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

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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)

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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

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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

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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.

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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).

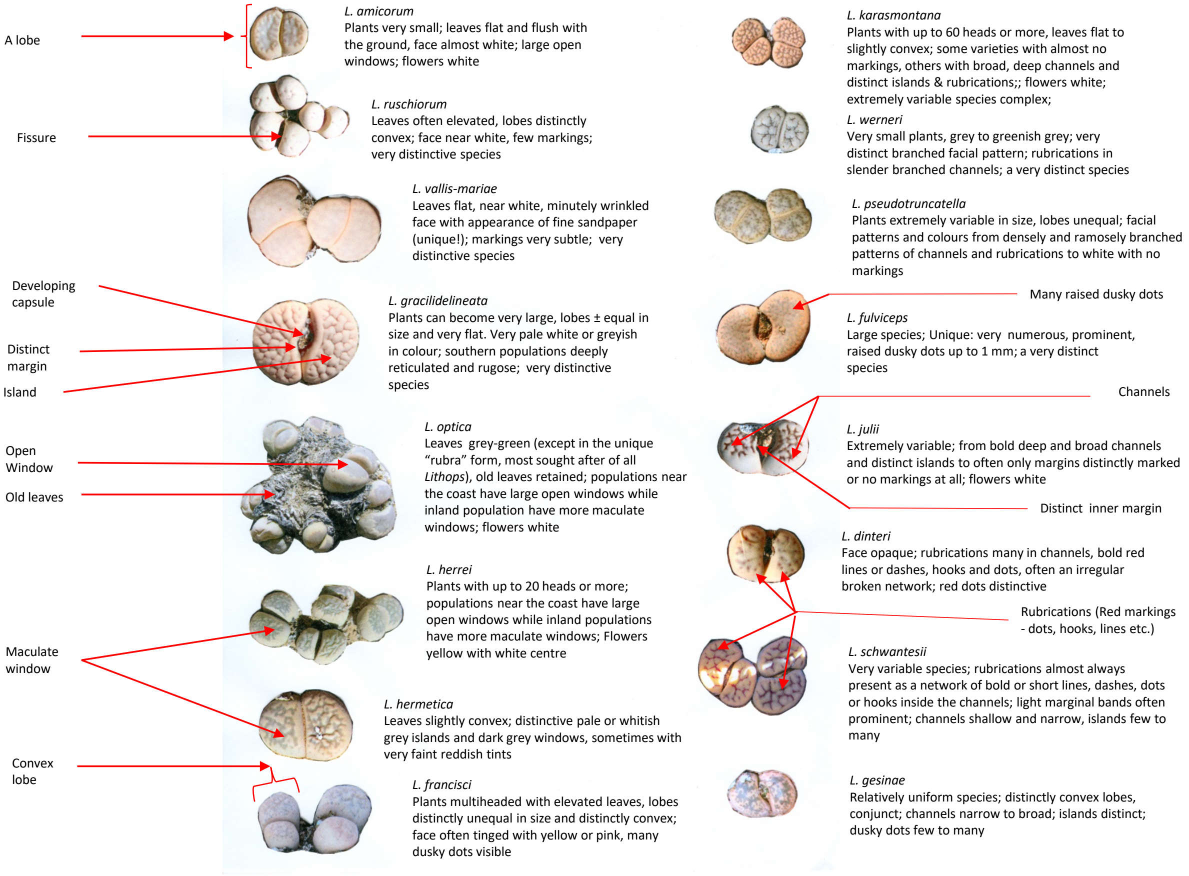
The ability of *Lithops* plants to remain undetected for a significant part of the season serves to defend them against herbivory. Being partially buried in the soil together with their tough epidermis prevents excessive water loss during dry periods. They are slow-growing in nature and can become 50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

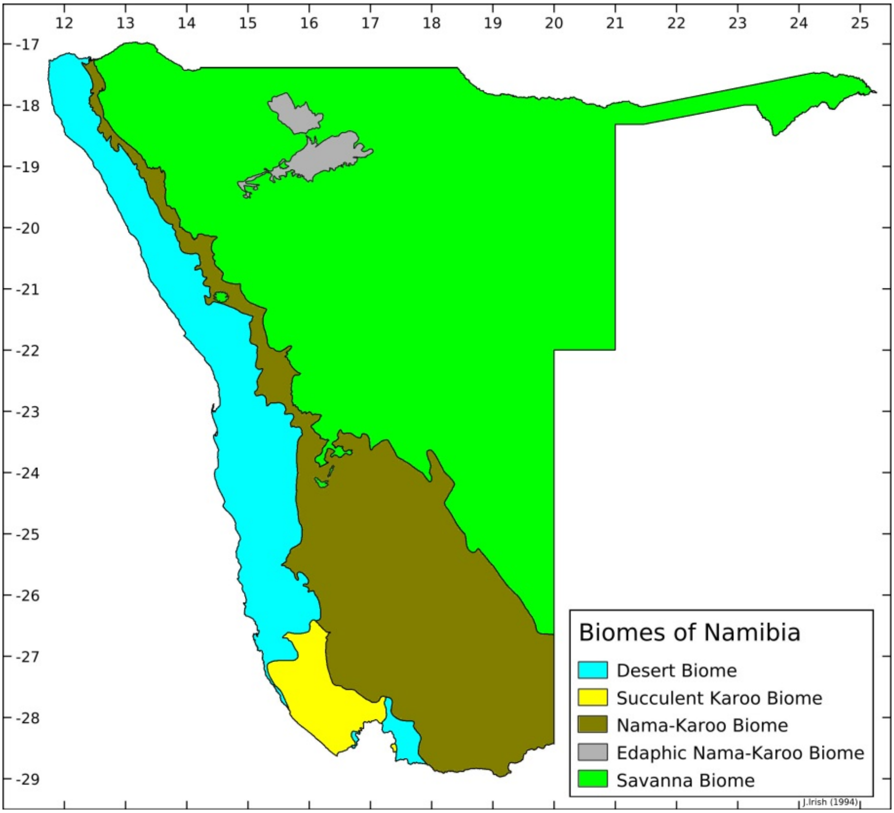


Figure 18b. Biomes of Namibia (Irish, 1994).

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).

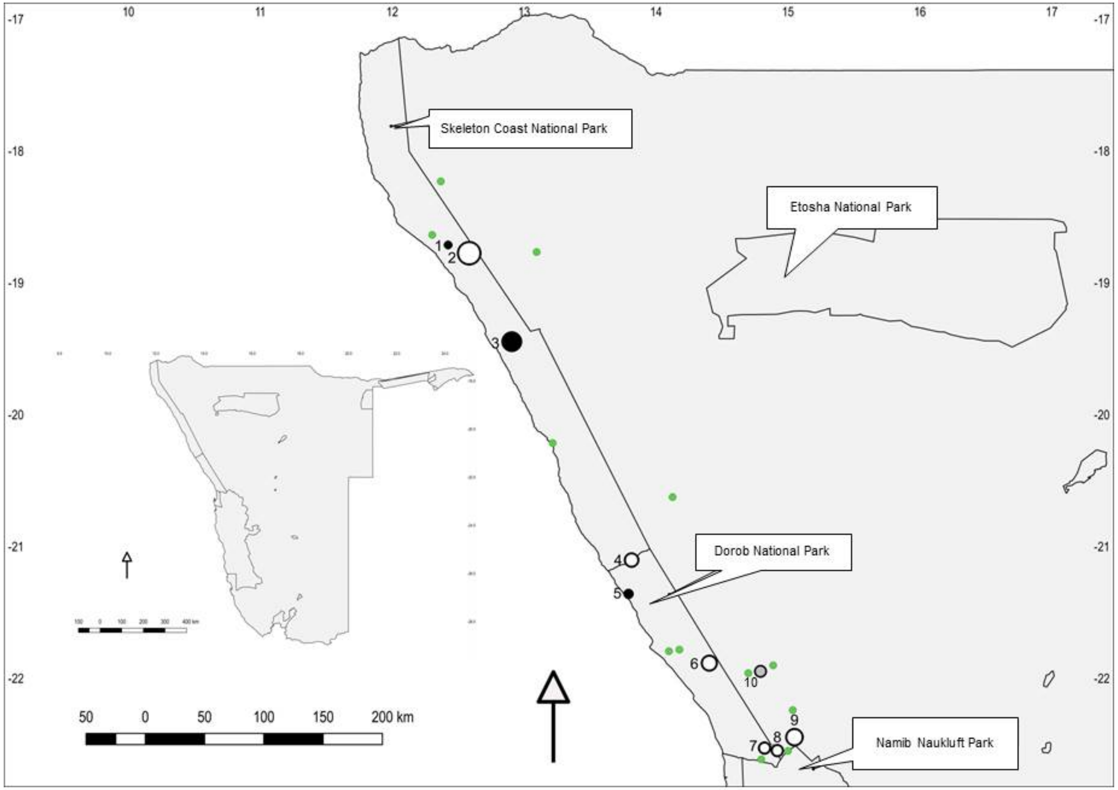


Figure 19. Distribution of surveyed *L. ruschiorum* populations. 1. View Point, 2. Khumib River, 3. Hoanib River, 4. Ugab River, 5. Ugab Salt Works, 6. Henties Bay–Uis Road, 7. Rössing Mountain, 8. Feldspar Ridge, 9. Rössing Uranium Limited license area (RUL), 10. Henties Bay–Usakos Road. Circle size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

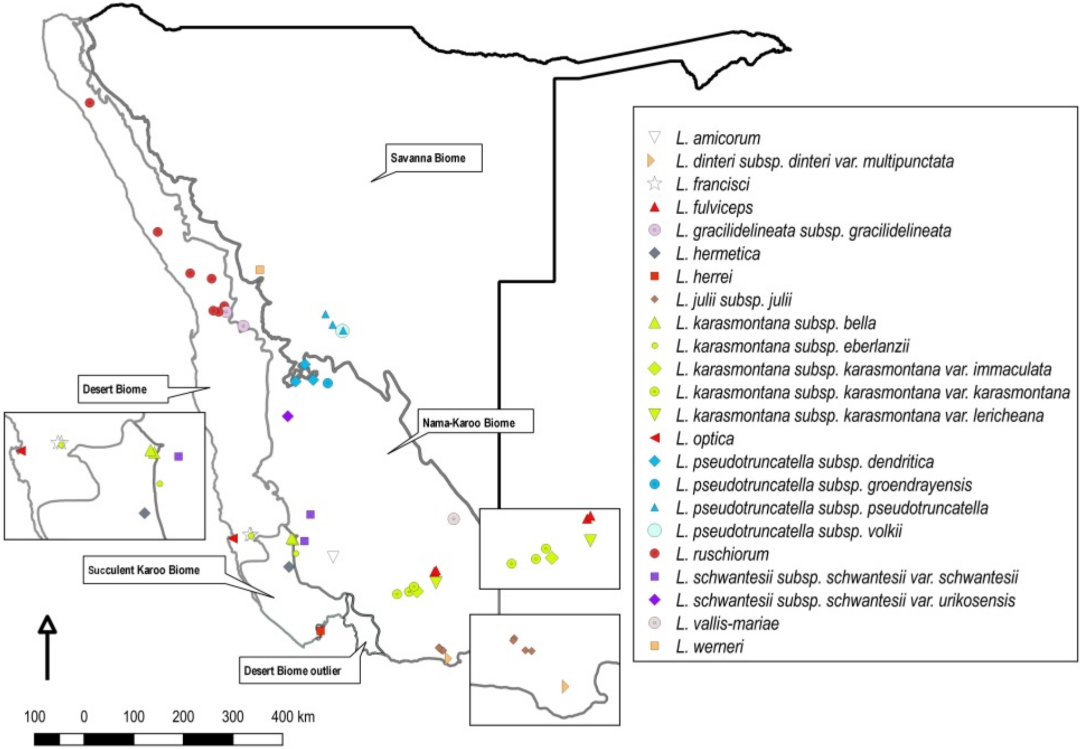


Figure 20. Geographic locations for the 44 analysed *Lithops* populations mapped on the biomes of Namibia, adapted from Irish (1994). Mapping done with QGIS version 2.18.25

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

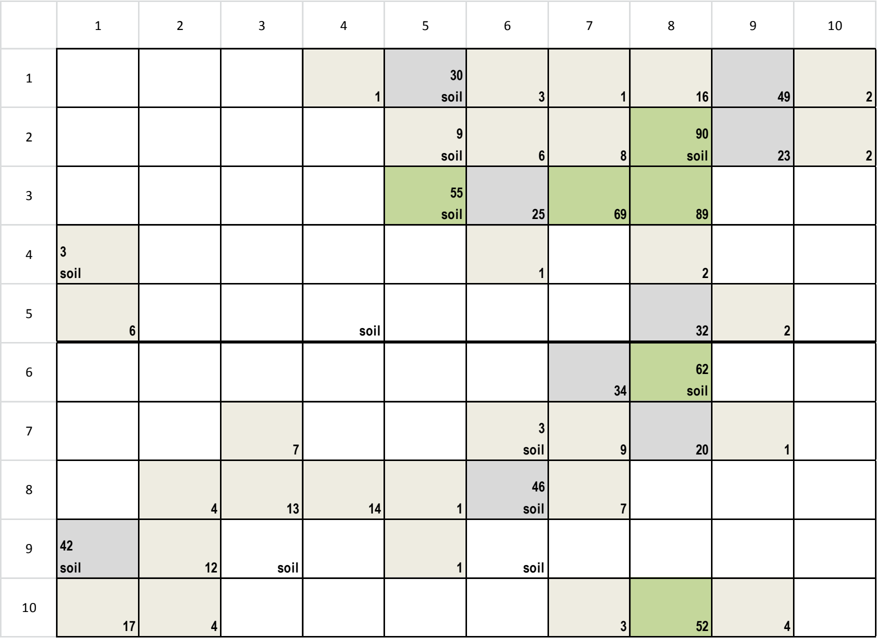
4.1.1 The *L. pseudotruncatella* population

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 soil	3	1	16	49	2
2					9 soil	6	8	90 soil	23	2
3					55 soil	25	69	89		
4	3 soil					1		2		
5	6			soil				32	2	
6							34	62 soil		
7			7			3 soil	9	20	1	
8		4	13	14	1	46 soil	7			
9	42 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 km^2.

Table 4. *Investigated populations with number of sites, number of plants and occupied area.*

Population	Sites	Plants	Area (m^2)
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).

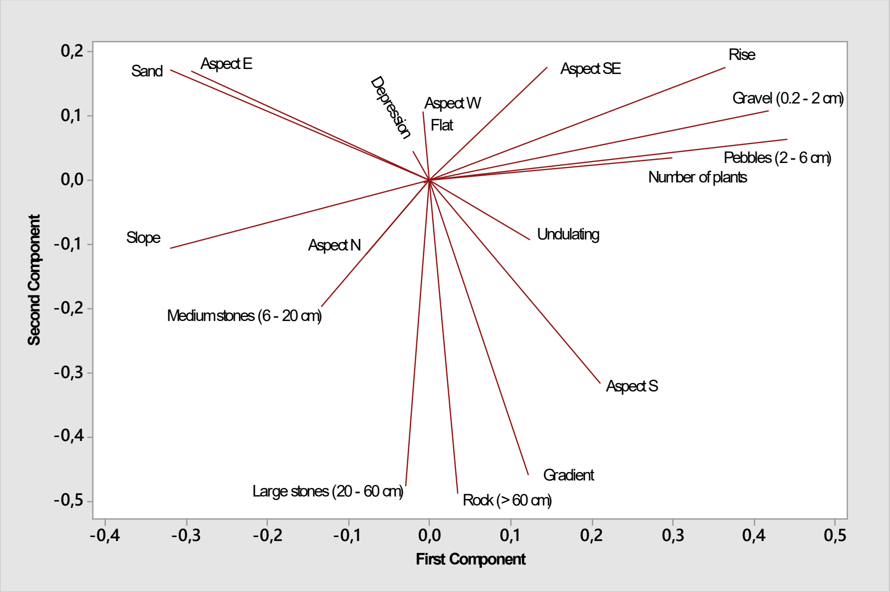


Figure 22. Principal Component Analysis showing the relationship between number of *Lithops* plants, substrate cover, slope (gradient), topography and aspect in 100 10×10 m test plots.

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.

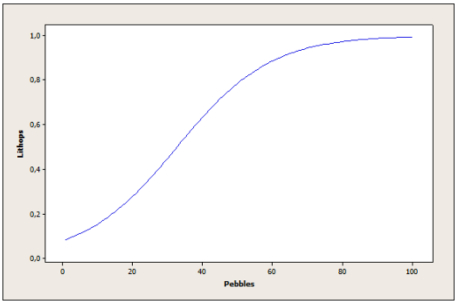


Figure 23a. Fitted line plot of number of *Lithops* plants as a function of percentage cover by pebbles in 100 10×10 m test plots

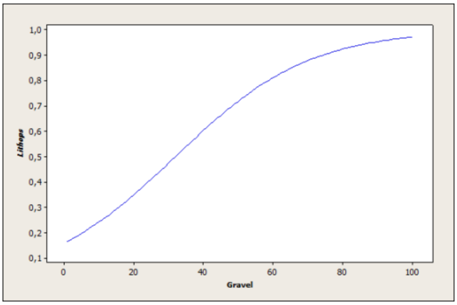


Figure 23b. Fitted line plot of number of *Lithops* plants as a function of percentage cover by gravel in 100 10×10 m test plots

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*

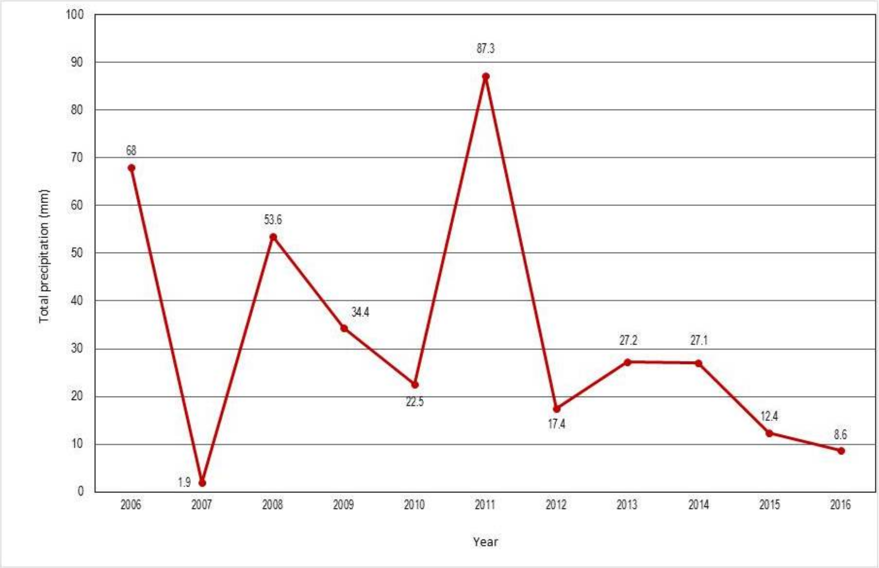


Figure 24. Total precipitation recorded for RUL from 2006 to 2016. This includes rain and fog.

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.

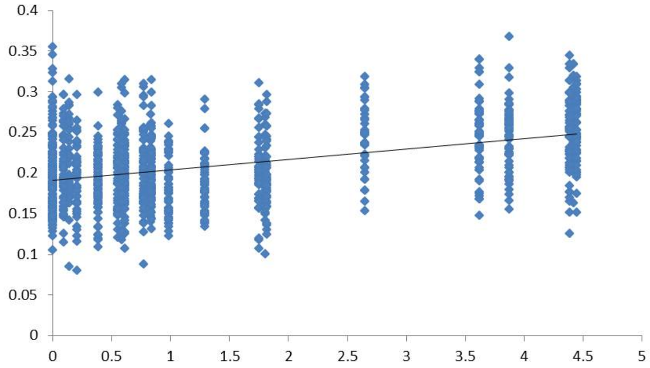


Figure 25. Mantel test with all 7 *L. ruschiorum* populations, r = 0.410.

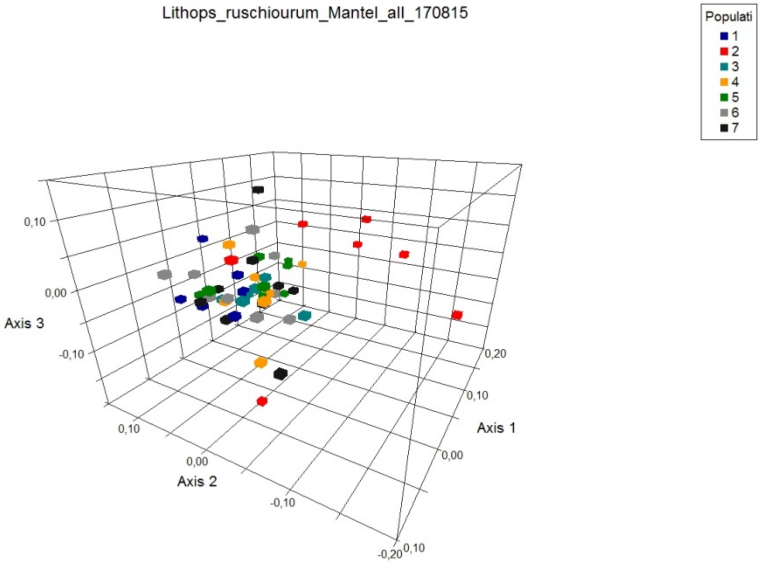


Figure 26. PCoA showing between population differentiation for all 7 *L. ruschiorum* populations. Population1. RUL, Population 2. Khumib River, Population 3. Ugab River, Population 4. Feldspar ridge, Population 5. Rössing Mountain, Population 6. Henties Bay–Uis Road, Population 7. Henties Bay–Usakos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

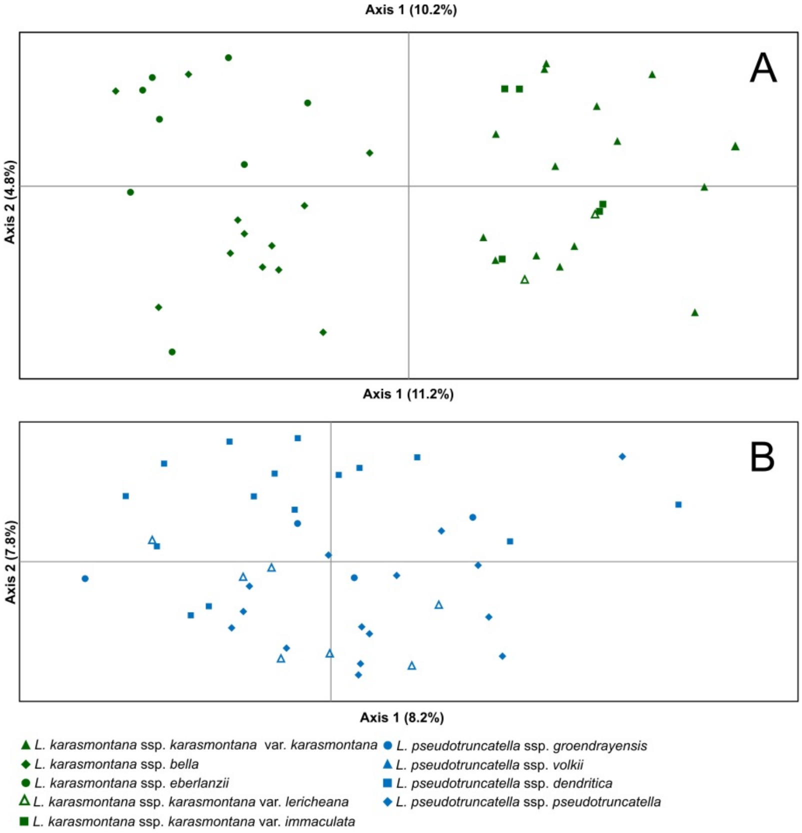


Figure 27. PCoA analyses based on AFLP data for 2 *Lithops* species. **A.** *L. karasmontana* including the 3 subspecies *bella*, *eberlanzii* and *karasmontana* with 3 varieties of the latter; var. *immaculata*, var. *karasmontana* and var. *lericheana*. Samples are plotted on the first 2 coordinates 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.

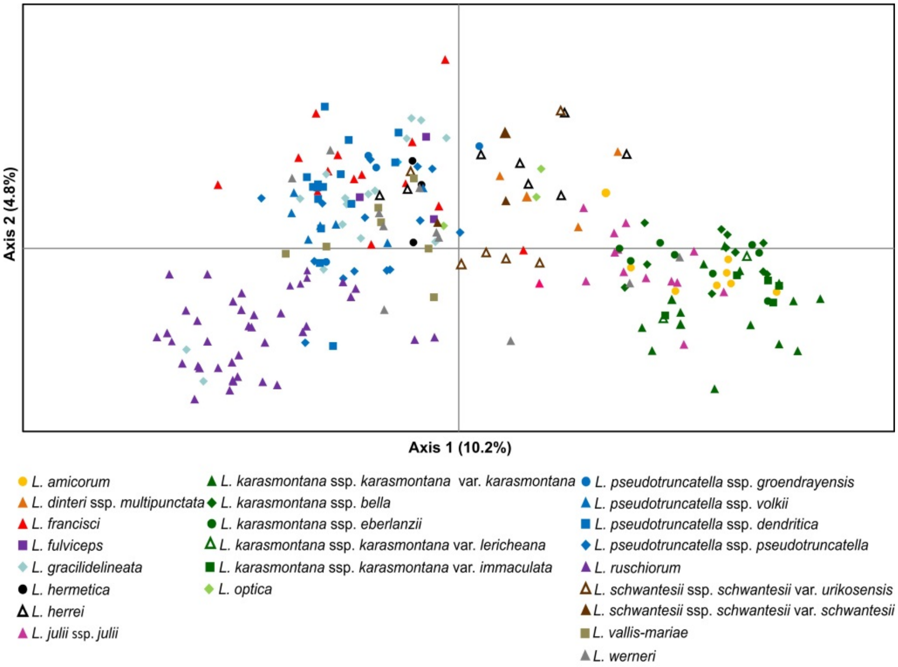


Figure 28. PCoA analysis based on AFLP data for 23 *Lithops* taxa. Samples are plotted on the first 2 coordinates which together explain 15% of the variability.

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.

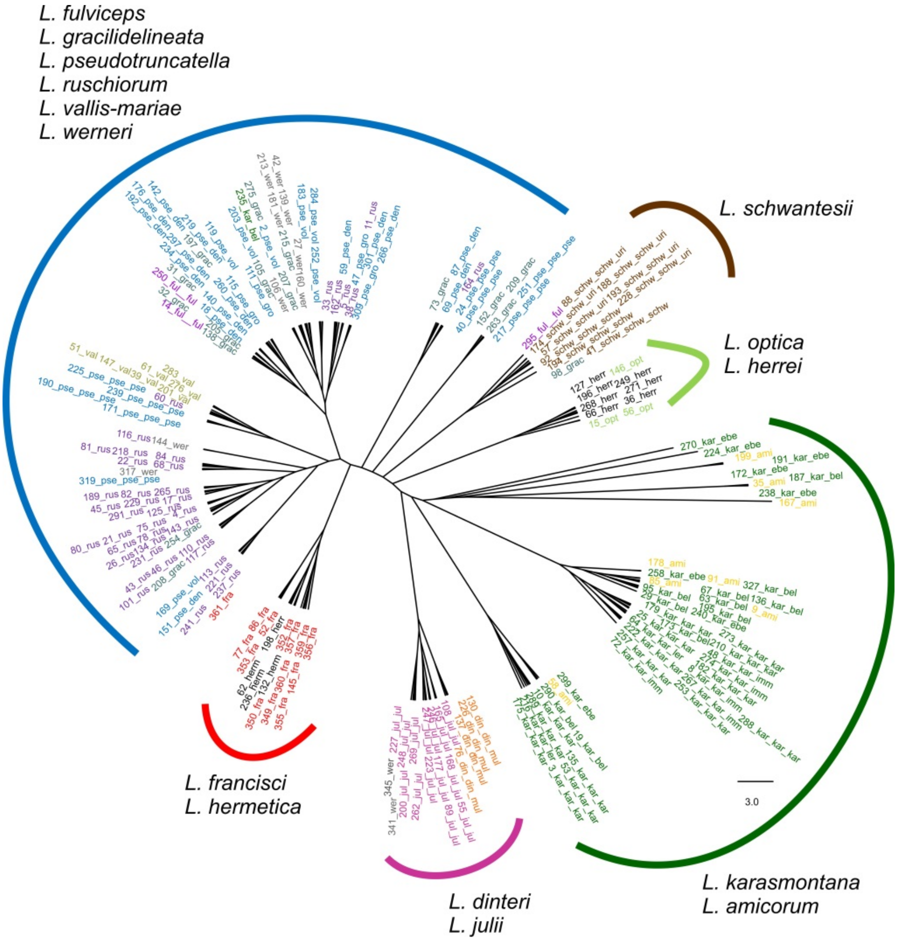


Figure 29. Unrooted AFLP-based neighbour-joining tree of 23 *Lithops* taxa.

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.

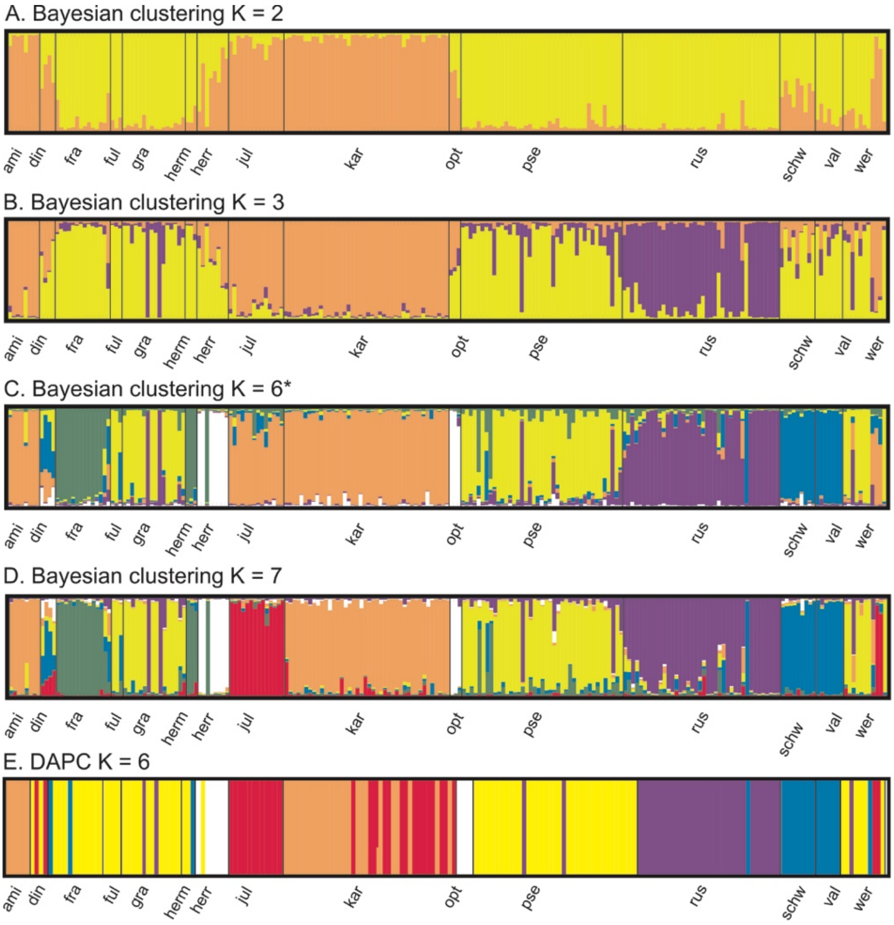


Figure 30. Clustering based on AFLP data for 15 *Lithops* species. Each plant is represented by a single vertical bar, which is partitioned into coloured segments representing different clusters. Length of the coloured segment corresponds to the probability (max 1.0) to belong to a certain cluster. A-C. 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.

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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.*

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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.

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