2716BB, 2004.05.03, *D.T. Cole,* C410 (WIND 000093697, holo).

***Lithops karasmontana*** N.E.Br. **subsp. *bella*** (N.E.Br.) D.T.Cole ≡ *Lithops bella* N.E.Br., Gard. Chron. Ser. III. (1922). – TYPE: Namibia, Damaraland, Aus, 21. August 1918, *I.B. Pole-Evans,* N.E.Brown 6110 (K000076619, holo). = *Lithops karasmontana* N.E.Br. subsp. *eberlanzii* (Dinter & Schwantes) D.T.Cole **syn. nov.** ≡ *Mesembryanthemum eberlanzii* Dinter & Schwantes, Z. Sukkulentenk. ii. 25 (1925). Since our searches in the herbaria BOL, B, HBG, M, NG, NH, PRE, SAM, WIND, Z, ZT, ZSS did not reveal any type specimen, we designate a neotype here. We chose the following specimen because its morphology matches with the description, it was sampled close one of the localities listed by Dinter & Schwantes: “Lüderitzbucht” and was genetically investigated by us: – NEOTYPE (**designated here**): Namibia, Karas, Lüderitz District, Hill N of Halenberg Station. Growing on W & SW-facing steep slopes on a kind of schist, 04. August 2011, *S. Loots & C.A. Mannheimer* (WIND 101798).

## Conservation status

The conservation status of *L. optica*: VU A4cd. This relates to a suspected global population reduction of at least 30%, where the time period includes the past and the future (up to a maximum of 100 years in the future) and where the causes of reduction may not have ceased and may not be reversible, based on a decline in habitat quality, area of occupancy (AOO) and actual or potential levels of exploitation (IUCN, 2001). The assessment is based on data that is available for 10 of the 24 known populations. South African populations are facing severe threats from the mining sector, poaching, off-road driving, small stock and live stock farming as well as future threats. Three populations have already disappeared and a further three have been reduced. The reduction seems to affect populations further from the coast. The Richtersveld populations are currently more exposed to habitat destruction and poaching, whereas most of the Namibian populations are still sheltered from poaching. However, the Tsau//Khaeb National Park in Namibia will be opened to tourism in the near future, and it is expected that this will impact more dwarf succulent populations. Prolonged droughts are believed to cause reductions in populations furthest away from the coast. The status of *L. karasmontana* subsp. *bella* (LC), after absorbing the populations of. *L. karasmontana* subsp. *eberlanzii* (LC), remains LC. The status of *L. karasmontana* subsp. *amicorum* (LC) also remains unchanged.

## Acknowledgements

Financial support was received from the SADC Plant Genetic Resources Centre (SPGRC) and Sida. Technical support was received from the Senckenberg Biodiversitäts- und Klimaforschungszentrum (SBik-F) and assistance with statistical analyses from V. Herklotz and advises in taxonomic treatments from V. Otte (Senckenberg Museum of Natural History Görlitz). Technical, administrative and financial support was received from the Ministry of Agriculture, Water and Forestry and especially staff at the National Botanical Research Institute. The Ministry of Environment and Tourism of Namibia granted research and entry permits into National Parks. Rössing Uranium Mine, Husab Uranium mine, Langer Heinrich Mine, Namdeb Diamond mine and Scorpion Zinc mine granted access and assistance in their license areas. The late Tok Schoeman, Hilde and Frikkie Mouton, Roy Earle and Keith Green provided information about localities. Lize von Staden of SANBI and the Threatened Species Programme, Pieter van Wyk from SANParks, and the Custodians of Rare and Endangered Wildlife are acknowledged for data to re-assess *Lithops optica*/ *L. herrei*. This work would not have been possible without the farm owners who granted access to their farms and provided assistance: Mr and Ms G.S. Berg, Ms B. Boehm-Erni, C. Buhrman, W. Diergaardt, L. Gessert, J. and S. Hopkins, B. and L. Eksteen, H. Esterhuizen, W. Itzko, Ms. Koch, A. Louw, J. and J. van Niekerk, Mr. I and Ms. du Plooy (Farm Garub), N. and C. Pretorius, H and O. Pretorius, A. Rusch, F. Snyman, B.N. and J. Steyn, P. and W. Swiegers, W. Teubner, G.G. Viviers, D. De Wet, R. and R. von Wielich.

## References

Arakaki M, Christin PA, Nyffeler R, Lendel A, Eggli U, Ogburn RM, Spriggs E, Moore MJ, Edwards EJ (2011) Contemporaneous and recent radiations of the world's major succulent plant lineages. Proc Nat Acad Sci USA 108:8379–8384.

Buys MH, Janse van Rensburg L-L, Mienie CMS, Barker N, Burgoyne PM, Mills L, Van Rensburg L, Hartmann H (2008) Applying AFLPs in Aizoaceae: The *Delosperma herbeum* complex as a case study. Biochem Syst Ecol 36:92–100.

Clark JY (1996) A key to *Lithops* N.E. Brown (Aizoaceae). Bradleya 14:1–9.

Cole DT (1988a) *Lithops* flowering stones. Acorn Books & Russel Friedman, Johannesburg, South Africa.

Cole DT (1988b) *Lithops* locality data. Numerical index C001–C392, and alphabetical species index). 3$^{rd}$ ed. Johannesburg.

Cole DT (2006) *Lithops* – two new taxa. Cactus & Co. 10:57–59.

Cole DT (2012) *Lithops karasmontana* subsp. *karasmontana* var. *immaculata*. Cactus & Co. 16:8–13.

Cole DT, Cole NA (2001) In Hartmann HEK (ed.) Illustrated Handbook of succulent plants: Aizoaceae, F-Z. Springer Verlag, Berlin.

Cole DT, Cole NA (2005) *Lithops* flowering stones. Cactus & Co, Tradate, Italy.

De Boer HW, Boom BK (1961) Notities over *Lithops*. Succulenta 4:41–42.

Dray S, Dufour A-B, Thioulouse J (2007**)** The ade4 package II: Two-table and K-table methods. R News 7:47–52.

Ellis AG, Weis AE, Gaut BS (2006) Evolutionary radiation of “stone plants” in the genus *Argyroderma* (Aizoaceae): unravelling the effects of landscape, habitat, and flowering time. Evolution 60:39–55.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molec Ecol 14:2611–2620.

Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molec Ecol Notes 7:574–578.

Chesselet P, Burgoyne PM, Klak C, Kurzweil H, Dold AP, Griffin NJ and Smith GF Mesembryanthemaceae (2003) In Germishuizen G, Meyer NL (eds.) Plants of Southern Africa: an annotated Checklist. Strelitzia 14. National Botanical Institute, Pretoria.

Hammer SA (1999) *Lithops* treasures of the veld. British Cactus and Succulent Society, Norwich.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

Irish J (1994) The biomes of Namibia, as determined by objective categorisation. Navorsinge van die Nationale Museum Bloemfontein 10:550–591.

IUCN (2001) IUCN Red List Categories and Criteria: Version 3.1. IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK.

Jainta H (2017). Wild *Lithops*. Klaus Hess publisher, Göttingen and Windhoek.

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11, 94.

Kellner A, Ritz CM, Schlittenhardt P, Hellwig FH (2011) Genetic differentiation in the genus *Lithops* L. (Ruschioideae, Aizoaceae) reveals a high level of convergent evolution and reflects geographic distribution. Plant Biol 13:368–380.

Klaassen ES, Kwembeya EG (eds) (2013) A Checklist of Namibian Indigenous and Naturalised Plants, Occasional Contributions No. 5, National Botanical Research Institute, Windhoek.

Klak C, Khunou A, Reeves G, Hedderson T (2003) A phylogenetic hypothesis for the Aizoaceae (Caryophyllales) based on four plastid DNA regions. Amer J Bot 90:1433–1445.

Klak C, Reeves G, Hedderson T (2004) Unmatched tempo of evolution in Southern African semi-desert ice plants. Nature 427:63–65.

Klak C, Bruyns PV, Hanáček P (2013) A phylogenetic hypothesis for the recently diversified Ruschieae (Aizoaceae) in southern Africa. Molec Phylogenet Evol 69:1005–1020.

Koopman WJM, Vosman B, Sabatino GJH, Visser D, Van Huylenbroeck J, De Riek J, De Cock K, Wissemann V, Ritz CM, Maes B, Werlemark G, Nybom H, Debener T, Linde M, Smulders MJM (2008) AFLP markers as a tool to reconstruct complex relationships in the genus *Rosa* (Rosaceae). Amer J Bot 95:353–366.

Loots S (2005) Red Data Book of Namibian plants. Southern African Botanical Diversity Report No. 38. SABONET, Pretoria and Windhoek.

Loots S (2011) National conservation assessment and management of two Namibian succulents, with specific reference to the Rössing Uranium Mine. Report on a partnership project between the National Botanical Research Institute of Namibia, Rössing Uranium Limited, the Rio Tinto Group and the Royal Botanic Gardens, Kew. Unpublished.

Loots S, Nybom H (2017) Towards better risk assessment for conservation of flowering stones: Plant density, spatial pattern and habitat preference of *Lithops pseudotruncatella* in Namibia. South Afr J Bot 109:112–115.

National Herbarium of Namibia (WIND) (2006–2011) SPMNDB Database. National Botanical Research Institute, MAWF, Windhoek.

Nel GC (1946) *Lithops*. University Press, Stellenbosch.

Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Molec Ecol 13:1143–1155.

Nybom H, Bartish IV (2000) Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Persp Pl Ecol Evol Syst 3:93–114.

Peakall R, Smouse P (2012) GENALEX 6.5: Genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatics 28:2537–2539.

Plant Red List Database of Namibia (2016) National Botanical Research Institute, Windhoek

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959.

QGIS Development Team (2018). QGIS Geographic Information System vers. 2.18.25. Open Source Geospatial Foundation Project, http://qgis.osgeo.org

R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Ritz CM, Fickenscher K, Foeller J, Hermann K, Mecklenburg R, Wahl R (2016) Molecular phylogenetic relationships of the Andean genus *Aylostera* Speg. (Cacataceae, Trichocereeae), a new classification and a morphological identification key. Pl Syst Evol 302:763–780.

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. Molec Ecol Notes 4:137–138.

Schuelke M (2000) An economic method for the fluorescent labelling of PCR fragments. Nature Biotech 18:233–234.

Schwantes G (1957) Flowering Stones and Mid-day Flowers. Ernest Benn Limited, London.

Swofford DL (2002) Paup* 4.0. Phylogenetic analyses using parsimony (and other methods). 4th ed. Sinauer Associates, Sunderland, Massachusetts.

Van Jaarsveld E (1987) The succulent riches of South Africa and Namibia. Aloe 24:45–92.

Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucl Acids Res 23:4407–4414.

Wallace RS (1990) Systematic significance of allozyme variation in the genus *Lithops* (Mesembryanthemaceae). Mitteilungen aus dem Institut für Allgemeine Botanik Hamburg 23:509–524.

Table 1. *The 23 taxa (species, subspecies and varieties) of Lithops included in the study. Number of populations, number of samples used in AFLP analysis as well as percentage of polymorphic loci (PPL) and expected heterozygosity ($H_e$) including standard deviation (SD) is given for each taxon.*

| Taxon | No. of populations | No. of samples | PPL [%] | He ± SD |
|---|---|---|---|---|
| *L. amicorum* D.T.Cole | 1 | 8 | 52.2 | 0.189 ± 0.022 |
| *L. dinteri* Schwant. subsp. *multipunctata* (H.W.de Boer) D.T.Cole | 1 | 4 | 40.2 | 0.157 ± 0.022 |
| *L. francisci* (Dint. & Schwant.) N.E.Br. | 2 | 14 | 79.4 | 0.290 ± 0.020 |
| *L. fulviceps* (N.E.Br.) N.E.Br. | 2 | 3 | 41.3 | 0.157 ± 0.021 |
| *L. gracilidelineata* Dint. subsp. *gracilidelineata* | 2 | 16 | 77.2 | 0.300 ± 0.020 |
| *L. hermetica* D.T.Cole | 1 | 3 | 28.3 | 0.113 ± 0.020 |
| *L. herrei* L.Bolus | 1 | 8 | 59.8 | 0.197 ± 0.021 |
| *L. julii* (Dint. & Schwant.) N.E.Br. subsp. *julii* | 4 | 14 | 66.3 | 0.243 ± 0.021 |
| *L. karasmontana* (Dint. & Schwant.) N.E.Br. subsp. *karasmontana* var. *karasmontana* | 3 | 15 | 79.4 | 0.298 ± 0.020 |
| subsp. *karasmontana* var. *immaculata* D.T.Cole | 1 | 5 | 48.9 | 0.190 ± 0.022 |
| subsp. *karasmontana* var. *lericheana* (Dint. & Schwant.) D.T.Cole | 1 | 2 | 30.7 | 0.086 ± 0.018 |
| subsp. *bella* (N.E.Br.) D.T.Cole | 2 | 12 | 78.3 | 0.292 ± 0.020 |
| subsp. *eberlanzii* (Dint. & Schwant.) D.T.Cole | 2 | 8 | 60.9 | 0.229 ± 0.022 |
| *L. optica* (Marloth) N.E.Br. | 1 | 3 | 22.9 | 0.095 ± 0.019 |
| *L. pseudotruncatella* (Berg.) N.E.Br. subsp. *pseudotruncatella* var. *pseudotruncatella* | 3 | 15 | 79.4 | 0.450 ± 0.028 |
| subsp. *dendritica* (G.C.Nel) D.T.Cole | 3 | 15 | 76.1 | 0.309 ± 0.020 |
| subsp. *groendrayensis* (H.Jacobsen) D.T.Cole | 1 | 4 | 54.4 | 0.217 ± 0.023 |
| subsp. *volkii* (Schwant. ex H.W.de Boer & B.K.Boom) D.T.Cole | 1 | 7 | 62.0 | 0.239 ± 0.022 |
| *L. ruschiorum* (Dint. & Schwant.) | 7 | 40 | 88.0 | 0.331 ± 0.017 |
| *L. schwantesii* Dint. subsp. *schwantesii* var. *schwantesii* | 2 | 3 | 40.2 | 0.161 ± 0.022 |

| Taxon | No. of populations | No. of samples | PPL [%] | He ± SD |
|---|---|---|---|---|
| subsp. *schwantesii* var. *urikosensis* (Dint.) H.W.de Boer & B.K.Boom | 1 | 6 | 56.5 | 0.223 ± 0.022 |
| *L. vallis-mariae* (Dint. & Schwant.) N.E.Br. | 1 | 7 | 60.9 | 0.225 ± 0.022 |
| *L. werneri* Schwant. & H.Jacobsen | 1 | 11 | 82.6 | 0.342 ± 0.019 |

Table 2. *Voucher specimens from sites where Lithops* DNA *collections were made for* AFLP *analysis. “None“ means that there is no voucher specimen for this particular locality.*

| Site number | Species | Collecting number |
|---|---|---|
| 1 | *L. julii* subsp. *julii* | SL 276 |
| 2 | *L. julii* subsp. *julii* | SB 1917 |
| 3 | *L. julii* subsp. *julii* | SL 275 |
| 4 | *L. julii* subsp. *julii* | No specimen collected |
| 6 | *L. karasmontana* subsp. *karasmontana* var. *lericheana* | SL 268 |
| 7a | *L. fulviceps* | SL 264 |
| 7b | *L. fulviceps* | SL 263 |
| 8 | *L. karasmontana* subsp. *karasmontana* var. *karasmontana* | SL 277 |
| 9 | *L. dinteri* subsp. *multipunctata* | SB 1954 |
| 10a | *L. karasmontana* subsp. *karasmontana* var. *karasmontana* | SL 278 |
| 10b | *L. karasmontana* subsp. *karasmontana* var. *immaculata* | No specimen collected |
| 11 | *L. karasmontana* subsp. *karasmontana* var. *karasmontana* | SL 279 |
| 13 | *L. schwantesii* subsp. *schwantesii* var. *schwantesii* | No specimen collected |
| 15 | *L. ruschiorum* | EK 1722 |
| 16 | *L. gracilidelineata* subsp. *gracilidelineata* | SL 281 |
| 17 | *L. gracilidelineata* subsp. *gracilidelineata* | SL 282 |
| 18 | *L. ruschiorum* | EK1918 |
| 19 | *L. ruschiorum* | EK 1772 |
| 20 | *L. schwantesii* subsp. *schwantesii* var. *urikosensis* | SL 283 |
| 21 | *L. amicorum* | C 410 |
| 22 | *L. schwantesii* subsp. *schwantesii* var. *schwantesii* | SL 286 |
| 23 | *L. karasmontana* subsp. *bella* | SL 284 |
| 24 | *L. karasmontana* subsp. *eberlanzii* | SL 285 |
| 25 | *L. karasmontana* subsp. *bella* | No specimen collected |
| 26 | *L. vallis-mariae* | SL 266 |
| 27 | *L. pseudotruncatella* subsp. *dendritica* | HK 1272 |
| 28 | *L. pseudotruncatella* subsp. *dendritica* | SL 287 |
| 29 | *L. pseudotruncatella* subsp. *dendritica* | SL 288 |
| 30 | *L. pseudotruncatella* subsp. *volkii* | No specimen collected |

| Site number | Species | Collecting number |
|---|---|---|
| 31 | *L. pseudotruncatella* subsp. *pseudotruncatella* var. *pseudotruncatella* | SL 297 |
| 32 | *L. pseudotruncatella* subsp. *groendrayensis* | SL 289 |
| 33 | *L. pseudotruncatella* subsp. *pseudotruncatella* var. *pseudotruncatella* | BS 2689 |
| 34 | *L. francisci* | SL 290 |
| 35 | *L. francisci* | SL 291 |
| 36 | *L. karasmontana* subsp. *eberlanzii* | SL 292 |
| 37 | *L. optica* | SL 293 |
| 38 | *L. herrei* | SL 294 |
| 39 | *L. hermetica* | SL 295 |
| 40 | *L. pseudotruncatella* subsp. *pseudotruncatella* | RUSCH E.F.T. 53533 |
| 41 | *L. werneri* | SL 296 |
| 42 | *L. ruschiorum* | SL 201 |
| 43 | *L. ruschiorum* | SL 237 |
| 44 | *L. ruschiorum* | EK 1777 |
| 45 | *L. ruschiorum* | SL 300 |

Table 3. *Differentiation of genetic variation with* AMOVA *at different hierarchical levels*

| Source of variation | d.f. | Sum of Squares | Estimated Variance | Percentage of Variation |
|---|---|---|---|---|
| AMOVA at species-level (all samples) | | | | |
| between species | 14 | 897.5 | 3.682 | 23* |
| within species | 208 | 2635.8 | 12.672 | 77 |
| AMOVA at taxon level (incl. subsp. and var.; all samples) | | | | |
| between taxa | 22 | 1077.1 | 3.902 | 24* |
| within taxa | 200 | 2456.1 | 12.281 | 76 |
| AMOVA within *L. karasmontana* | | | | |
| between subspecies | 2 | 72.915 | 1.880 | 13* |
| within subspecies | 39 | 485.966 | 12.461 | 87 |

| Source of variation | d.f. | Sum of Squares | Estimated Variance | Percentage of Variation |
|---|---|---|---|---|
| AMOVA within *L. karasmontana* subsp. *karasmontana* | | | | |
| between varieties | 2 | 25.791 | 0.098 | 1* |
| within varieties | 19 | 235.3 | 12.384 | 99 |
| AMOVA within *L. karasmontana* | | | | |
| between populations | 8 | 157.819 | 1.648 | 12* |
| within populations | 33 | 401.062 | 12.153 | 88 |
| v within *L. pseudotruncatella* | | | | |
| between subspecies | 3 | 59.4 | 0.738 | 5* |
| within subspecies | 37 | 474.0 | 12.810 | 95 |
| AMOVA within *L. pseudotruncatella* | | | | |
| between populations | 7 | 130.7 | 1.272 | 9* |
| within populations | 33 | 402.6 | 12.201 | 91 |
| AMOVA within *L. franciscii* | | | | |
| between populations | 1 | 24.6 | 2.389 | 18* |
| within populations | 12 | 131.4 | 10.950 | 82 |
| AMOVA within *L. gracilidelineata* | | | | |
| between populations | 1 | 19.6 | 0.895 | 7* |
| within populations | 14 | 174.5 | 12.464 | 93 |
| AMOVA within *L. julii* | | | | |
| between populations | 2 | 27.8 | 0.644 | 5* |
| within populations | 10 | 112.3 | 11.233 | 95 |
| AMOVA within *L. ruschiorum* | | | | |
| between populations | 6 | 82.0 | 0.294 | 2* |
| within populations | 33 | 396.8 | 12.025 | 98 |
| AMOVA within *L. schwantesii* | | | | |
| between subspecies | *1* | *15.8* | *0.881* | *7** |
| within subspecies | *7* | *86.2* | *12.310* | *93* |

Supplementary Table 1. *Populations of Lithops in Namibia where material for DNA samples were collected. Exact location not reported on purpose to avoid illegal collecting), approximative size, habitat and taxon*

| Site number | Species | Nearest town | Habitat |
|---|---|---|---|
| 1 | *L. julii* subsp. *julii* | Warmbad | Medium-sized population on level gravel plain; red-brown clay loam; granite and quartz; 947 masl. |
| 2 | *L. julii* subsp. *julii* | Warmbad | Medium-sized population; aspect SW; red-brown clay loam; granite; gentle slope; 932 masl. |
| 3 | *L. julii* subsp. *julii* | Warmbad | Medium-sized population; aspect W; reddish-brown soil; quartz and pegmatite; gentle slope; 959 masl. |
| 4 | *L. julii* subsp. *julii* | Warmbad | Medium-sized populationon on level gravel plain; red-brown clay loam; quartz and granite; 923 masl. |
| 6 | *L. karasmontana* subsp. *karasmontana* var. *lericheana* | Karasburg | Small population; aspect SE to E; red clay; quartz, granite and quartzite; very gentle slope; 1405 masl. |
| 7a | *L. fulviceps* | Karasburg | Medium-sized population on level terrain; red-brown clay loam; gneiss; 1545 masl. |
| 7b | *L. fulviceps* | Karasburg | Small population; red-brown clay-loam; gneiss; very gentle slope; 1560 masl. |
| 8 | *L. karasmontana* subsp. *karasmontana* var. *karasmontana* | Karasburg | Medium-sized population; red-brown clay; quartz; 1284 masl. |
| 9 | *L. dinteri* subsp. *dinteri* var. *multipunctata* | Warmbad | Medium-sized population; aspect S; red-brown clay; quartz, quartzite and schist; slope 5–10°; 781 masl. |
| 10a | *L. karasmontana* subsp. *karasmontana* var. *karasmontana* | Grünau | Medium-sized population on level terrain; 1233 masl. |
| 10b | *L. karasmontana* subsp. *karasmontana* var. *immaculata* | Grünau | Medium-sized population; aspect W; red-brown clay; white and pink quartz; slope 22°; 1283 masl. |
| 11 | *L. karasmontana* subsp. *karasmontana* var. *karasmontana* | Grünau | Medium-sized population on almost level terrain; aspect NW; red-brown clay-loam; quartz and granite; 1365 masl. |

| | | | |
|---|---|---|---|
| 13 | *L. schwantesii* subsp. *schwantesii* var. *schwantesii* | Helmeringhausen | Medium-sized population; aspect E to NE; red-brown loam; granite; slope 5–30°. |
| 15 | *L. ruschiorum* | Swakopmund | Large population; 604 masl. |
| 16 | *L. gracilidelineata* subsp. *gracilidelineata* | Swakopmund | Medium-sized population; aspect E to W; marble; slope 5–7°; 558 masl. |
| 17 | *L. gracilidelineata* subsp. *gracilidelineata* | Walvis Bay | Small population; aspect S; light-brown clay-loam; feldspar, quartz and basalt; slope 10–15°; 705 masl. |
| 18 | *L. ruschiorum* | Khorixas | Large population; light-brown clay; schist and pegmatite. |
| 19 | *L. ruschiorum* | Khorixas | Medium-sized population; aspect W. |
| 20 | *L. schwantesii* subsp. *schwantesii* var. *urikosensis* | Maltahöhe | Medium-sized population; aspect S to SW; light-brown clay; calcrete and limestone; gentle slope. |
| 21 | *L. amicorum* | Aus | Medium-sized population; redish-brown clay; gentle slope. |
| 22 | *L. schwantesii* subsp. *schwantesii* var. *schwantesii* | Aus | Small population; aspect W; red-brown clay; quartzite; gentle slope;1580 masl. |
| 23 | *L. karasmontana* subsp. *bella* | Aus | Small population; red-brown clay; granite; gentle slope. |
| 24 | *L. karasmontana* subsp. *eberlanzii* | Aus | Small population; red-brown clay-loam; calcrete; 1195 masl. |
| 25 | *L. karasmontana* subsp. bella | Aus | Small population; aspect NE; red-brown clay-loam; quartz, granite and pegmatite; slope 0–10°; 1402 masl. |
| 26 | *L. vallis-mariae* | Keetmanshoop | Small population on level terrain; red-brown clay; quartz and calcrete; 1003 masl. |
| 27 | *L. pseudotruncatella* subsp. *dendritica* | Rehoboth | Medium-sized population; red-brown clay; quartz and quartzite; gentle slope; 1743 masl. |
| 28 | *L. pseudotruncatella* subsp. *dendritica* | Rehoboth | Medium-sized population; aspect SW; red-brown clay; quartz and sandstone; slope 0–10°; 1565 masl. |
| 29 | *L. pseudotruncatella* subsp. *dendritica* | Rehoboth | Medium-sized population on level terrain; red-brown clay; quartz and schist; 1704 masl. |

| | | | |
|---|---|---|---|
| 30 | *L. pseudotruncatella* subsp. *volkii* | Windhoek | Small population; aspect N; brown loam-clay; quartz and dolomite; slope 10–15°. |
| 31 | *L. pseudotruncatella* subsp. *pseudotruncatella* | Windhoek | Large population; quartz; gentle slope. |
| 32 | *L. pseudotruncatella* subsp. *groendrayensis* | Rehoboth | Medium-sized population; red-brown clay; mainly quartz; slope 0–10°; 1469 masl. |
| 33 | *L. pseudotruncatella* subsp. *pseudotruncatella* | Windhoek | Small population on level terrain; red-brown clay; quartz; 1899 masl. |
| 34 | *L. francisci* | Lüderitz | Medium-sized population; aspect S to W; brown loam; calcrete and schist; slope 5–10°; 727 masl. |
| 35 | *L. francisci* | Lüderitz | Medum-sized population; aspect W to SWt; light red-brown gravelly loam; calcrete and schist; slope 1–25°; 554 masl. |
| 36 | *L. karasmontana* subsp. *eberlanzii* | Lüderitz | Medium-sized population; aspect S to W; light-brown loam; feldspar, schist and quartz; slope 5–20°; 607 masl. |
| 37 | *L. optica* | Lüderitz | Medium-sized population; aspect S to SW; brown clay; gneiss; gentle slope; 31 masl. |
| 38 | *L. herrei* | Oranjemund | Medium-sized population; aspect S to SW; red-brown loam sand; gentle slope; 98 masl. |
| 39 | *L. hermetica* | Aus | Medium-sized population; aspect W; red-brown sand; calcrete and limestone; gentle slope; 883 masl. |
| 40 | *L. pseudotruncatella* subsp. *pseudotruncatella* | Windhoek | Small population on level terrain; brown clay-loam; quartz and schist; 1992 masl. |
| 41 | *L. werneri* | Usakos | Medium-sized population; aspect E; brown loam-clay; granite; gentle slope; 1109 masl. |
| 42 | *L. ruschiorum* | Swakopmund | Medium-sized population; aspect S; light-brown clay; feldspar and granite; slope 0–30°; 256 masl. |
| 43 | *L. ruschiorum* | Swakopmund | Large population; aspect W; brown soil; schist with quartz intrusion; 458 masl. |

|  |  |  |  |
|---|---|---|---|
| 44 | *L. ruschiorum* | Henties Bay | Large population; aspect W; light-brown clay-loam; quartz and felpspar; slope 0–10°; 214 masl. |
| 45 | *L. ruschiorum* | Henties Bay | Small population; aspect N to S; light-brown clay-loam; quartz; very gentle slope; 627 masl. |

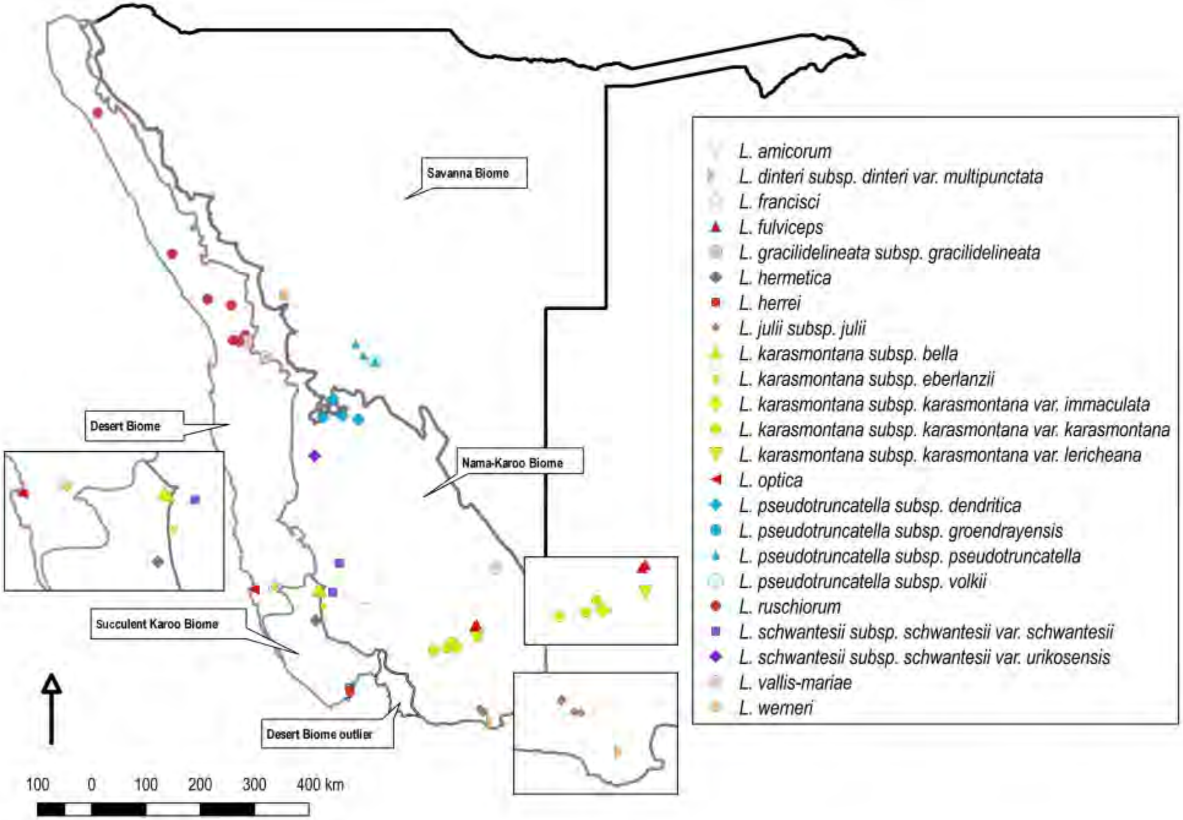


*Figure 1.* Geographic locations for the 44 analysed *Lithops* populations mapped on the biomes of Namibia, adapted from Irish (1994). Mapping done with QGIS version 2.18.25.

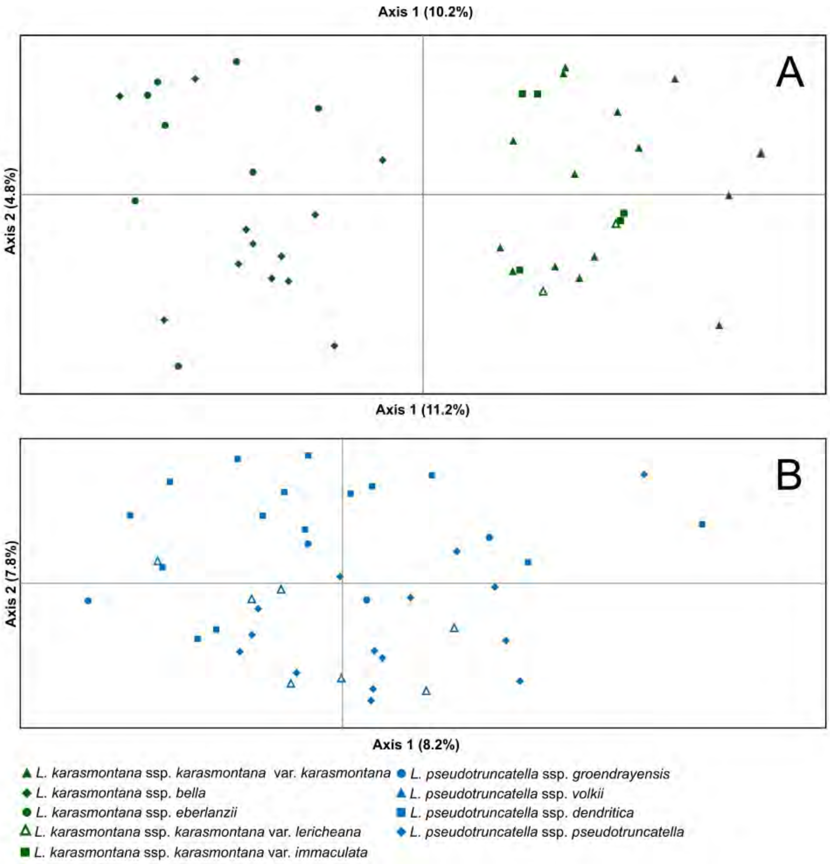


*Figure 2.* PCoA analyses based on AFLP data for two *Lithops* species. A. *L. karasmontana* including the three subspecies *bella*, *eberlanzii* and *karasmontana* with three varieties of the latter; var. *immaculata*, var. *karasmontana* and var. *lericheana*. Samples are plotted on the first two coordinates which together explain 20% of the variability. B. *L. pseudotruncatella* including the four subspecies *dendritica*, *groendrayensis*, *pseudotruncatella* and *volkii*. Samples are plotted on the first two coordinates which together explain 16% of the variability.

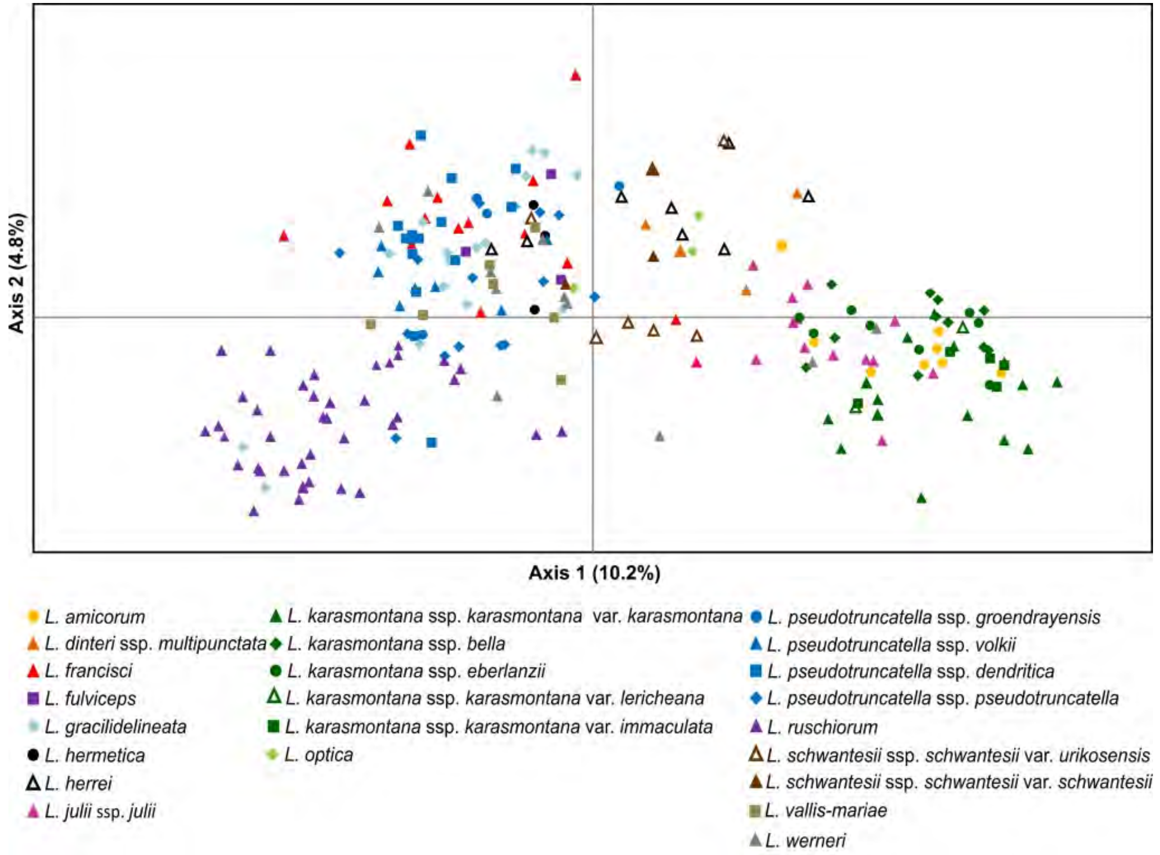


*Figure 3.* PCoA analysis based on AFLP data for 23 *Lithops* taxa. Samples are plotted on the first two coordinates which together explain 15% of the variability.

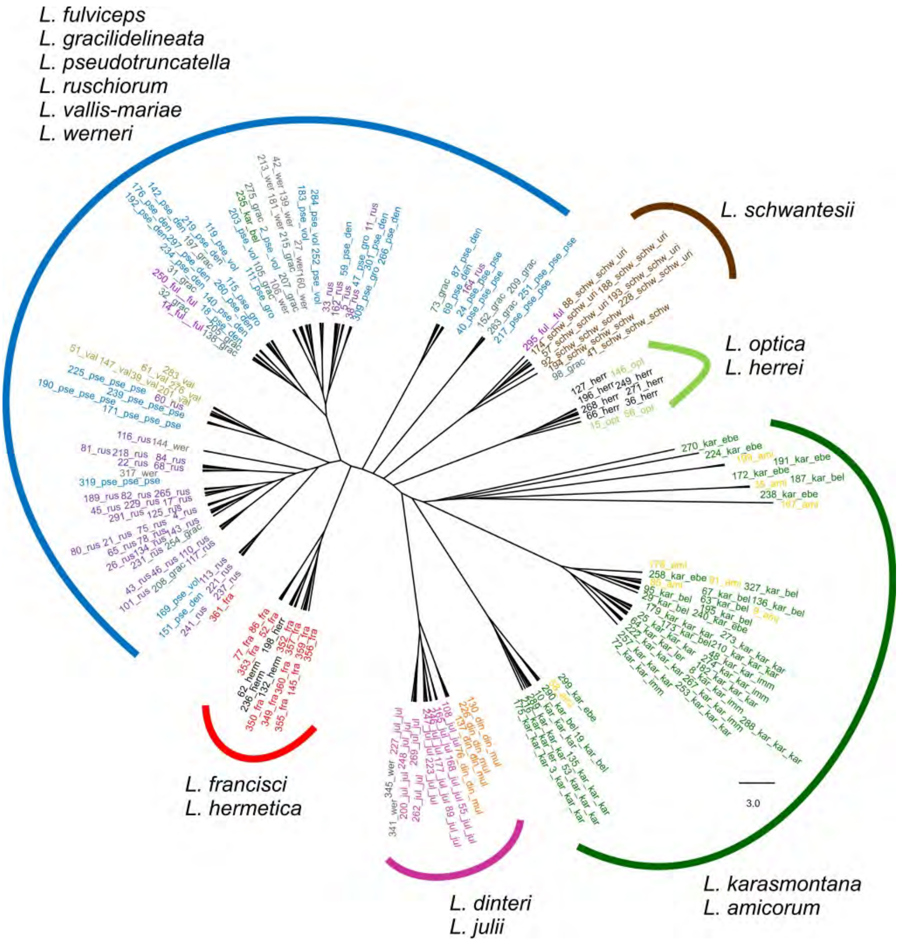


*Figure 4.* Unrooted AFLP-based neighbour-joining tree of 23 *Lithops* taxa.

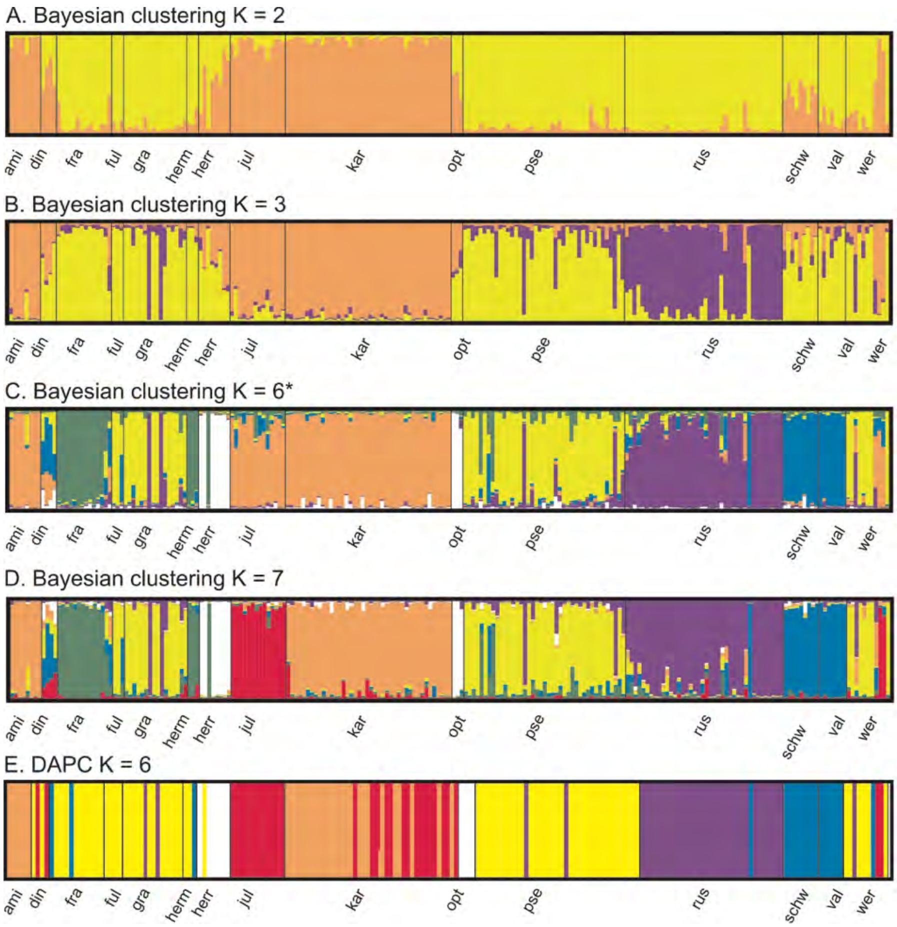
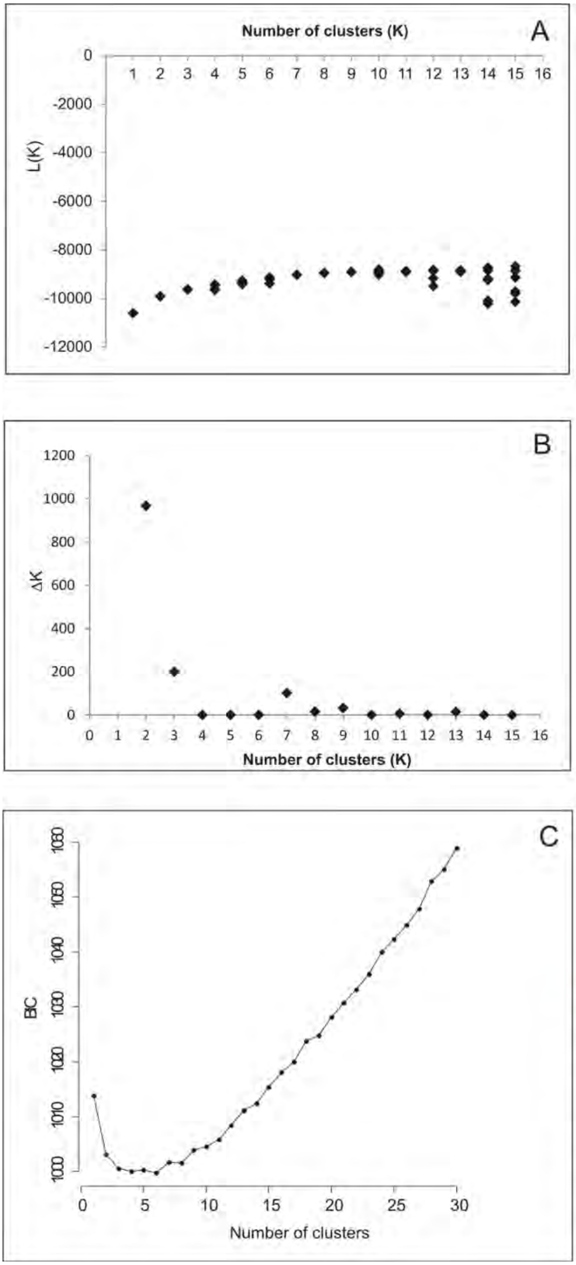
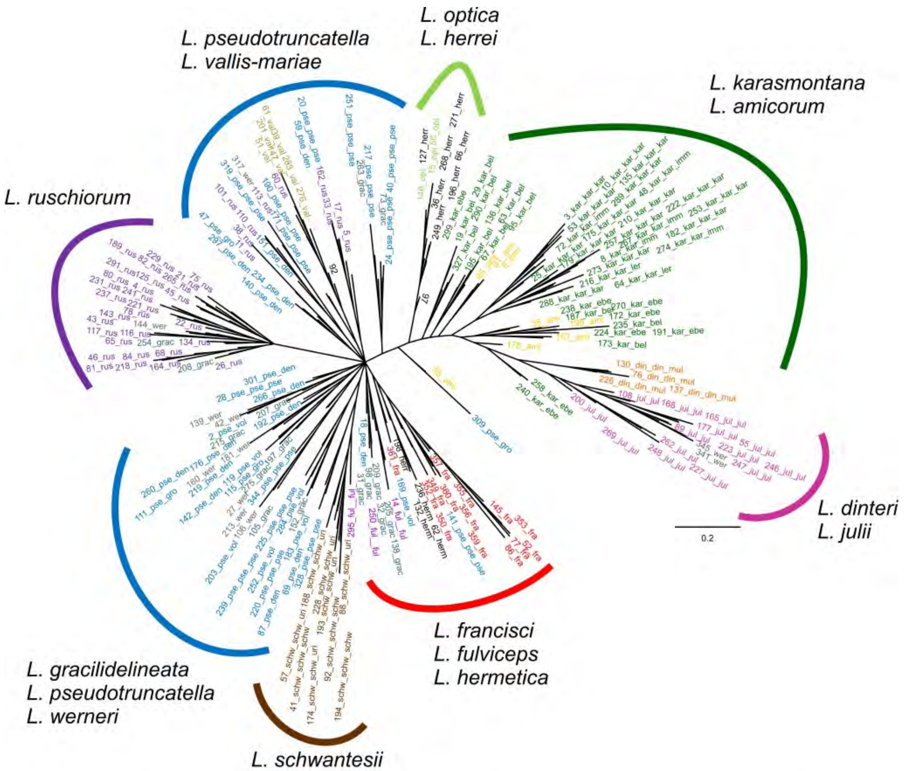


*Figure 5.* Clustering based on AFLP data for 15 *Lithops* species. Each plant is represented by a single vertical bar, which is partitioned into coloured segments representing different clusters. Length of the coloured segment corresponds to the probability (max 1.0) to belong to a certain cluster. A-C. Results from Bayesian clustering for models consisting of 2, 3 or 7 clusters. D. Result from DAPC analysis based on 6 clusters.



*Supplementary figure S1.* A. Distribution of log likelihood of 10 replicates across different models (*K* = 1–15) computed by Bayesian clustering. B. Distribution of Δ*K* based on the rate of change in log probability according to the method described by Evanno et al. (2005). C. Bayesian Information Criterion (BIC) across different models of the DAPC analysis.



Suplplement material xx. Unrooted Bayesian phylogeny of *Lithops* based on 92 polymorphic AFLP fragments. Posterior probabilities > 0.9 ar given.

*Supplementary figure S2*. Unrooted Bayesian phylogeny of *Lithops* based on 92 polymorphic AFLP fragments. Posterior probabilities > 0.9 are given.

# Appendix 4

# Changes in plant abundance for the endemic succulent *Lithops ruschiorum* (Aizoaceae)

Sonja Loots[1,2], Hilde Nybom[3]

[1]Swedish University of Agricultural Sciences, Department of Plant Breeding, Box 101, 23053 Alnarp, Sweden. Tel. +46(0)40415565. Email: sonja.loots@slu.se
[2]National Botanical Research Institute, Ministry of Agriculture, Water and Forestry, Private Bag 13184, Windhoek, Namibia. Tel. +264612022020. Email: Sonja.Loots@mawf.gov.na
[3]Swedish University of Agricultural Sciences, Department of Plant Breeding, Balsgård, Fjälkestadsvägen 459, 29194 Kristianstad, Sweden, Tel. +46(0)44265802. Email: hilde.nybom@slu.se

## Abstract

Increased desertification due to global warming is a possible threat to the future survival of some of the endemic dwarf succulents in Southern Africa, such as the genus *Lithops*. For well-informed plant conservation decisions, data is needed on plant abundance as well as the impact of various habitat variables. We conducted a survey based on 36 permanent 10×10 m monitoring plots that were laid out in 8 natural populations of *L. ruschiorum* in Namibia. Inter-populational distances varied between 10 and 520 km. For each plot, number of *Lithops* plants was recorded in 2007, as well as altitude, aspect, gradient, soil texture and rock substrate. Influence of habitat parameters on plant density, as revealed in a PCA (principal component analysis) is consistent with the important role of precipitation, received mainly as fog in the 7 coastal populations and as both fog and rain in the population at Rössing Uranium Limited (RUL), situated 60 km inland. Plant number dropped by 50% in the 21 plots monitored at RUL during a nine-year period, probably caused by the prolonged draught in 2012–2016. Plant number declined also in some of the 15 monitoring plots in the other populations, especially between 2012 and 2016 but it is not clear whether, e.g., human activity or changes in weather patterns were responsible.

## Introduction

Increased desertification due to global warming is a possible threat to the future survival of some of the dwarf succulents presently occurring in the desert biomes of Southern Africa. Among the many endemic plants in this region, the genus *Lithops* N.E.Br. is well-known for its unusual plant morphology. These “flowering stones” or “stone plants” blend in well with the soil cover and are often almost impossible to spot in their natural habitat. Individual plants can reach an age of at least 50 years (Schwantes, 1957). Population turnover data has never been reported in the genus, but surprisingly short generation spans were noted for shrubby leaf succulents in a desert in South Africa (Jürgens et al., 1999). The *Lithops* plants have a shallow root system, reaching only 5 cm down into the soil and are vulnerable to competing plants. Insects and larger herbivores, both wild and domestic, also threaten plant survival. In addition, human interference, evidenced as habitat disruption and illegal collection, has had a negative impact on many *Lithops* populations (Loots, 2005, 2011; Jainta, 2017). A number of *Lithops* species are listed as endangered or vulnerable (IUCN, 2001; Klaassen & Kwembeya, 2013) but more information on plant abundance as well as factors affecting their survival is needed for making well-informed plant conservation decisions. A considerable impact of habitat variation on plant density was demonstrated within a carefully surveyed population of *L. pseudotruncatella*, which grows in the savanna biome and receives winter rains (Loots & Nybom, 2017). Long-term demographic studies are, however, missing for natural populations of *Lithops* and there are few reports on changes in plant abundance over time.

One of the most widely distributed *Lithops* species in Southern Africa is *L. ruschiorum* (Dinter & Schwantes) N.E.Br. This species grows in the central and northern Desert biome of Namibia along 600 km of the Atlantic coast, and approximately 75 km inland (Cole and Cole, 2005) in an area know as the high fog zone. Here, fog may produce five times as much precipitation as rain and is also more predictable, and therefore affects the distribution of many plant species (Olivier, 1995; Hachfeld and Jürgens, 2000; Seely and Pallet, 2008). *Lithops ruschiorum* usually begins to flower in January. Rain is apparently needed for seed germination as well as for the survival of young seedlings. By contrast, adult plants in populations within the high fog zone can probably survive with very little rain for several years. In a survey of 9 *L. ruschiorum* populations, plant density showed a strong association with habitats suitable for intercepting fog-based precipitation (Loots et al., manuscript submitted). The importance of specific habitat characteristics has been described also for another genus of dwarf succulents in the Namib Desert, namely *Conophytum* N.E.Br. (Young and Desmet, 2016).

The current study was undertaken to assess changes in plant abundance for *L. ruschiorum* during a 9-year period and to investigate possible associations with habitat and weather in the monitored plots.

## Material and Methods

### *Field work*

Eight *L. ruschiorum* populations were surveyed in 2007 (Fig. 1). For the most intensely studied population, RUL (the license area of Rössing Uranium Limited), one 10×10 m (100 m²) long-term monitoring plot was established in each of 21 different sites (at least 500 m apart) containing a substantial number of plants. The four corners of each plot were permanently marked with a cemented dropper and the GPS coordinates were recorded in the centre. Plants inside the monitoring plots were marked with temporary markers and recorded as mature, damaged (with extensive predatory damage) or juvenile (plants having no distinctive heart-shaped profile, but being instead flat on top and with as yet un-separated facial lobes). Altitude, aspect (the compass direction that a slope faces), gradient (degrees of slope), soil texture and rock substrate were recorded for each site (Table 1). The plots were monitored again in 2008, 2010 and 2016, and plants (mature, damaged and juveniles) were similarly counted and recorded.

Another 15 plots were laid out in sites with many *Lithops* plants in the other 7 populations, with two plots in each population except Henties Bay–Uis Road (four plots) and Hoanib River (one plot). Plants were counted and habitat variables scored as described above. Six plots (two at Rössing Mountain and four at Henties Bay–Uis Road) were monitored again in 2012. All plots were monitored in 2016 except three (two at Khumib River and one at Hoanib River).

### *Statistical analysis*

Normality of plant and habitat parameters was evaluated with the Anderson Darling test, and transformation into natural logarithms was applied when needed. A Spearman rank correlation test was used to study the impact of altitude and gradient on plant number while analysis of variance (ANOVA) was used for the category habitat parameters (aspect, soil texture and substrate), together with Tukey pairwise comparisons. A principal component analysis (PCA) was undertaken to reveal possible relationships between plant number and all the habitat parameters. A partial Spearman rank correlation test was performed to detect significant changes in plant number in the monitoring plots at RUL and at the other 7 populations. This was achieved by doing a randomization test through a series of calculations involving standardizing the data in each plot, ranking the standardized observations, performing a one-way ANOVA, calculating correlations, randomising the number of plants observed, repeating the calculations many times and finding a p-value using a two-tailed test. Spearman rank correlation test, ANOVA and PCA were performed using Minitab 17, while the partial Spearman correlation and the randomization test was performed using the programme “R” (R Core Team, 2016).

## Results and Discussion

### *Populations and plant counts*

Most *Lithops* species grow in small colonies with large distances in between, resulting in overall very low plant densities. Plant number at the outset of this study (2007) varied between 8 and 70 (mean = 24.1) in the 21 10×10 m plots laid out in the RUL population (Table 1). In the remaining 15 plots, plant number varied from 10 to 117 (mean = 41.2). Each plant was defined as mature, juvenile or damaged. Juveniles are, however, exceptionally hard to spot and some may have been overlooked. The plot with the highest percentage juveniles (12%) was located in the overall largest population, Khumib River, while the second largest population, RUL, contained the plot with the highest percentage damaged plants (42.9%). There was, however, no indications that percentage juveniles or percentage damaged plants were associated with any of the habitat variables (results not shown) and therefore all plants are treated together in the following analyses.

### *Impact of habitat characteristics*

The 21 plots in the RUL population were characterized by an altitude of 556 to 705 m (mean 633 m) and a gradient of 0 to 35° (mean 8.5°) (Table 2). The most common aspect was S+SE+E recorded for 10 plots, and then there were 3 plots with N+NE+NW and SW, respectively. The predominant soil texture was light clay (7 plots) followed by loam (6 plots), while the predominant substrate was granite+quartz (13 plots) followed by feldspar+quartz (6 plots). Corresponding values for the 15 plots laid out in the other 7 populations were

17–613 m (mean 273 m) for altitude, 0–20° (mean 9°) for gradient, a S+SE+E aspect (5 plots) followed by W (4 plots), clay loam in 10 plots while the remaining had loamy sand+sand, and quartz+other in 6 plots followed by schist+other in 3 plots.

The PCA provides an overview of the impact of the different habitat variables (Fig. 2). Neither altitude nor soil texture appear to have any influence on plant number in these plots. By contrast, a western aspect and a steep gradient often co-occurs with high plant number, as expected in habitats where fog precipitation has a major role in plant survival; the fog arrives by winds from the Atlantic coast, and is more easily trapped by rather steep, west-facing slopes. Finally, pegmatite+other and schist+other appear to have a positive impact on plant number whereas quartz+other, in spite of being the most common substrate, seems to be associated with a lower plant number. No significant relationships could, however, be determined using correlation analyses for altitude and gradient (results not shown). Among the category variables, only substrate affected plant number according to the ANOVA, $F = 3.36$, $p = 0.022$, with pegmatite+other having a significantly higher plant number than quartz+other (Table 3).

Importance of specific habitat parameters was investigated in larger areas (“sites”, up to 23,000 $m^2$) in the same populations of *L. ruschiorum* in a previous study (Loots et al., manuscript submitted). These results suggest that habitats with a high ability to attract fog-based precipitation (a short distance from the coast, relatively steep SW-facing slopes) are especially beneficial for populations in the high fog zone. For RUL, where most sites occurred on S- to E-facing slopes, a substantial influence of rain-based precipitation was suggested since this population is situated in the outskirts of the fog zone.

### *Long term monitoring of plants*

The 21 monitoring plots at RUL were observed in 2007, 2008, 2010 and 2016 (Table 1). During this time, total number of plants decreased from 506 (mean number of plants per plot 24.1) to 259 (mean 13.0). The decline started already in 2008 (mean number of plants per plot = 18.9) and remained unchanged in 2010 (mean 18.7). A partial Spearman rank correlation analysis indicated a strong decline in plant number with time ($Rho = 0.63$, $p < 0.001$). The RUL population is situated approximately 60 km inland in the outskirts of the high fog zone. Rainfall and fog precipitation data recorded by RUL personnel in 2006–2016 show that there were peaks in 2006, 2008 and 2011 (Fig. 3), suggesting that a prolific rainfall event at least every two to three years may be required to sustain this population. It is doubtful, however, whether plants have simply shrunk back in later years due to lack of water, or whether other factors also apply. There was considerable evidence of animal disturbance in some plots and an unusual number of large herbivores were sighted during the 2016 monitoring session. The prolonged dry period from 2012 to 2016 most likely compelled the large herbivores to move into the mining area to find food and water, thus destroying many plants.

For shrubby leaf succulents in South Africa, the detrimental impact of livestock grazing is mainly caused by the suppression of flower and fruit production with serious effets even in areas of comparatively light grazing regimes (Riginos and Hoffman, 2003). Similarly, grazing by wild ungulates (and occasional livestock) in the Namib Desert has the potential to severely reduce reproduction in *Lithops*. If repeated over several years, this grazing may thus have long-term effects on population survival.

In the other 7 populations, data was available both in 2007 and 2016 for 12 monitoring plots. In these plots, average number of plants decreased from 33.8 to 25.9. This decline was not statistically significant when the 12 plots were analysed together (Rho = -0.460, p = 0.11). Nervertheless, plant number decreased notably in some populations, especially in the two plots at Rössing Mountain; from 60 to 12, and from 89 to 39, respectively. These plots showed signs of disturbance by humans, but it is not clear whether the drastic decline, which took place between 2012 and 2016, can be ascribed to human activity and/or changes in weather patterns. Plant number declined also in two of the four plots at Henties Bay–Uis Road (from 18 to 5, and from 23 to 8) while the other two plots in this population remained stable. Since no disturbances were noted here, the recorded declines could be due to plants being over-looked during counting (or having shrunk back into the soil) or natural mortality although no remains were recorded here. Additional surveys of the *Lithops* monitoring plots in our study should be made, especially for investigating possible effects of the large 2018 rains in the southern populations such as RUL and Rössing Mountain.

Previous vegetation analyses in desert biomes have shown that plant number in succulent shrubs and dwarf shrubs is significantly affected by dry spells. Jürgens et al. (1999) and Schmiedel et al. (2012) examined various species in the Succulent Karoo Desert biome (which receives sparse winter rainfall as well as fog and dew) in South Africa, including several leaf succulent shrubs (e.g. *Argyroderma* spp. and *Conophytum* spp.) and found that inter-annual changes in plant numbers were associated with periods of rain and draught, respectively. The present climate change projections predict increased variability in both rainfall and temperatures. The overall warming and resulting raise in evapotranspiration could be hypothesized to seriously impede survival and reproduction of *Lithops* populations in the future. Whether a particular species is vulnerable to climatic changes depends, however, not only on the changes themselves but also on the life history and capacity for demographic buffering (Doak et al., 2010). Based on a super-high-resolution model for climatic projections together with available demographic data for the short-lived dwarf shrub *Cryptantha flava* (Utah, US) and the annual herb *Carrichtera annua* (Negev, Israel), a positive view on the survival of plant species in arid environments was presented by Salguero-Goméz et al. (2012). Detailed demographic studies would thus be highly desirable but unfortunately also extremely time-consuming in *Lithops* due to difficulties with, e.g., detecting the plants in the field, and outfitting them with permanent markings.

## Acknowledgements

Rio Tinto and Rössing Uranium Limited provided funds and logistical support to conduct the population surveys. Staff from NBRI assisted in the field, and MAWF provided transport and financial support of this staff. J.-E. Englund is acknowledged for statistical help and M. Fatih for logistical support in Sweden. Financial support was received from Sida (Swedish International Development Cooperation Agency) as part of Nordic support to SADC Plant Genetic Resources Project.

## References

Cole, D.T., Cole, N.A. 2005. *Lithops* Flowering Stones. Cactus & Co., Tradate, Italy.

Doak, D.F., Morris, W.F. 2010 Demographic compensation and tipping points in climate-induced range shifts. Nature 46: 959–962. (doi:10.1038/ nature09439)

Hachfeld, B., Jürgens, N. 2000. Climate patterns and their impact on the vegetation in a fog driven desert: the central Namib Desert in Namibia. Phytocoenologia 30: 567-589

IUCN. 2001. IUCN Red List Categories and Criteria: Version 3.1. IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK.

Jürgens, N., Gotzmann I.H., Cowling, R.M. 1999. Remarkable medium-term dynamics of leaf succulent Mesembryanthemaceae shrubs in the winter-rainfall desert of northwestern Namaqualand, South Africa. Journal of Ecology 142: 87–96.

Klasseen, E.S., Kwembeya, E.G., (Eds). 2013. A Checklist of Namibian Indigenous and Naturalised Plants. Occasional Contributions No. 5, National Botanical Research Institute, Windhoek, Namibia.

Loots, S. 2005. Red Data Book of Namibian plants. Southern African Botanical Diversity Report No. 38. SABONET, Pretoria and Windhoek. 124 pp.

Loots, S. 2011. National conservation assessment and management of two Namibian succulents, with specific reference to the Rössing Uranium Mine. Report on a partnership project between the National Botanical Research Institute of Namibia, Rössing Uranium Limited, the Rio Tinto Group and the Royal Botanic Gardens, Kew.

Loots, S., Nybom, H. 2017. Towards better risk assessment for conservation of flowering stones: Plant density, spatial pattern and habitat preference of *Lithops pseudotruncatella* in Namibia. South-African Journal of Botany 109: 112-115.

Loots, S., Ritz, C., Schwager, M., Sehic, J., Garkava-Gustavsson L., Nybom, H. Distribution, habitat profile and AFLP-based analysis of Namibian succulent *Lithops ruschiorum.* Manuscript submitted.

Mannheimer, C.A., Loots, S. 2012. A simple aid to assessing cryptic succulents in the field. Dinteria 32: 78-80.

Olivier, J. 1995. Spatial distribution of fog in the Namib. Journal of Arid Environments 29: 129-138.

R Core Team 2016: R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing, <https://R-project.org>.

Riginos, C., Hoffman, T.M. 2003. Changes in population biology of two succulent shrubs along a grazing gradient. Journal of Applied Ecology 40: 615–625.

Salguero-Gómez, R., Siewert, W., Casper, B.B., Tielbörger, K. 2012. A demographic approach to study effects of climate change in desert plants. Phil. Trans. R. Soc. B 367: 3100–3114.

Schmiedel, U., Dengler, J., Etzold, S. 2012. Vegetation dynamics of endemics-rich quartz fields in the Succulent Karoo, South Africa, in response to recent climatic trends. Journal of Vegetation Science 23: 292–303.

Seely, M.K., Pallet, J. 2008. Namib Secrets of a Desert Uncovered. Venture Publications. Windhoek, Namibia.

Young, A.J., Desmet, P.G. 2016. The distribution of the dwarf succulent genus *Conophytum* N.E.Br. (Aizoaceae) in southern Africa. Bothalia 46(1), a2019. http://dx.doi.org/10.4102/abc.v46i1.2019

Table 1. *Population, plot and number of plants detected in the 36 monitoring plots.*

| Population | Plot | 2007 | 2008 | 2010 | 2012 | 2016 |
|---|---|---|---|---|---|---|
| RUL | 1 | 33 | 44 | 45 | * | 41 |
| | 2 | 36 | 36 | 29 | * | 18 |
| | 4 | 8 | 6 | 7 | * | 2 |
| | 5a | 21 | 17 | 14 | * | 18 |
| | 6 | 16 | 10 | 11 | * | 3 |
| | 8 | 31 | 22 | 20 | * | * |
| | 9d | 17 | 16 | 18 | * | 11 |
| | 10 | 10 | 9 | 15 | * | 7 |
| | 13b | 16 | 16 | 15 | * | 2 |
| | 15a | 15 | 12 | 14 | * | 0 |
| | 17a | 38 | 29 | 38 | * | 4 |
| | 18b | 18 | 9 | 7 | * | 0 |
| | 19a | 29 | 11 | 7 | * | 3 |
| | 21 | 10 | 9 | 4 | * | 4 |
| | 46 | 8 | 2 | 3 | * | 3 |
| | 47 | 16 | 7 | 1 | * | 2 |
| | 48a | 29 | 17 | 28 | * | 7 |
| | 48b | 28 | 17 | 18 | * | 5 |
| | 49 | 70 | 55 | 30 | * | 46 |
| | 50 | 15 | 8 | 5 | * | 12 |
| | 68 | 42 | 44 | 63 | * | 70 |
| Rössing Mountain | 51b | 60 | * | * | 75 | 12 |
| | 51c | 89 | * | * | 78 | 39 |
| The Ridge | 52a | 18 | * | * | * | 11 |
| | 52b | 10 | * | * | * | 9 |
| Ugab Salt Works | 53a | 12 | * | * | * | 11 |
| | 53b | 18 | * | * | * | 18 |
| Ugab River | 84a | 36 | * | * | * | 46 |
| | 85b | 24 | * | * | * | 34 |
| Henties Bay-Uis Road | 88a | 24 | * | * | 15 | 22 |
| | 89 | 18 | * | * | 7 | 5 |
| | 90 | 23 | * | * | 6 | 8 |

| Population | Plot | 2007 | 2008 | 2010 | 2012 | 2016 |
|---|---|---|---|---|---|---|
| | 91 | 73 | * | * | 91 | 96 |
| Khumib River | 96a | 63 | * | * | * | * |
| | 96b | 117 | * | * | * | * |
| Hoanib River | 98 | 33 | * | * | * | * |

*no data recorded for this plot in this year

Table 2. *Population, plot and habitat variables recorded for the 36 monitoring plots.*

| Population | Site | Altitude | Aspect | Gradient | Soil texture | Substrate |
|---|---|---|---|---|---|---|
| RUL | 1 | 648 | N+NW+NE | 7 | light clay | feldspar + other |
| | 2 | 672 | undetermined | 1 | loam | quartz + other |
| | 4 | 594 | undetermined | 5 | loamy sand + sand | quartz + other |
| | 5a | 633 | level | 0 | silt loam | granite + other |
| | 6 | 647 | N+NW+NE | 9 | silt loam | quartz + other |
| | 8 | 667 | SW | 20 | clay loam | quartz + other |
| | 9d | 636 | S+SE+E | 18 | loam | quartz + other |
| | 10 | 667 | undetermined | 3 | light clay | quartz + other |
| | 13b | 704 | S+SE+E | 8 | clay loam | quartz + other |
| | 15a | 611 | S+SE+E | not recorded | light clay | quartz + other |
| | 17a | 611 | S+SE+E | 13 | clay loam | feldspar + other |
| | 18b | 573 | S+SE+E | 17.5 | light clay | feldspar + other |
| | 19a | 556 | S+SE+E | not recorded | silt loam | feldspar + other |
| | 21 | 562 | S+SE+E | 35 | loam | quartz + other |
| | 46 | 659 | N+NW+NE | 10 | light clay | quartz + other |
| | 47 | 608 | SW | 7 | loam | granite + other |
| | 48a | 703 | S+SE+E | 3 | loam | quartz + other |
| | 48b | 705 | S+SE+E | 3 | loam | quartz + other |

| Population | Site | Altitude | Aspect | Gradient | Soil texture | Substrate |
|---|---|---|---|---|---|---|
| | 49 | 608 | undetermined | 14 | light clay | feldspar + other |
| | 50 | 630 | S+SE+E | 7.5 | light clay | feldspar + other |
| | 68 | 599 | SW | 20 | clay loam | quartz + other |
| Rössing Mountain | 51b | 613 | W | 12.5 | loamy sand + sand | schist + other |
| | 51c | 604 | W | 17.5 | loamy sand + sand | schist + other |
| The Ridge | 52a | 470 | S+SE+E | 15 | clay loam | feldspar + other |
| | 52b | 470 | SW | 20 | loamy sand + sand | feldspar + other |
| Ugab Salt Works | 53a | 17 | N+NW+NE | 8 | loamy sand + sand | quartz + other |
| | 53b | 19 | S+SE+E | 1.5 | loamy sand + sand | quartz + other |
| Ugab River | 84a | 97 | W | 11 | clay loam | granite + other |
| | 85b | 105 | S+SE+E | 21 | clay loam | granite + other |
| Henties Bay-Uis Road | 88a | 182 | W | 5 | clay loam | quartz + other |
| | 89 | 189 | level | 0 | clay loam | quartz + other |
| | 90 | 195 | N+NW+NE | 7.5 | clay loam | quartz + other |
| | 91 | 202 | N+NW+NE | 12 | clay loam | quartz + other |
| Khumib River | 96a | 344 | S+SE+E | 15 | clay loam | pegmatite + other |
| | 96b | 378 | SW | 18 | clay loam | pegmatite + other |
| Hoanib River | 98 | 213 | S+SE+E | 10 | clay loam | schist + other |

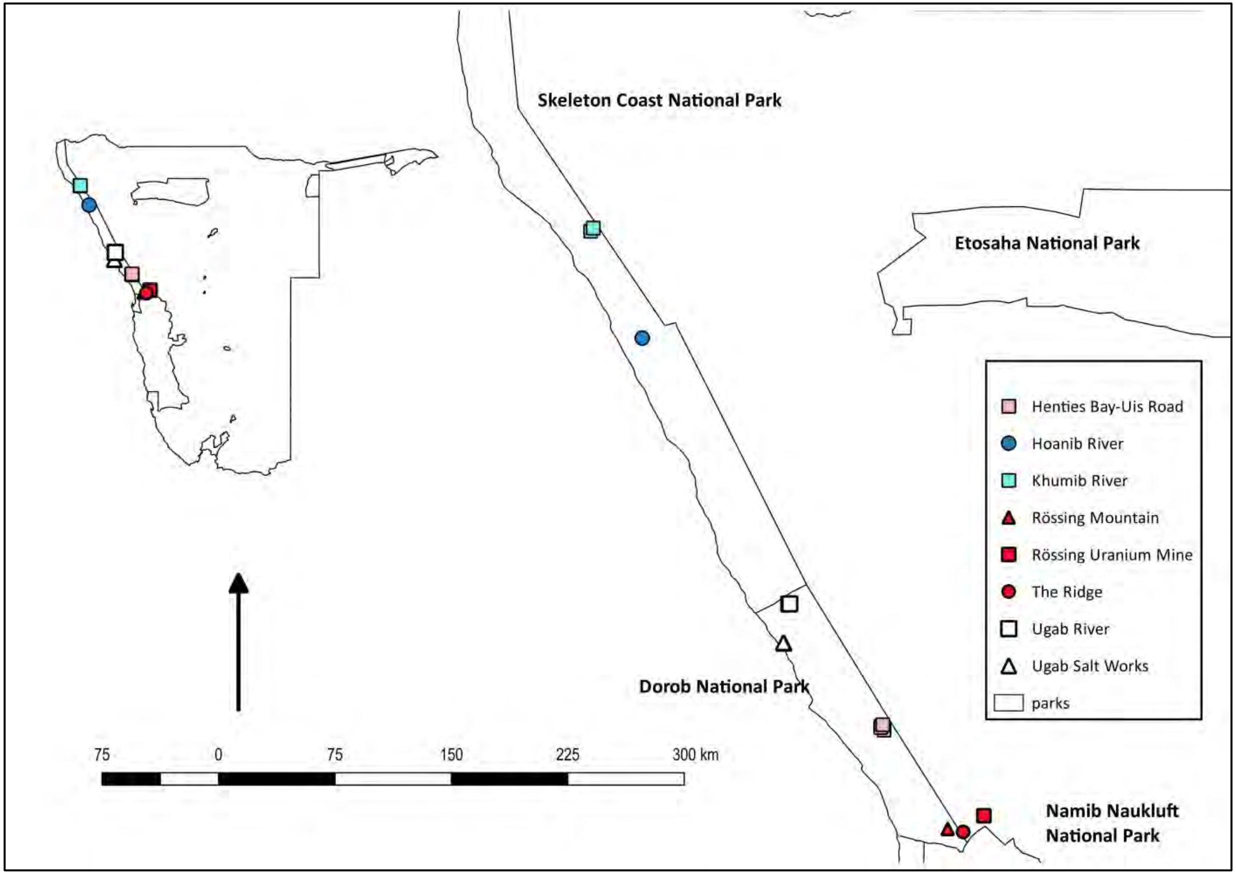


*Figure 1.* Map of the eight populations where monitoring plots were laid out.

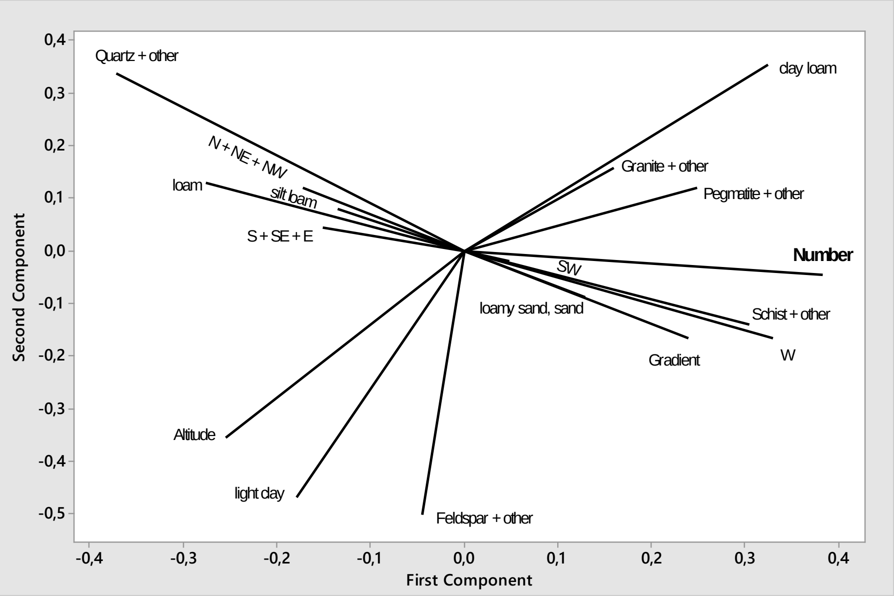


*Figure 2.* PCA showing the associations between plant number and numerical habitat variables (Altitude and slope) as well as categorical habitat variables (aspect, soil texture and substrate) in the 36 monitoring plots. Gradient = slope.

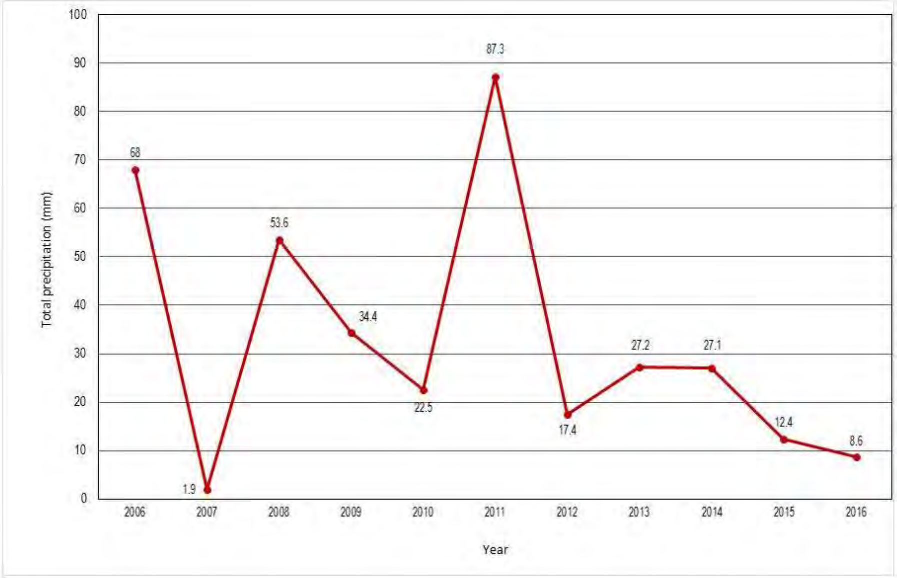


*Figure 3.* Annual precipitation measured at RUL (licence area of Rössing Uranium Limited)

Acta Universitatis Agriculturae Sueciae
Doctoral Thesis No. 2019:28

The dwarf succulent genus Lithops, with 16 species in Namibia is of conservation concern and also over-classified. Habitat profile, spatial arrangement and abundance were investigated in 1 population of L. pseudotruncatella. Plant abundance and habitat variables in addition to long-term monitoring data were analysed in 9 L. ruschiorum populations. Genetic variability within and among 15 species (23 taxa in total) was studied with AFLP markers to elucidate relationships between them and reveal any isolation by distance patterns.

**Sonja Loots** received her graduate education at the Department of Plant Breeding, SLU, Balsgård and Alnarp. She received her M.Sc. in Conservation and Utilisation of Plant Genetic Resources, School of Biological Sciences, University of Birmingham, UK and her B.Sc at the University of Namibia.

ISSN 1652-6880
ISBN (print version) 978-91-7760-374-0
ISBN (electronic version) 978-91-7760-375-7