CHAPTER 1

INTRODUCTION

Clivia Lindl. (1928), a genus endemic to southern Africa, is classified in the sub-Saharan African tribe Haemantheae (Pax) Hutch. (1934) of the family Amaryllidaceae J. St-Hil. (1805). At present the genus comprises five described species, namely *Clivia nobilis* Lindl. (1828), *Clivia miniata* (Lindl.) Regel (1854), *Clivia gardenii* Hook. (1856), *Clivia caulescens* R.A.Dyer (1943) and *Clivia mirabilis* Rourke (2002) (Chapter 3).

All the species, but notably *Clivia miniata*, are currently the subject of considerable horticultural attention (reviewed in Chapter 5). The genus is also extensively utilised by traditional healers (Chapter 4). Despite all this attention, essentially nothing is known regarding the genetic diversity within natural populations of *Clivia*. A principal objective of the present study was to investigate the genetic diversity and structure of *Clivia miniata* populations in nature. With no previous work having been done at population level on any member in the genus, the first step was to develop the necessary molecular tools needed for such a study.

Existing molecular studies on both *Clivia* and Amaryllidaceae focus mainly on clarifying the taxonomic structure (phylogeny) of the taxa (Chapter 3). No infraspecific studies, i.e. on the population level, could be traced. Hence the need for the present study to develop the necessary molecular tools for further population work within the group. Whilst involved in the molecular work, other gaps in our knowledge of *Clivia* were revealed. To address some of these questions, the present study also focuses on the taxonomy, ecology, phytogeography, conservation status and horticultural significance of the genus.

Chapters are presented as self-contained units. Initial chapters address questions regarding the genus as a whole, with increased focus being placed in later chapters on the development of molecular tools for *Clivia miniata*. It is believed that the research

presented here will contribute towards an improved understanding of the genus *Clivia* and would lay the foundation for more intensive future research into the genetic structure and diversity of *Clivia* species in nature, especially *Clivia miniata*.

General materials and methods employed during the study are set out in Chapter 2. Selected molecular techniques, buffers and reagents are described in some detail, with alterations to existing protocols indicated. The general section of this chapter also covers aspects of terminology and lists all voucher specimens used in the molecular part of the study. Methods described in this chapter are only briefly referred to in subsequent chapters.

The genus *Clivia* is introduced in Chapter 3. This chapter provides a historical background on the taxonomy of *Clivia*, including its current taxonomic status and proposed phylogenetic relationships. Diagnostic characteristics for the different infrageneric taxa and an identification key, down to the variety level, are presented for the first time. Included in the chapter is a new, as yet undescribed species, and a new variety. Interspecific hybridisation within the genus and the cultivar-group classification system for *Clivia* hybrids, are discussed.

Chapter 4 presents the results of a study on the geographical distribution of the various members of the genus *Clivia*. The geographical distribution of each species is mapped and described based on the analysis of numerous herbarium and other records, supplemented by extensive fieldwork. Notes on the ecology of different populations of the species, based on the literature, field observations and herbarium records are presented. To explain the current distribution of the various species, an hypothesis is offered, exploring ancient migratory routes induced by historical environmental changes.

The trade in and horticultural significance of *Clivia*, with the focus on *Clivia miniata*, is investigated in Chapter 5. Notes are provided on the history of the industry, including the aims of *Clivia miniata* breeders in different regions of the world. Estimates are presented

which highlight the considerable size and monetary value of *Clivia* markets worldwide, especially the horticultural significance of *Clivia miniata*.

Chapter 6 addresses the lack of knowledge regarding the diversity of natural *Clivia miniata* populations and describes the development and application of chloroplast PCR-RFLP molecular markers. Biogeographic structure within the distribution range of this species is indicated.

The development of microsatellite markers for *Clivia miniata* is described in Chapter 7. An overview of the technology is given and the isolation method is described in detail. The first ever microsatellite markers developed for any member of the genus *Clivia* are presented in this chapter.

Chapter 8 gives an overview of the work presented in this dissertation. Included are new questions resulting from this study, potential future applications utilising the developed molecular tools and general and specific conclusions reached as a result of this study.

Data used in the construction of biogeographic distribution maps for the different species (Chapter 4), are presented in Appendix I. A RFLP data matrix, the experimental results of Chapter 6, is included in Appendix II. Sequenced data, used in Chapter 7 for the development of microsatellite markers, are presented in Appendix III.

CHAPTER 2

MATERIALS & METHODS

2.1 Introduction

Chapters in this dissertation are presented as individual units, but some techniques are common to two or more of them. Techniques used are given here in more detail to prevent unnecessary duplication. The principal aim of this chapter is to give an account of the materials and methods tested and used during the course of this study. Methods, tried but not used, are summarised and the reasons for discarding them are given. The selected methods that were used during the course of this study are briefly described, with all alterations indicated.

This chapter is divided into two sections: a general section, covering all the chapters and a molecular section, covering specifically Chapters 6 and 7.

2.2 General techniques

2.2.1 Descriptors

In many scientific fields qualitative terms are used to express approximate values. These descriptors, being subjective, indicate different values depending on the authors' viewpoint. Descriptors used in this dissertation are based mainly on the objective guidelines proposed by Schmid (1982). Table 2.1 is a summary from Schmid (1982) and gives an indication of the approximated percentages used.

Table 2.1 Descriptors as expressed by approximate percentages.[1]

0%	Absent; completely absent; lacking; never; not at all; wanting	31–54%	Often; fairly commonly; frequently; in many cases; repeatedly
V<2%	Very[2] rarely; almost never; in a very few cases; not very commonly/ frequently/often; uniquely; very exceptionally/seldom/unusually	55–64%	Very often; very frequently; in very many cases
2–4%	Rarely; exceptionally; extraordinary; in a few cases; seldom; uncommonly; unusually	65–94%	Usually; as a rule; characteristically; chiefly; commonly; consistently; customarily; generally; in most cases mainly; mostly; normally;
5–10%	Very occasionally; very sporadically	95–99%	Nearly[3] always; almost always; very commonly
11–30%	Occasionally; at times; often; now and then; on occasion; sometimes; sporadically	100%	Always; absolutely; all; all the time; completely; entirely; universally; with no exception

[1] From Schmid (1982).

[2] Very = exceeding, exceptionally, extraordinary, extremely, highly, etc.

[3] Nearly = all but, almost, not quite, very nearly.

2.2.2 Voucher specimens

Vouchers are required for any serious questioning or re-examination of results and conclusions. They are essential to reassess the identity of a sample and, if necessary, to duplicate the result (Goldblatt *et al.* 1992).

Vouchers were made of all plant samples (*Clivia miniata*) used during this study with the exception of material obtained from Kirstenbosch National Botanical Garden[1] (Table 2.2). All material collected or donated during the course of this study was deposited in the H.G.W.J. Schweickerdt Herbarium, University of Pretoria (Table 2.2 & Appendix I). Voucher numbers are subdivided according to the number of samples collected/obtained for that specific locality, with different voucher numbers assigned for each collector/donor.

[1] National Botanical Garden, Kirstenbosch, Private Bag X7, Claremont 7735, South Africa

Table 2.2 Voucher specimens for *Clivia miniata* material collected or donated during the course of this study.

Collection and voucher/ accession numbers	Collector/donor	Number of samples	Locality
ZH5, PRU92693, 2096, PRU92682	Z.H. Swanevelder; J.T. Truter	5	Bearded Man Mountain
431/99	Kirstenbosch NBG	9	Broedershoek Farm
327/00	Kirstenbosch NBG	9	Donkeni
ZH3, PRU92687	M. Exelby	1	Howick Falls
ZH4, PRU92699, 3014, PRU92687	Z.H. Swanevelder; J.T. Truter	5	Karkloof
3107, PRU92688	J.T. Truter	1	Kei River Mouth
3108, PRU92684, 3284, PRU92685	J.T. Truter	2	Koek Koek River
3611, PRU92683	J.T. Truter	1	Kentani Area, Transkei
3213, PRU92689	J.T. Truter	1	Lebombo Mountains
524/98	Kirstenbosch NBG	14	Mbashe
520/98	Kirstenbosch NBG	10	Nqobara River
435/99	Kirstenbosch NBG	11	Ntomeni Forest
3619, PRU92686	J.T. Truter	1	Oribi Gorge
ZH1, PRU92697, PSJ729/96	Z.H. Swanevelder; J.T. Truter; Kirstenbosch NBG	31	Port St Johns
720/96	Kirstenbosch NBG	10	Qora
436/99	Kirstenbosch NBG	10	Ngoye Forest
PRU91194, 515/98	A. Hardinge; Kirstenbosch NBG	26	Umtamvuna Nature Reserve
PRU91195	A. Hardinge	6	Mzamba River

Plant collection was conducted in accordance with the rules and regulations of the particular provinces. The necessary permits were obtained from Ezemvelo KwaZulu-Natal Wildlife (permits 27110/2001, 30443/2002 & 966/2003), Mpumalanga Parks Board (permits MPB. 1039 & MPB. 1056) and Department of Economic Affairs, Environment and Tourism, Province of the Eastern Cape (General Permit 01/07/2001).

2.3 Molecular techniques

2.3.1 DNA isolation and purification

Meerow *et al.* (1999) proposed the CTAB (cetyltrimethylammonium bromide) method of Doyle & Doyle (1987) for DNA (deoxyribonucleic acid) extraction from *Clivia* leaves. Several subsequent publications described DNA extraction procedures for *Clivia*, including the CTAB method (Ran *et al.* 2001c; Conrad & Reeves 2002) and the Nucleon Phytopure kit of Amersham Pharmacia Biotech (used on root tips) (Ran *et al.* 2001a; Ran *et al.* 2001b).

Initially the CTAB method (Doyle & Doyle 1987) was attempted on *Clivia* leaves. This method was discarded even though DNA was obtained, largely due to poor PCR (Polymerase Chain Reaction) results using these samples as templates and the lengthiness of the protocol. The Plant DNAzol™ Reagent (GibcoBRL, LifeTechnologies™) for genomic DNA isolation from plants and the monocot DNA isolation method (Edwards *et al.* 1991) were also tested, but both isolated less DNA than the CTAB method.

The genomic DNA isolation method of Raeder & Broda (1985) for fungal mycelia was tried. Though this method isolated large quantities of genomic DNA, PCR results with these templates were still unreliable. The higher amounts of DNA isolated, however, compensated for DNA losses that occurred during additional purification steps. Together with alterations made to the extraction buffer, better DNA templates were isolated.

The altered DNA isolation method, based on that of Raeder & Broda (1985), consisted of 200 mM Tris-HCl pH 8, 150 mM NaCl, 25 mM ethylenediaminetetra-acetic acid (EDTA) pH 8, 0.5% (w/v) SDS (sodium dodecyl sulphate) and 1% (v/v) 2-mercaptoethanol. Fresh leaf disks are homogenized in the extraction buffer (700 µl), as dried leaves produced poorer DNA templates.

Homogenisation was either done in a Bio 101 FastPrep machine at setting 2 for one minute or by hand in an eppendorf tube till material was fully macerated. Phenol-chloroform (3:5) purifications were performed till no interface was visible (centrifugal

steps performed at 10 000 rpm, 4°C). Chloroform-phenol in a 1:1 ratio or chloroform-isoamyl alcohol (24:1) can be used as substitutes in the purification steps. DNA in the aqueous layer was precipitated with two volumes of ice-cold absolute ethanol, after a final chloroform purification step. The addition of 0.1 volume 3M sodium acetate (pH 5) to the two volumes of absolute ethanol, further promotes the precipitation of the DNA. DNA forms a small, whitish pellet after centrifuging (11 000 rpm) at 4°C. Salts were removed from the DNA pellets by washing them with 70% (v/v) ethanol. DNA pellets were dried under vacuum, before re-suspending them in sterile, double-distilled water (ddH_2O).

2.3.2 Visualising and separating DNA

Agarose gels

DNA was separated in 1–3% (w/v) agarose gels and run in 1× (v/v) TAE buffer (50× TAE buffer: 2 M Tris-acetate and 0.05 M EDTA, pH 8) at 5 V/cm for 60 min (Sambrook *et al.* 1989).

Staining

DNA fragments were visualised by the addition of ethidium bromide (EtBr) at 0.5 µg/ml to the melted gel. The DNA, with the chelated EtBr, was viewed under UV light and photographed (Sambrook *et al.* 1989).

Loading buffers

As standard protocol, DNA samples were loaded with a 6× loading buffer (15% w/v ficoll and 0.25% w/v bromophenol blue indicator dye, ddH_2O). Bromophenol blue migrates through agarose gels at approximately the same rate as linear double-stranded DNA of 300 bp (base pair) in length (Sambrook *et al.* 1989).

Commercial blue/orange 6× loading dye (10% w/v ficoll, 0.25 % w/v xylene cyanol FF, migrating at ± 4 kb, 0.25% w/v bromophenol blue and 0.25% w/v orange G, migrating at 50 bp, in water) was used in conjunction with a 100 bp ladder (Promega Corporation, Madison, WI) (Sambrook *et al.* 1989).

Polyacrylamide gel electrophoresis (PAGE)

As standard, 6% polyacrylamide gels were used for the testing of microsatellite markers. Gels (75 ml) contained 2 % (v/v) Long Ranger® gel solution (BioWhittaker Molecular Applications, Rockland, ME), 1× (v/v) TBE buffer (10× TBE buffer: 0.9 M Tris-borate and 0.02 M EDTA, pH 8), 10% (w/v) APS (ammonium persulfate) and 0.004% (v/v) TEMED (N,N,N',N'-tetramethylethylene-diamine) (Sambrook *et al.* 1989). APS and TEMED are added last because they activate polymerisation.

Gel solutions were evacuated for 5 min before pouring commenced. A 30 min setting time preceded a pre-run of 7 V/cm for 30 min. All PAGE were run 1× (v/v) TBE buffer. Wells were washed with 1× TBE buffer before samples were loaded.

Samples (10 µl) were loaded into the wells with a mixture of 2 µl SYBR Green I, diluted 1:500 (Roche Diagnostics GmbH, Mannheim, Germany) and 2 µl commercial 6× loading buffer (10% w/v ficoll, 0.25 % w/v xylene cyanol FF, 0.25% w/v bromophenol blue and 0.25% w/v orange G) (Promega Corporation, Madison, WI). Because SYBR Green I dye is light sensitive, the four-hour runs were done in darkness at a constant 7 V/cm. Gels were visualised under UV light and photographed.

2.3.3 Molecular markers

DNA Molecular weight marker III

λ DNA (250 ng/µl) was restricted for three hours with *Eco*RI (24 U) and *Hind*III (20 U) (Promega Corporation, Madison, WI) in 10% (v/v) buffer B (Promega Corporation, Madison, WI), resulting in fragment sizes of 21 226, 5 148, 4 973, 4 268, 3 530, 2 027, 1 904, 1 584, 1 375, 947, 831 and 564 base pairs. Restricted λ DNA was denatured at 60°C for 10 min and 6× (v/v) loading buffer (previous section) was added to obtain a final 1× (v/v) concentration. The resulting DNA molecular weight marker III (50 ng/µl) is stored at –20°C till needed. Five microlitres of this marker is used as size standard in agarose gels.

100 bp ladder

The 100 bp ladders were obtained commercially (Promega Corporation, Madison, WI) and used in conjunction with 2% (and higher) agarose gels and PAGE.

2.3.4 Polymerase chain reaction (PCR)

PCRs were either performed on Applied Biosystems GeneAmp 2700 or the 9700. Reactions work best with Super-Therm DNA polymerase, magnesium chloride (25 mM $MgCl_2$ stock) and 10× PCR reaction buffer (composition unknown), all supplied by Southern Cross Biotechnology (Pty) Ltd. (Cape Town, South Africa). The $MgCl_2$ concentrations varied between the different reactions. Primers stocks (100 pmol/µl) were diluted to a working stock of 10 pmol/µl and deoxynucleotide triphosphates (dNTPs, 100 mM) to a working stock of 50 µM each. Template DNA was diluted to a final concentration of 30 ng/µl.

Problems with amplifications were addressed by changing the reaction conditions, proposed by Innis & Gelfand (1990). Additional additives such as 2-pirrolidinone (usually at 480 mM) and cosolvents like butaine (0.5–2.0 M), formamide (1–5%, v/v) and DMSO (1–5%, v/v), were sometimes added (in different concentrations) in an attempt to facilitate a successful reaction.

Reactions were started with a warm block (hotstart) to prevent any artefacts from being produced. Touchdown PCRs were performed during the optimisation steps, in accordance to the protocol of Don *et al.* (1991). Detailed reaction conditions for PCRs are presented in Chapter 6 and 7.

2.3.5 Cloning

Vector

The pGEM®-T Easy vector (Promega Corporation, Madison, WI, USA) was used for cloning. This vector contains single 3'-thymine (T)-overhangs that greatly increases the cloning efficiency of PCR products with an adenosine (A)-overhang. The Super-Therm DNA polymerase (Southern Cross Biotechnology (Pty) Ltd., Cape Town, South Africa) used has no proofreading capability and therefore adds the A-overhang needed for successful ligation (Promega Corporation, Madison, WI, USA).

Li & Guy (1996) reported that by increasing the final extension time (60–120 min) of the PCR, greater cloning efficiency of PCR products could be obtained. The extension time of 7 min that was used gave sufficient colonies with the fragment of interest.

The pGEM®-T Easy vector contained T7 and SP6 primer sites, one on each side of the insert. This allows for amplification of fragments out of the plasmid (colony PCRs) without going though lengthy plasmid extractions. The insertion site is located within the enzyme β-galactosidase. Inserted fragments that inactivate this enzyme allow for direct colour screening of recombinant clones. The vector also contains an ampicillin resistance gene that only allows transformed colonies to grow on the selective medium (Technical manual: pGEM®-T and pGEM®-T Easy Vector Systems, Promega Corporation, Madison, WI, USA).

Ligation and transformation

The ligation and transformation protocol of Promega (Technical manual: pGEM®-T and pGEM®-T Easy Vector Systems, Promega Corporation, Madison, WI, USA) were used with the following alterations: total reaction volumes were lowered to 10 µl, with 2× Rapid Ligation Buffer and PCR products lowered in accordance, 50 ng pGEM®-T Easy Vector and 3 units T4 DNA ligase were used (pGEM®-T and pGEM®-T Easy Vector Systems, Promega Corporation, Madison, WI, USA). Reactions were done overnight.

The JM109 high efficiency competent cells provided with the pGEM®-T Easy Vector Systems (Promega Corporation, Madison, WI, USA) were used in transformations. The standard transformation protocol supplied with the cell line JM109 was used (Promega Corporation, Madison, WI, USA). A brief summary of the protocol is as follows: 100 µl competent *E. coli* cells (JM109) were heat shocked (42°C), together with 50 ng vector (containing the PCR insert) for a period of 45–50 s in a water bath, after an ice treatment of 10 min; heat shock was followed with a 2 min ice treatment and SOC medium (900 µl, 4°C) was added before incubating at 37°C (60 min); the cells were plated out onto LB/ampicillin/ IPTG/X-Gal plates and grown overnight.

The clones with inserted PCR products generally produce white colonies on the LB/ampicillin/IPTG/X-Gal plates, but fragments cloned in-frame may produce blue colonies. The white colonies were first selected for further analysis and only if fragments were absent, were the blue colonies randomly chosen and tested for those fragments.

2.3.6 Growth media

LB medium with ampicillin, IPTG and X-Gal

LB (Luria-Bertani) medium contains 10 g/l tryptone, 5 g/l yeast extract, 5 g/l sodium chloride (NaCl) and 15 g/l agar, pH 7.0 (Sambrook *et al.* 1989). The medium was autoclaved and cooled to 50°C before adding ampicillin to a final concentration of 100 µg/ml, 0.5 mM IPTG (isopropylthio-β-D-galactoside) and 80 µg/ml X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside).

The medium was poured into petri dishes, allowed to solidify and stored at 4°C for no longer than 1 month. Plates were allowed to reach room temperature prior to plating out of bacterial colonies (Technical manual: pGEM®-T and pGEM®-T Easy Vector Systems, Promega Corporation, Madison, WI, USA).

SOC medium

The SOC medium contained 2 g/100ml tryptone, 0.5 g/100ml yeast extract, 10 mM NaCl, 2.5 mM KCl, 1 ml filter-sterilised Mg^{2+} stock (20.33 g/100ml $MgCl_2.6H_2O$ and 24.65 g/100ml $MgSO_4.7H_2O$) and 20 mM glucose, with the final volume adjusted to 100 ml, pH 7. The medium was filtered-sterilised (Technical manual: pGEM®-T and pGEM®-T Easy Vector Systems, Promega Corporation, Madison, WI, USA).

2.3.7 Sequencing reactions

Quarter volume sequencing reactions were performed with 2 µl ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit II (Perkin-Elmer, Warrington, UK), 3.2 pmol primer T7 or SP6 (stock 10 pmol/µl), ≤ 200 ng DNA/PCR template, with the final volume adjusted to 5 µl with ddH_2O.

Cycle conditions were in accordance to those proposed by Perkin-Elmer (Warrington, UK) for GeneAmp PCR Systems 9600 and 2400. Amplification consisted of 25 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 4 min.

The PCR products were precipitated using the ethanol sodium acetate precipitated protocol of Perkin-Elmer (Warrington, UK). A 20 µl reaction mixture (quarter reactions adjusted to this volume with ultra pure water) was precipitated by 0.1 volume (v/v) 3M sodium acetate, pH 4.6 and 2.5 volumes (v/v) absolute ethanol. DNA was 'pelleted' (10 000 rpm, 30 min) before the ethanol mixture was aspirated with a micropipette. Salts were removed with a 70% (v/v) ethanol (250 µl) washing step and the invisible pellet dried under vacuum after the ethanol was removed.

Sequencing were conducted on an ABI PRISM™ 377 automated DNA sequencer.

2.3.8 Cleaning of PCR products

PCR reactions that were selected for cloning or sequencing were first purified using either the QIAquick PCR Purification Kit (Qiagen, GmbH, Germany) or the ethanol sodium acetate protocol (previous section). The protocol of Qiagen (GmbH, Germany) was used for the QIAquick PCR Purification Kit.

2.4 References

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CHAPTER 3

TAXONOMIC NOTES ON THE GENUS *CLIVIA* LINDL.

3.1 Introduction

The taxonomic history of the genus *Clivia* is, like most other taxonomic stories, full of twists and turns. What makes this tale more interesting is that it is one of those rare cases in history where the same genus was described—on the same day—by two different authors (Hooker 1828; Lindley 1828; Obermeyer 1972; Duncan 1985).

At present the genus *Clivia* Lindl. consists of five recognised species, *C. nobilis* Lindl. (1828), *C. miniata* (Lindl.) Regel (1854), *C. gardenii* Hook. (1856), *C. caulescens* R.A.Dyer (1943) and *C. mirabilis* Rourke (2002). The genus is endemic to southern Africa, i.e. South Africa and Swaziland (Vorster 1994; Meerow & Snijman 1998; Duncan 1999; Snijman 2000; Koopowitz 2002; Rourke 2002a).

The aim of this chapter is to supply background information on the historical and current classification of the genus *Clivia* Lindl. Diagnostic features and habitat information required to identify the various species are also noted and used in a key. Current evidence on the molecular phylogeny and interspecific hybridisation of the genus will also be reviewed.

3.2 Taxonomic notes

3.2.1 Suprageneric classification

A comparison between different suprageneric classifications of *Clivia* is supplied in Table 3.1. The suprageneric classification of Kubitzki (1998b) was adopted in this thesis and is as follows:

Class	'Monocotyledons'
Superorder	Lilianae Takhatajan (1967)
Order	Asparagales Huber (1969)
Informal group	'Higher' Asparagales Kubitzki (1998a)
Family	Amaryllidaceae J. St-Hil. (1805)
Tribe	Haemantheae (Pax) Hutchinson (1934)
Genus	*Clivia* Lindl. (1828)

Table 3.1 A comparison of different suprageneric classifications for the genus *Clivia* Lindl.

Rank	Snijman (2000)	Meerow *et al.* (1999)	Kubitzki (1998b)[1]	Takhtajan (1997)	Dahlgren *et al.* (1985)	Cronquist (1981)
Division	-	-	-	-	-	Magnoliophyta
Class	'Monocotyledons'	-	'Monocotyledons'	-	'Monocotyledons'	Liliopsida
Subclass	-	-	-	Liliidae	-	Liliidae
Superorder	Lilianae	-	Lilianae	Lilianae	Liliiflorae	-
Order	Asparagales	-	Asparagales	Amaryllidales	Asparagales	Liliales
Group (informal)	-	-	'Higher' Asparagales	-	-	-
Family	Amaryllidaceae	Amaryllidaceae	Amaryllidaceae	Amaryllidaceae	Amaryllidaceae	Liliaceae[2]
Tribe/Tribus	-	Haemantheae	Haemantheae	Haemantheae	Haemantheae	-
Genus	*Clivia*	*Clivia*	*Clivia*	*Clivia*	*Clivia*	*Clivia*

[1] Chapters by Kubitzki 1998a; Kubitzki *et al.* 1998; Meerow & Snijman 1998 were used to compile this classification.

[2] Cronquist (1981) lumps into Liliaceae amongst others the classical families Liliaceae and Amaryllidaceae.

The superorder Lilianae comprises four orders, of which the order Asparagales is the largest containing 31 families (Kubitzki *et al.* 1998; Meerow *et al.* 1999). Kubitzki (1998a) proposed the informal group 'Higher Asparagales' for those families in which microsporogenesis is successive and steroidal saponins are common (except in Amaryllidaceae).

The family Amaryllidaceae occurs worldwide, mainly throughout warm temperate and tropical regions. It encompasses approximately 60 genera, representing an estimated total of 850 species. About 18 of the genera are represented in southern Africa—one of the three centres of diversity—with about 280 species. The other two centres of diversity are the Andean region in South America (28 genera) and the Mediterranean (8 genera) (Meerow & Snijman 1998).

Amaryllidaceae inflorescences are pseudoumbels. In the case of the flowers, the perigone consists in all cases of two whorls, each with three petaloid tepals. The tepals are usually connected basally to form a tube, with those of the outer whorl usually slightly longer than those of the inner whorl. Stamens are opposite the tepals and occur usually in two whorls (3 + 3). The gynoecium is syncarpous and tricarpellate. All genera have septal nectaries in their ovaries. Seedlings are distinguished by a bifacial cotyledon (Meerow & Snijman 1998, and references therein).

Haemantheae is an African baccate-fruited tribe and the only tribe of the Amaryllidaceae that contains rhizomatous genera, namely *Clivia*, *Scadoxus* and *Cryptostephanus*. Members of this tribe have fleshy, recalcitrant seeds that lack phytomelan (Meerow & Snijman 1998; Meerow *et al.* 1999). Meerow *et al.* (1999) showed that the tribe is strongly supported by both molecular and morphological evidence, thus rendering the subtribe Cliviinae (for *Clivia* and *Cryptostephanus*) of Müller-Doblies, paraphyletic.

The genus *Clivia* is characterized by evergreen, rhizomatous plants with distichous, firm, strap-shaped leaves. Inflorescences are pseudo-umbels borne on solid, compressed scapes that usually produce red (yellow for some forms of *C. miniata* and *C. caulescens*)

subglobose berries with fleshy, ivory-white seeds embedded in a soft yellow pulp (Meerow & Snijman 1998; Snijman 2000; Rourke 2002a).

3.2.2 Infrageneric classification

The taxonomic history of the different *Clivia* species is briefly described in this section.

Clivia nobilis Lindl. (1828)

The first scientific collection of a member of the genus *Clivia* dates back to September 1815. The collection was made near the mouth of the Great Fish River, Eastern Cape, South Africa by William J. Burchell (Vorster 1994; Duncan 1999).

It was James Bowie, however, who brought the new species to the attention of botanists in England. He collected specimens in the same area as Burchell in the early 1820s and brought them to England in 1823 (probably to Kew Gardens and Syon House) (Hooker 1828; Lindley 1828; Obermeyer 1972; Duncan 1992; Vorster 1994; Duncan 1999). According to the review by Smith & Van Wyk (1989) of the collecting journeys of Bowie, he most probably collected *C. nobilis* during the course of his fourth journey (May 1821–December 1822). Bowie, being notorious for insufficient and misleading details, gave the Orange River as the locality of *C. nobilis*. Commercial interests—with Cape plants being highly fashionable in Europe at that time—could have been the reason for his misleading notes (Smith & Van Wyk 1989). In correspondence to Hooker, Mr. Aiton (patron to Bowie) gave the Great Fish River as the locality (Hooker 1828).

Bowie wrote to W.J. Hooker (before returning to the Cape) informing him of a 'Cyrtanthus-like plant' that he had collected previously in the Cape. He included specimens of flowers and a leaf, both collected in the wild. In his letter he requested Hooker that if the plant flowered, it should bear as specific name, that of his patron, Mr. Aiton (Hooker 1828).

A specimen indeed flowered that year (October 1827?). Mr. Forrest, who was in charge of the plant collection at Syon House, requested that a drawing of the flowering plant be

made. The Duke of Northumberland granted permission for the plant to be sketched. At the same time, Mr. Aiton, sent a drawing, some fruits, and some extracts of Bowie's notes on the habitat of the new genus, to Hooker. The sketch ordered by the Duke was copied and together with the other information supplied by Mr. Aiton, was used in the article by W.J. Hooker (October 1828) in which he described the new genus. He named it *Imatophyllum aitoni* as requested by Bowie (Hooker 1828). *Imatophyllum* was later miss-spelled by Hooker, when he described *Imantophyllum ? miniatum* (Koopowitz 2002)

Mr. Forrest meanwhile, had discussed the interesting plant that was in his care with the botanist John Lindley. The plant flowered for the second time (July 1827?) at Syon House and Mr. Forrest informed Lindley (Lindley 1828) of this. A drawing of the plant was made (probably the same one as used by Hooker) and in October of 1828, John Lindley described the species as *Clivia nobilis* (Lindley 1828; Duncan 1999). In Lindley's article (1828) it is clear that he was uncertain about the collector and original locality. He nevertheless credited Bowie as the collector but the exact locality remained speculative. He named the genus *Clivia* after the Duchess of Northumberland, Lady Charlotte Florentine Clive. In his article he thanked her for the opportunity to publish the new species (Lindley 1828; Duncan 1999). Lindley commented that, at first, the new species appeared to be a member of *Cyrtanthus*, but later questioned whether it should be placed in the family Amaryllidaceae [=Amaryllideae]. He classified it in this family because it resembles *Haemanthus*, some members of which have imperfect bulbs (Lindley 1828).

Lindley and Hooker's publications did not only appear in the same year, but also on the same day in October 1828 (Obermeyer 1972; Duncan 1992). In 1830, Roemer and Schultes, according to literature, chose *Clivia nobilis* as the type species, thus reducing Hooker's *Imatophyllum aitoni* to a synonym (Obermeyer 1972; Duncan 1992). *Clivia nobilis* is illustrated in Figure 3.1.

Obermeyer (1972) stated that rumours had it that Lindley obtained the plant he used to describe the new genus, 'surreptitiously' from Kew. In Lindley's article describing the new genus *Clivia*, he does not mention once the word Kew (Lindley 1828). He refers to

Mr. Forrest and Syon House as sources of information, but doesn't state clearly from where he obtained the material he used to describe the new species. He thanked the Duchess for allowing him to name the species after the family Clive (Lindley 1828). I suspect that Lindley's request regarding the naming of the genus was probably either directed at the Duchess (Lady Clive) or was intercepted by her. Mr. Forrest could have acted on Lindley's behalf, asking the Duchess rather than the Duke, for permission. Mr. Forrest knew that Hooker was busy naming the new species. At the same time Aiton,

Figure 3.1 *Clivia nobilis* in cultivation.

Bowie and probably the Duke gave permission to Hooker to describe his new species.

***Clivia miniata* (Lindl.) Regel (1854)**

Clivia miniata* var. *miniata

This species was discovered in the early 1850s and due to its beautiful flowers, it was on public display even before it was named (Duncan 1985, 1992; Koopowitz 2002).

Initially, flower shape led different taxonomists to place it in various genera. According to Koopowitz (2002), Lindley described it in 1854 as a possible species of *Vallota*, namely *Vallota ? miniata*, the question mark indicating his uncertainty (Koopowitz 2002). Hooker

described the same species—again in the same year as Lindley—but placed it in his genus *Imantophyllum*. He described it as *Imantophyllum* ? *miniatum* in Curtis's Botanical Magazine, 1854 (Duncan 1985, 1992; Pole Evans 1921; Koopowitz 2002). Again, he was unsure about the generic identity of the taxon. Koopowitz (2002) refers to another name change made by Koch, during which the species were placed in yet another genus, namely *Himantophyllum miniatum*.

Regel discarded Hooker's name *Imantophyllum miniatum* ten years later on the basis of the recognition by Roemer & Schultes (1830) of Lindley's *Clivia nobilis* as type species. According to Duncan (1985, 1992) Pole Evans (1921) and Koopowitz (2002), Regel changed the name of Hooker's species to *Clivia miniata* (Lindl.) Regel in *Gartenflora*, 1864, p. 131, t. 434. He argued that the different flower form (mainly perianth shape) was insufficient to place the species in its own genus and that Lindley's earlier description had priority, therefore justifying the reduction of both Koch's *Himantophyllum miniatum* and Hooker's *Imantophyllum* ? *miniatum*, to synonyms under *Clivia miniata* (according to Koopowitz 2002). *Clivia miniata* cultivar is depicted in Figure 3.2.

Figure 3.2 *Clivia miniata* cultivar.

***Clivia miniata* var. *citrina* Watson (1899)**

The earliest date for the discovery of a yellow form of *C. miniata* is given to be around 1888 in Zululand, KwaZulu-Natal, by Phillips (1931) when he described the variety *C. miniata* Regel var. *flava* E.Phillips. Subsequently, one or two yellow-flowered specimens of *C. miniata* were collected in the Eshowe Forest, Zululand, by Mr. C.R. Saunders of Melmoth, Zululand. A specimen was send by Mr. B. Nicholson to the National Herbarium, Pretoria, from which Phillips described the new variety (Phillips 1931).

Gooding (1964), however, mentions a yellow/cream form of *C. miniata* discovered in Eshowe, Zululand in 1892. Sir Melmoth Osborne's (the Commissioner at the time) Zulu cook discovered a pale yellow-coloured *Clivia* while searching for firewood. According to Gooding (1964), Sir Osborne shared offsets of the plant with his friends, giving one to his assistant, Sir Charles Saunders. Sir Charles sent some of the 'bulbs' (rhizomes), including a flower, to his mother. Mrs. J.H. Saunders. She made a drawing of the flower and sent it with a 'bulb' (rhizome) to the Royal Botanical Gardens at Kew where notes of the drawing were made before it was returned to her. A first collection date of around 1888 claimed by Duncan (1985, 1999) and Phillips (1931), therefore may well be nearer 1892 and it is likely the collector accredited should either be the Zulu cook or Sir Melmoth Osborne.

According to a communication between Wessel Marais (Herbarium, Royal Botanical Gardens, Kew) and Mrs. Mauve (Botanical Research Institute, Pretoria) in 1964, an earlier note reporting a yellow form of *C. miniata* exists, namely in *Wien. Illustr. Gartenzeit*, 1888: 275. The yellow form was referred to as *Clivia sulphurea*, a novelty displayed at some show. According to the communication, this name is a *nomen nudum* and therefore has no nomenclatural standing. This, however, places the date of discovery definitely before 1892 and almost certainly before 1888.

Subsequently, various other yellow-flowered specimens of *C. miniata* (Figure 3.3) were discovered in both the Eastern Cape and KwaZulu-Natal, but in most cases precise

localities are not known. The picture is further clouded by spontaneous mutations and hybrids that were produced in cultivation over the years (Duncan 1985, 1992, 1999).

Formal description of a yellow form of *C. miniata* followed some years after its first collection. W. Watson published an article containing reference to the yellow variety of *Clivia miniata* in volume 25 of *The Gardener's Chronicle* in 1899. He formally described the variety as *C. miniata* Regel var. *citrina* W.Watson in *The Garden* 1899:56 (Duncan 1985, 1999).

Varietal names like *C. miniata* Regel var. *flava* E.Phillips (Phillips 1931) and *C. miniata* var. *aurea* Hort. (described in *The Gardener's Chronicle* 35: 301 and *The Garden* 65: 330, both dated 7th May 1904—according to the communication between Mauve and Marias) are later homonyms and should be discarded. As already mentioned, *C. sulphurea* is a *nomen nudum* and should also be discarded.

Figure 3.3 *Clivia miniata* yellow cultivar.

***Clivia gardenii* Hook. (1856)**

Clivia gardenii (Figure 3.4) was discovered and collected by Major Robert J. Garden of the 45th Regiment. He collected specimens while stationed in Natal and sent them to the Royal Botanical Gardens at Kew. It was here that Sir W. Hooker, being third time lucky, named the new species *Clivia gardenii* when it flowered in 1856, in honour of the collector Major Garden (Hooker 1856; Obermeyer 1972; Duncan 1985, 1992, 1999; Koopowitz 2002).

Figure 3.4 *Clivia gardenii* var. *gardenii*.

Clivia gardenii* var. *citrina* Swanevelder et al. *ined.

In this dissertation a yellow form of *Clivia gardenii* Hook. is described as a variety (Figure 3.5).

Clivia gardenii var. **citrina** *Swanevelder, A.E.van Wyk & Truter*, var. nov. floribus pallide luteis vel citrinis, non aurantiacus vel rubris ut in varietate typico distinguitur. TYPE.—KwaZulu-Natal, 2731 (Louwsburg): Ngome Forest (–CD), *Swanevelder & Truter* ZH10 (PRU, holo.).

The holotype was collected in Ngome Forest (Ngotshe District, KwaZulu-Natal) by Z.H. Swanevelder and J.T. Truter on 22 June 2002 (permit no. 30443/2002, collection no. ZH10) The visit confirmed previous reports of a population of yellow-flowered *C. gardenii*. This stands in contrast to the single specimen of *Clivia miniata* var. *citrina* Watson that was available when the variety *citrina* was described. We therefore consider the establishment of a new yellow-flowered variety *Clivia gardenii* var. *citrina* as fully justified.

Figure 3.5 *Clivia gardenii* var. *citrina* Swanevelder, A.E.van Wyk & Truter, *ined.*

The first published reference to the yellow-flowered form of *Clivia gardenii* is by Obermeyer (1972). In that publication a specimen of a yellow-flowered plant, collected by Dr. L.E.W. Codd in April 1956, was used to illustrate *C. gardenii*. The specimen was also from Ngome Forest and was planted in the Pretoria Botanical Gardens where it flowered in September 1965. A plate was prepared by the artist C. Letty in that same year. It was used by Obermeyer (1972) in her publication '*Clivia gardenii*' in *The Flowering Plants of Africa* 42: t.1641. A second publication—a photograph by G. Duncan—was published on page 37 of the *Clivia Yearbook 2* (*Clivia* Club, Kirstenbosch Botanical Garden, 2000).

***Clivia caulescens* R.A.Dyer (1943)**

The first *Clivia* species to be scientifically described and named in its country of origin was *Clivia caulescens* (Figure 3.6). This pendulous-flowering species, similar to *C. gardenii* and *C. nobilis*, was under observation for several years before it was formally described as a new species, by Dr. R.A. Dyer in 1943 (Dyer 1943; Duncan 1999).

Figure 3.6 *Clivia caulescens.*

In his publication Dr. Dyer acknowledged two collectors of the new species, namely Dr. F.Z. van der Merwe and E.E. Galpin. The specimen collected by Dr. Van der Merwe was used to prepare the plate in Dyer's publication. Galpin was credited with collecting a specimen as early as October 1890 (Dyer 1943).

***Clivia mirabilis* Rourke (2002)**

Clivia mirabilis is the most recent species described in the genus and the second to be described in South Africa. Mr. J. Afrika, a game guard at the Oorlogskloof Nature Reserve near Nieuwoudtville, Northern Cape Province, drew the attention of Mr. Wessel Pretorius, the Reserve Manager, to this species. Mr. Pretorius collected and sent a

specimen to the Compton Herbarium, Cape Town, as part of a batch of material that needed identification. In February 2001, Dr. J.P. Rourke identified the genus and determined that it was an undescribed species after visiting a flowering population of the plants in the Oorlogskloof Nature Reserve. The new species was subsequently described as *Clivia mirabilis* Rourke in May 2002 (Rourke 2002a, b).

Clivia 'Robust' *gardenii*

The so-called 'Robust' form of *C. gardenii* (Figure 3.7) was first proposed as a new species after genetic data analyses of several collections of *Clivia* by Ran *et al.* (2001a, 2001b). According to Ran *et al.* (2001b), the RAPD results showed enough statistical significant differences between *C.* 'Robust' *gardenii* and the other species, to merit a new taxon. This taxon only occurs in a specific phytogeographical region, the Pondoland Centre of Endemism (Chapter 4, Van Wyk 1994; Van Wyk & Smith 2001).

Figure 3.7 *Clivia* 'Robust' *gardenii* in habitat.

3.3 Notes on the identification of *Clivia* species

Though the genus *Clivia* comprises of five species, *C. miniata* is the only one that is quite readily distinguishable with its large trumpet-shaped flowers, arranged unmistakably in an upright umbel. The four pendulous-flowered species all appear very similar at a glance and a combination of features must therefore be used to make a positive identification. Natural variation further complicates identification because the diagnostic features are not constant and can vary greatly within a species (Duncan 1999). The inadequacy of existing distribution maps for the various species also renders the identification difficult (Chapter 4, Winter 2000).

These factors have led to much confusion and wrong identifications abound in herbaria. Publications based on such wrong identifications have further led to confusion among both the scientific community and the public. This snowball effect is clearly evident in a publication of Vorster (1994) in which he attributed the so-called *C.* 'Robust' *gardenii* to *C. nobilis*. Though the *C.* 'Robust' *gardenii* was not yet recognised as a potentially new taxon at the time, his specimen should at least have been called *C. gardenii* and not *C. nobilis*. Diagnostic characters and habitat information required for positive identification of the different species of *Clivia* are summarised in Table 3.2 and are also supplied in the form of a key. With this information it should be possible to identify at least most specimens of *Clivia* from the wild to species or infraspecific taxon.

Table 3.2 Key diagnostic characters for the identification of *Clivia* species.[1*]

Character	*Clivia caulescens*	*Clivia gardenii*	*Clivia miniata*	*Clivia mirabilis*	*Clivia nobilis*	*Clivia* 'Robust' *gardenii*
Flowering time	September–November (Spring)[2*]	May–July (late Autumn–mid Winter)	August–November (Spring–early Summer)[2*]	October–mid-November (late Spring)	August–January (Spring–Summer)[2*]	Late March–early August (Autumn–Winter)
Flower: number	14–50	10–20	10–40	20–48	20–50	15–40
Umbel: form	Usually tight & flattened on one side	Usually loose, flattened to one side, slightly rounded on other side	Forming big round umbels, almost globose	Forming a tight umbel, judged from published pictures	Dense, compact, round umbel	Variable, usually loose, slightly globose
Distance stigma protrudes from tip of perianth tube	< 7 mm	Prominent, > 7 mm	Variable	Slight, judged from published pictures	< 6 mm	Variable, stigma pushed out beyond anthers
Degree anthers protrudes from tip	Slight	Always prominent	Variable	Slight	Variable	Slight–prominent
Flower length (perianth and ovary length)	30–35 mm	40–52 mm	Variable, depending on flower shape	35–50 mm	24–40 mm	30–55 mm
Pedicels: orientation	Stiff, erect, drooping near flower	Stiff, erect/sub-erect	Stiff and erect	Drooping	Slightly curved along length/drooping	Stiff, erect/sub-erect
Pedicels: colour	Usually green	Usually tinged red or orange	Green	red/orange during flowering, green when fruiting	Usually green	Variable

Character	*Clivia caulescens*	*Clivia gardenii*	*Clivia miniata*	*Clivia mirabilis*	*Clivia nobilis*	*Clivia* 'Robust' *gardenii*
Pedicels: length	15–35 mm	20–40 mm	± 30–70 mm	25–40 mm	20–40 mm	15–60 mm
Flowers: Orientation	Drooping	Drooping on stiff pedicels	Erect	Drooping	Drooping	Drooping on stiff pedicels
Flowers: Perianth shape	Tubular and curved; inner petals re-curved	Tubular and curved (falcate) downwards; inner petals re-curved	Open, funnel-shaped with spreading flower segments	Tubular, linear to curved, tubular with increasingly flaring at the apex	Tubular and linear with straight inner petals	Tubular, somewhat falcate with an increasingly flaring apex
Leaf sheath: colour	Green–light red	Green–light red	Green–light red	Prominent, flushed deep carmine maroon	Purplish	Green–light red
Leaves: Orientation	Arching	Recurved	Arching	Stiff, erect	Stiff, sub-erect	Arching–erect
Leaves: length × width (mm)	300–400(–900) × 35–50(–70)	350–450(–900) × 25–50(–60)	400–500(–900) × (25–)50–65(–70)	600–1200 × 30–50	300–700(–1000) × 25–45	300–800(–1200) × 30–70(–90)
Leaves: margin	Rarely serrated	Cartilaginous, minutely toothed	Usually entire	Entire, cartilaginous, usually smooth	Serrated	Cartilaginous and dentate
Leaves: apex	Obtuse–acute	Obtuse–acute	Acute	Obtuse-acute	Retuse and oblique	Abruptly rounded/ retuse
Leaves: special characteristics	-	-	-	Prominent white stripe in centre of leaf	White stripe absent or present	White stripe absent or present
Aerial stem	Usually present when mature; up to 3 m long	Rarely present; very old specimens	Rarely present; very old specimens	Not yet reported	Absent	Usually present for swamp forms

Character	*Clivia caulescens*	*Clivia gardenii*	*Clivia miniata*	*Clivia mirabilis*	*Clivia nobilis*	*Clivia* 'Robust' *gardenii*
Seed: number	1–4	Usually 1 or 2	1–4(–25)	(1)2–4(–7)	1 or 2(–6)	1 or 2(–4)
Seed: maturation time (months)	± 9	± 9–12	± 9–12	± 4–6	± 9–12	± 9–12
Seed: size (diameter)	Medium, ± 12 mm	Large, ± 18 mm	Medium, ± 12 mm, Transkei and Eastern Cape forms larger	Small, ± 10 mm	Small, ± 9 mm	Large, 10–18 mm
Endocarp: colour	Colourless	Colourless	Colourless	Colourless	Red-pigmented	Colourless
Distribution	Limpopo Province (Soutpansberg) Mpumalanga Province and Swaziland	KwaZulu-Natal Province	Eastern Cape Province (Transkei), KwaZulu-Natal Province, Swaziland, Mpumalanga Province	Northern Cape Province	Eastern Cape Province	Southern KwaZulu-Natal Province, Eastern Cape Province (Pondoland Centre of Endemism)

[1*] Information included in this table based on: Mr. J. Truter pers. comm.[1]; Mr. A. Hardinge pers. comm.[2]; Mr. R. Dixon pers. comm.[3] Hooker 1828, 1856; Pole Evans 1921; Dyer 1943; Obermeyer 1972; Duncan 1999; Malan 2000; Koopowitz 2002; Rourke 2002a, b; personal observations by author.

[2*] May flower sporadically throughout the year.

[1] J.T. Truter, PO Box 5085, Benoni South 1502, South Africa
[2] A. Hardinge, PO Box 14964, Margate 4275, South Africa
[3] R. Dixon, PO Box 1041, Pyramid 0120, South Africa

3.4 Key to the identification of *Clivia* species

1a Perianth lobes open to form a trumpet-/funnel-shaped flower; flowers erect (Eastern Cape Province, KwaZulu-Natal Province, Mpumalanga Province and Swaziland).... **2**

2a Flowers yellow ..... ***C. miniata* var. *citrina***

2b Flowers orange, red or pastel shades of these colours..... ***C. miniata* var. *miniata***

1b Perianth lobes parallel to form tubular flowers; flowers pendulous **3**

3a Leaves stiffly erect or sub-erect; leaf sheath prominently coloured (carmine-maroon or purplish); seed diameter small (< 10 mm); umbel dense and compact with high flower numbers (usually 30 or more)..... **4**

4a Endocarp pigmented (reddish); leaf apex retuse and oblique; leaf sheath purplish; white stripe in centre of leaf present or absent; pedicels green at anthesis; summer rainfall area (Eastern Cape Province) ..... ***C. nobilis***

4b Endocarp unpigmented; leaf apex obtuse-acute; leaf sheath prominent, flushed deep carmine; white stripe in centre of leaf present or absent; pedicels orange-red at anthesis; winter rainfall area (Northern Cape Province)

..... ***C. mirabilis***

3b Leaves flexible, arching, recurved or arching-erect; leaf sheath not prominently coloured; seed diameter medium to large (> 10 mm); umbel densities vary, variable flower numbers..... **5**

5a Flowering in spring or early-summer; older specimens with prominent stems; seeds usually with a diameter of ± 12 mm; perianth tube rarely curved (falcate); stigma and anthers may protrude slightly from perianth (Limpopo Province (Soutpansberg), Mpumalanga Province and Swaziland)

..... ***C. caulescens***

5b Flowering in autumn or winter; prominent stems present or absent; seed usually large with a diameter of ± 18 mm; perianth tube usually curved (falcate); stigma and anthers usually protrude from perianth..... **6**

6a Flowering in autumn and winter; sometimes in swampy habitat—with a prominent stem; high flower numbers (15–40); leaf apices becomes abruptly rounded/retuse; plants robust (southern Kwazulu-Natal Province, Eastern Cape Province: Pondoland Centre of endemism)

..... ***C.* 'Robust' *gardenii***

6b Flowering in late autumn and mid winter; usually in a well-drained habitat, rarely with a prominent stem; flower numbers low (10–20); leaf apices obtuse-acute; plants less robust (KwaZulu-Natal Province) 7

7a Flowers yellow ..... ***C. gardenii* var. *citrina***

7b Flowers shades of orange and red ..... ***C. gardenii* var. *gardenii***

3.5 Interspecific hybrids

The genus *Clivia*, notably *C. nobilis* and *C. miniata*, has received much horticultural attention since being introduced to Britain during the early and mid 1800s. With only the two species known at the time, horticulturists were quick to attempt interspecific hybrids. According to Vorster (1994) the first hybrid between these two species was published in 1856 in *Revue Horticole* 8: 258–260. The author(s) of this interspecific hybrid, *C. miniata* × *C. nobilis*, named it *Himantophyllum cyrthanthiflorum* (Vorster 1994). *Himantophyllum miniatum*, a synonym for *C. miniata*, was used as source for genus name during the naming of this hybrid (Koopowitz 2002). Another well known interspecific hybrid, possibly between *C. miniata* and *C. nobilis*, was produced by Charles Raes in the late 1850s and published by Van Houtte as *Clivia* Cyrtanthiflora in 1869 (Duncan 1999).

Koopowitz (2000) refers to *C.* Cyrtanthiflora—published as *Imantophyllum* Cyrtanthiflorum (1877)—as the only valid 'grex name' published at the time. According to Article 4.6 (including Note 4, Art. 4) of the *International Code of Nomenclature for Cultivated Plants* 1995 (ICNCP), the designation 'grex' is reserved for the Orchidaceae (Trehane *et al.* 1995). Article 4 (ICNCP) stipulates that the designation 'cultivar-group' should be used for assemblages of similar cultivars—either within a genus, hybrid genus (nothogenus), species, hybrid species (nothospecies) or other denomination class (Trehane *et al.* 1995). It is here recommended that the cultivar-group name, *Clivia* Cyrtanthiflora Group, be used for all hybrids between these two species.

It is also recommended here that the series of 'grex names', erroneously designated as such by Koopowitz (2000), be replaced by the designation cultivar-group. The cultivar-group names for the existing hybrids, including the names of the parent taxa, are therefore as follows:

Parentage	Cultivar-group name
C. gardenii × *C. caulescens*	*Clivia* Caulgard Group
C. miniata × *C. nobilis*	*Clivia* Cyrtanthiflora Group
C. Cyrtanthiflora × *C. miniata*	*Clivia* Minicyrt Group
C. gardenii × *C. miniata*	*Clivia* Minigard Group
C. miniata × *C. caulescens*	*Clivia* Minilescent Group
C. nobilis × *C. caulescens*	*Clivia* Nobilescent Group
C. gardenii × *C. nobilis*	*Clivia* Noble Guard Group

Though a particular cultivar-group name is used for any cross between two particular taxa (Trehane *et al.* 1995; Koopowitz 2000), there are uncertainties as to which parents have been used as berry- or pollen parent to date. Literature research has yet to produce proof of the following hybrids (berry parent listed first): *C. caulescens* × *C. gardenii*, *C. caulescens* × *C.* 'Robust' *gardenii*, *C.* 'Robust' *gardenii* × *C. nobilis* and *C.* 'Robust' *gardenii* × *C. gardenii* (Table 3.3 and references therein). Conflicting statements between different sources, poor record keeping and misidentification of the pendulous species, prompts one to question the reversal of some of the yet unsuccessful hybrids, listed as successful (e.g. *C. gardenii* × *C. caulescens*, Table 3.3). The extent to which interspecific hybridisations have been attempted on numerous individuals from different localities, are not normally indicated. Hybrids produced between very fertile individuals of different species, which might be separated hundreds of kilometres from each other, may produce offspring. Such hybrids would probably not occur in nature.

Clivia miniata appears to be very compatible with the other species and is the only one that has been successfully hybridised with all the known species—the one exception being *C. mirabilis*, which was only described in 2002 (Rourke 2002a). Attempts to use *C. mirabilis* in hybridisation will undoubtedly be attempted soon. Natural hybrids between *C. miniata* and other *Clivia* species are also known for some populations where two *Clivia* species occurs together. These include *C. miniata* × *C. nobilis* (*C.* × *cyrtanthiflora*),

C. miniata × *C. gardenii* and *C. miniata* × *C. caulescens* (J.T. Truter pers. comm.[1]; Winter 2000).

Table 3.3 Known interspecific hybrids in the genus *Clivia*.

♂ Species ► / ▼ ♀ Species	*C. caulescens*	*C. gardenii*	*C. miniata*	*C. mirabilis*	*C. nobilis*	*C.* 'Robust' *gardenii*
C. caulescens	-	NO[1]	YES[1]	?	YES[1, 4]	NO[1]
C. gardenii	YES[1, 2, 4]	-	YES[1, 2]	?	YES[1, 2]	YES[1]
C. miniata	YES[1, 2, 4]	YES[1, 4, 3]	-	?	YES[1, 2, 4]	YES[1]
C. mirabilis	?	?	?	-	?	?
C. nobilis	YES[1, 2]	YES[1]	YES[1]	?	-	YES[1]
C.* 'Robust' *gardenii	NO/YES[1]	NO/YES[1]	YES[1]	?	YES[1]	-

[1]J.T. Truter pers. comm.; [2]Koopowitz (2000); [3]Ran (2001c) & [4]Anderson (2001).

3.6 Systematics of the genus *Clivia* Lindl.

In recent years, molecular evidence became increasingly popular as the method of choice for reconstructing phylogenetic relationships amongst taxa. This can clearly be seen when one reviews the number of publications published yearly in the field of plant systematics. At least three such studies on *Clivia* have already been published (Ran *et al.* 2001a; Ran *et al.* 2001b; Conrad & Reeves 2002).

Meerow *et al.* (1999) placed the genus *Clivia* into its family context by using sequence data, generated from the rbcL and trnL-F regions, of the chloroplast genome. The results suggested that *Cryptostephanus* is the genus nearest to *Clivia* and further supports the tribe Haemantheae.

The work by Ran *et al.* (2001a; 2001b) was the first to look at the infrageneric relationships of *Clivia*. In a phylogenetic analysis of the genus (Ran *et al.* 2001a), they utilized the ITS and the 5S non-transcribed spacer regions of the genome. The trees generated by the two data sets were identical. This corresponds with another publication

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by this research group (Ran *et al.* 2001b), in which RAPDs (randomly amplified polymorphic DNAs) were used to infer relationships within the genus. Ran *et al.* (2001a; 2001b) showed with their molecular work that the so-called *C.* 'Robust' *gardenii*, is sufficiently different from the other species to justify naming it as a new taxon. The general relationships between the different species as indicated by the work of Ran *et al.* (2001a; 2001b), are shown in Figure 3.8.

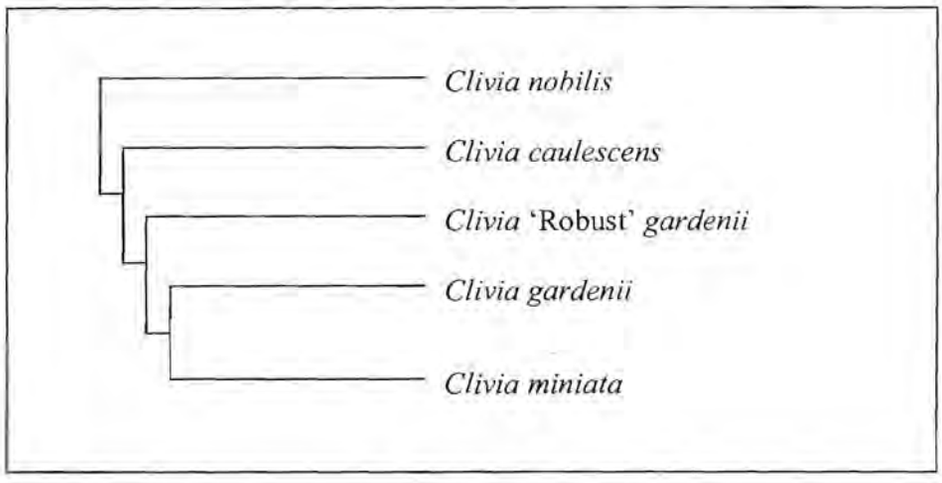

Figure 3.8 A tree proposed by Ran *et al.* (2001a; 2001b).

Conrad & Reeves (2002), like Meerow *et al.* (1999), targeted the plastid genome. They used sequence data generated by two intergenic spacers (rpoB-trnC and trnL-F) and two introns (rps16 and trnL) in their analysis. They showed that *C. mirabilis* was sister to all the other taxa (Figure 3.9) followed by *C. nobilis* and then *C. gardenii*. Unfortunately they did not include *C.* 'Robust' *gardenii* into their analysis. *C. caulescens* and *C. miniata*, according to the resultant tree, are more closely related, with *C. gardenii* sister to these. This pattern is different from relationships suggested by the trees of Ran *et al.* (2001a; 2001b), all of which grouped *C. miniata* and *C. gardenii* together, with *C. caulescens* being sister in three of their four trees. These differences in the trees could be due to numerous factors, including different methods of analysis, different mutation rates of the regions under investigation, intraspecific variation among localities from different areas (Chapter 6), the latter which is not accounted for by the small sample sizes usually used in molecular studies.

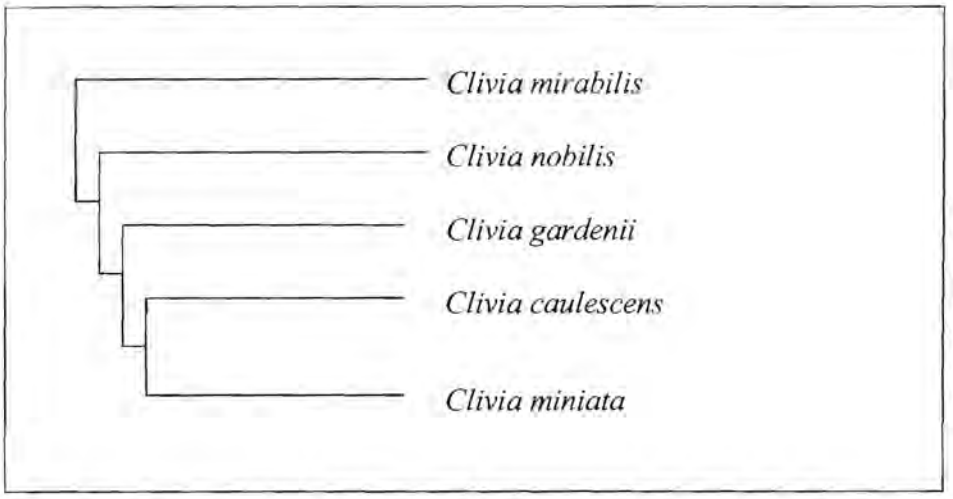

Figure 3.9 Tree proposed by Conrad & Reeves (2002) using plastid regions in their analysis.

A hypothetical tree (Figure 3.10), based on the distribution records (Table 3.2 and Chapter 4), proposes the possible relationships between the different species within the genus *Clivia*.

Figure 3.10 Hypothetical tree based on phylogenetic and distribution records of the different species within the genus *Clivia*.

Clivia mirabilis is placed basal, followed by *C. nobilis*, *C.* 'Robust' *gardenii* and *C. gardenii*, with *C. miniata* and *C. caulescens* filling the terminal clade. This tree corresponds best with the tree proposed by Conrad & Reeves (2002). This could be

attributed to the maternally inherited chloroplast regions they used (Chapter 6). Maternally inherited regions will only be distributed though seed. With speciation occurring through isolation, one expects higher nuclear variation (2001a; Ran *et al.* 2001b), but lower variation in more highly conserved regions like the chloroplast genome.

3.7 Acknowledgements

Special thanks to Dr. L.J. Viljoen and Mr. A. Hardinge for the photographs used in this chapter. Mr. J.T. Truter and Mr. A. Hardinge are thanked for the detailed locality information and lengthy discussions.

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CHAPTER 4

CLIVIA POPULATIONS: DISTRIBUTION, ECOLOGY & CONSERVATION STATUS

4.1 Introduction

The recent horticultural attention received by *Clivia miniata* (Chapter 5) has emphasised our lack of knowledge regarding the natural ecology of the genus. In one of the few reports on the distribution and ecology of *Clivia*, Winter (2000) emphasized the lack of herbarium records of this group in the herbaria of the National Botanical Institute of South Africa. He believes that due to the inaccessibility of most *Clivia* populations, the discovery of additional localities for the various species is highly likely.

This chapter aims to contribute towards our understanding of *Clivia* ecology and its distribution range. A brief historical overview of early Africa and its climate precedes an hypothesis, aimed at explaining the current distribution pattern of *Clivia* species. The conservation status of the genus is also reviewed using existing/future threats and present distribution patterns. The distribution of infraspecific taxa (e.g. yellow-flowered varieties) will not be considered in this chapter.

4.2 Materials and methods

Maps presented in this chapter are compiled from more than 250 herbarium records, confirmed visual reports and records in living collections (Appendix I). Herbarium records in the C.E. Moss Herbarium (University of the Witwatersrand), Compton Herbarium (National Botanical Institute, Cape Town), Lowveld National Botanical Gardens Herbarium (National Botanical Institute, Nelspruit), H.G.W.J. Schweickerdt Herbarium (University of Pretoria), Natal Herbarium (National Botanical Institute, Durban), National Herbarium, including PRECIS (National Botanical Institute, Pretoria) and Selmar Schonland Herbarium (Rhodes University, Grahamstown) were used.

All records were sorted according to the different species before analysis. Grid and GPS references available were plotted using the program *Arc View GIS*. Records lacking this information were located using the locality descriptions, maps and the search function of *Arc View GIS*.

The difficulty experienced by most people in identifying the pendulous-flowered species prompted the questioning of some locality records. Record identity was deemed unreliable when no duplicate record existed for that locality (either visual, herbaria or living collection) and the record fell outside a grid already containing a known record or it was outside the previously accepted distribution range/habitat of that species. Collections made before species description, were deemed incorrectly identified (species unknown at time of collection) when they did not fulfil the above criteria. Sightings were only included as records if they were confirmed by at least two individuals or by a herbarium/living collection record. Records lacking the necessary information to establish the grid references were discarded.

4.3 Distribution and ecology of Clivia

4.3.1 Introduction

Clivia is known to grow in diverse habitats, from coastal forest and secondary coastal dunes, to swamps, riverbanks and rock screes. Specimens are even found as epiphytes in some localities (Duncan 1999; Winter 2000). *Clivia* populations normally occur as rather inaccessible, isolated colonies. According to Winter (2000), these populations are extremely old, with some populations producing very little seed. The only hope for these populations to survive is their ability to regenerate vegetatively.

Species prefer to grow in cool, shaded, well drained habitats, located in the summer rainfall area, with the exception of *Clivia mirabilis*, which has a localized distribution in a semi-arid area, with Mediterranean climate and winter rainfall (Duncan 1999; Winter 2000; Rourke 2002a, b).

Clivia is believed to be endemic to South Africa and Swaziland, with unconfirmed reports of sighting in Mozambique and as far north as Kenya and Uganda (Winter 2000; Rourke 2002a). The distribution of *C. miniata*, *C. nobilis*, *C. caulescens* and *C. gardenii* is along the coastal and inland Afromontane forests of southern Africa, with *C. mirabilis* growing among relictual evergreen Afromontane forest elements in the southwestern corner of the Northern Cape (Rourke 2002a). The genus' distribution extends in an

eastwards direction, from the coastal areas of the Eastern Cape Province in the south, through KwaZulu-Natal Province, Swaziland and Mpumalanga Province to the Soutpansberg in Limpopo (=Northern) Province (Duncan 1985, 1992; Vorster 1994; Duncan 1999; Snijman 2000; Winter 2000; Rourke 2002a). *Clivia mirabilis*, however, is only known to occur in one area near Nieuwoudtville in the Northern Cape Province (Rourke 2002a).

4.3.2 *Clivia nobilis* Lindl.

The distribution of *Clivia nobilis* has been described as within the Eastern Cape (including Transkei) and southern KwaZulu-Natal (Vorster 1994; Duncan 1999). Malan (2000) reported localities from west of Grahamstown, in the Albany and Bathurst Districts, along the Eastern Cape coastal belt to the east of Kei River Mouth. Winter (2000) described the distribution as from Alexandria Forest, northwards along the coast to Nqabara River, with occasional populations inland as far as Grahamstown.

The present study found that *Clivia nobilis* is only distributed in the Eastern Cape (Malan 2000; Winter 2000) and not in southern KwaZulu-Natal as previously reported by Vorster (1994) and Duncan (1999). Populations are concentrated towards the coast, from just north of the Sundays River Mouth, extending up along the coast to the Mbashe River area, with colonies occurring as far inland as the vicinity of Grahamstown (Figure 4.1).

The distribution range of *C. nobilis* is located in the Albany Centre (AC) and the southern part of the Maputaland-Pondoland Region (MPR) of endemism (Figure 4.1). The Albany Centre is a mosaic of different vegetation types, displaying floristic elements of the five different phytochoria converging on it. Biomes such as Savanna, Forest, Thicket, Grassland, Fynbos and Nama-Karoo are found in this centre (reviewed by Van Wyk & Smith 2001). According to Van Wyk (1994), the AC represents a southwards extension of the MPR, but with the presence of Cape floristic and Karroid elements giving the centre its own distinct character. The MPR and AC also contain enclaves of Afromontane forests (Van Wyk & Smith 2001).

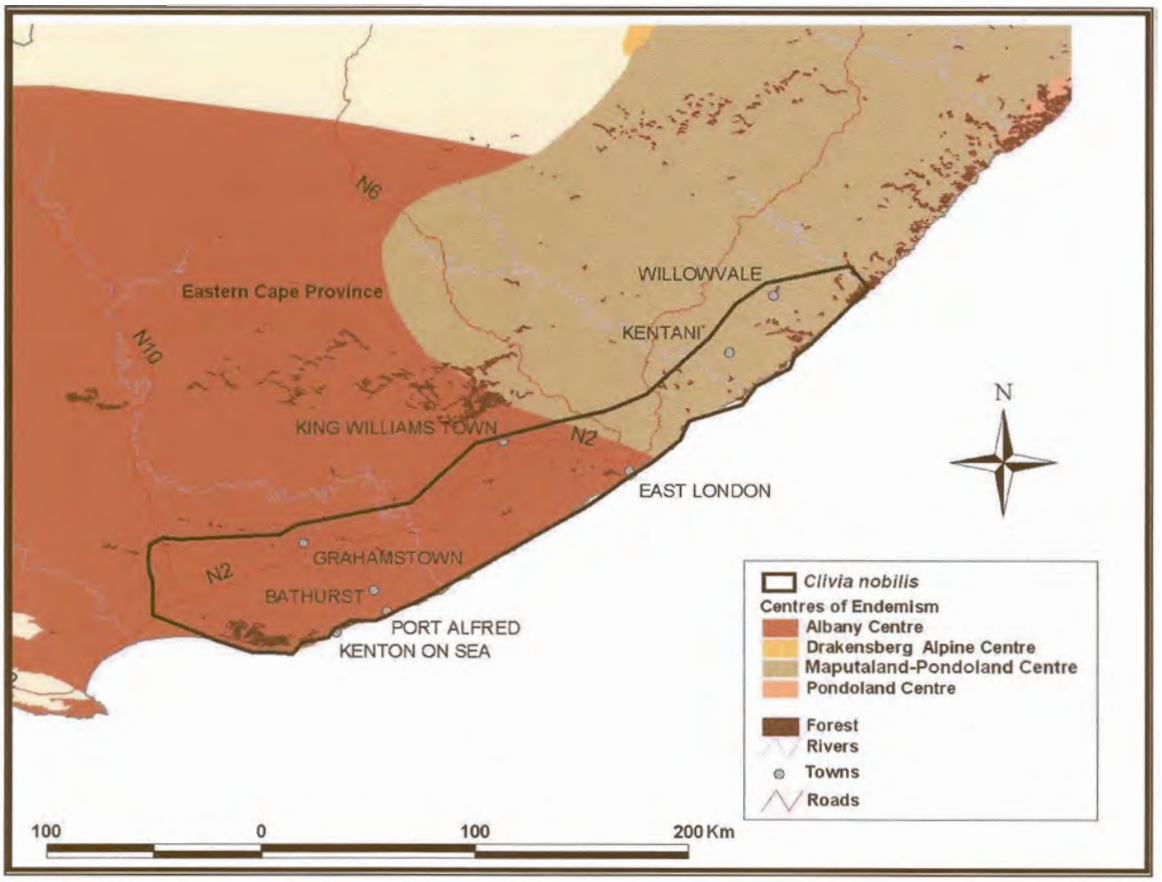

Figure 4.1 Natural distribution range of *Clivia nobilis*.

The Afromontane Archipelago-like Region of Endemism is a series of isolated floristic areas distributed from the Cape Peninsula, northwards along the southern and East African uplands, to northeastern Africa (White 1983). The region has a temperate climate and receives mostly high rainfall, anything from 700 mm to 2000 mm annually. In southern Africa, this region is centred in the Lesotho and KwaZulu-Natal Drakensberg and Midlands, extending north- and southwards along the Great Escarpment. A second centre, containing the largest contiguous block of forest in the subcontinent, is located on the coastal platform of the southern Cape (Lubke & Mckenzie 1996; Cowling & Hilton-Taylor 1997). Dominant canopy species in Afromontane forest are 30–40 m high, with those in the Albany Centre 2–10 m in height (Lubke & Mckenzie 1996; Midgley *et al.* 1997).

The MPR encompasses, like the AC, a diverse array of vegetation types, including grassland, forest (tropical/sub-tropical and Afromontane), savanna, thicket and aquatic elements (Goldblatt 1978; Cowling & Hilton-Taylor 1997; Van Wyk & Smith 2001). Van Wyk (1994) mentions that the southern part of this region contains mainly grasslands, with indigenous forest covering less than 1% of the entire MPR. The coastal forests are floristically related to the coastal forests of East Africa and secondarily to the tropical rainforests of Guineo-Congolian Region (Goldblatt 1978; White 1983; Cowling & Hilton-Taylor 1997). Midgley *et al.* (1997) mapped Subtropical Thicket as a vegetation type mainly confined to the Eastern Cape. Climate in this region ranges from subtropical/tropical in the low-lying areas, to more temperate on the higher ground (with frost in winter). Annual precipitation varies from 400–1 200 mm or more and occurs predominantly during summer (Goldblatt 1978; Van Wyk 1994; Cowling & Hilton-Taylor 1997; Van Wyk & Smith 2001).

The coastal areas of the Albany Centre have a mild climate (9–25°C) and receive 600–900 mm rainfall annually. Inland areas have frost/snow in the winter to 45°C maximum summer temperatures and ± 250 mm mean annual precipitation (Midgley *et al.* 1997; Van Wyk & Smith 2001). *Clivia*, being a shade-loving genus, will grow in the higher rainfall areas where there is appropriate canopy cover—clearly evident in the distribution pattern and habitat of the species (Figure 4.1).

Clivia nobilis is found under evergreen forest, low bush (thicket) and amongst dune vegetation (Duncan 1999; Malan 2000; Winter 2000). Inland populations are found in wooded kloofs where they grow on riverbanks, rocky outcrops and along forest margins (Malan 2000; Winter 2000). Indications are that *C. nobilis* is not competing very successfully with the current climax forest communities. Populations are usually more exposed on primary coastal dunes with their low canopy cover (2–5 m) (Duncan 1999; Malan 2000).

A visit to the Kei River area confirmed the habitat of a *C. nobilis* coastal population. This locality appears to contain two ecotypes. At the base of the dune away from the sea, long-leaved, large *C. nobilis* plants with long extended root systems, growing under a high, closed canopy of 5–10 m, are found. Mid-way up the dune, short leaved plants with smaller rooting systems are found under a low 2–3 m canopy. Plants were in flower or in bud. The colony grows in sea sand with lots of humus/decomposing leaves originating from the canopy overhead. Some plants on the top of dunes grow in full sunlight with no apparent damage.

Many seedlings, growing either together in groups or scattered, gives a seedling to mature plant ratio of 3:1. Some plants even grow epiphytically. Seedlings of every size were seen in the colony. Single, 2, 3 and multi-stemmed mature plants were recorded. There were a number of scapes and leaves damaged by presumably insects (caterpillars). During the time of the visit (October 2001), at least 40% of the adult plants were in flower, with more still in bud. Individuals that flowered earlier (out of season) produced fruit with seed on almost every pedicel; fertilization and seed-set are high. This population appears to be actively growing and reproducing, sexually and especially asexually.

Leaves of the plants were fairly narrow (25–45 mm), with plants not markedly stunted. There was some flower colour variation, generally light orange with some green at the tips to darker red with/without green tips, pastel orange and pink-red. From 10 to 40 flowers per umbel were noted. No significant signs of large herbivory were detected, but some plants had been removed from the colony. The plants were well spaced and there appeared to be little competition with undergrowth. The above observations are similar to those of Malan (2000).

The accuracy of some herbarium specimens in NBI herbaria was questioned by Winter (2000). *Clivia gardenii* (*C.* 'Robust' *gardenii*) and *C. caulescens* specimens were sometimes misidentified as *C. nobilis*.

4.3.3 *Clivia gardenii* Hook. & *Clivia* 'Robust' *gardenii*

The Eastern Cape Province and KwaZulu-Natal Province are currently regarded as the distribution area of *Clivia gardenii*. Scattered populations occur from Port St Johns (Eastern Cape) in the south, to Ngome Forest (KwaZulu-Natal) in the north (Hooker 1856; Obermeyer 1972; Vorster 1994; Duncan 1999; Winter 2000).

The distribution pattern presented in this study coincides more or less with the currently accepted distribution for *Clivia gardenii* (Figure 4.2). However, known records clearly divide the distribution into three separate areas. The southernmost of these, extends from Port St. Johns in the south to the Mzimkulu River in the north. The second area begins around Durban, progressing northwards to Empangeni and inland as far as the Howick area. The most northern area is confined to Ngome Forest. No linking records were found to connect the different areas.

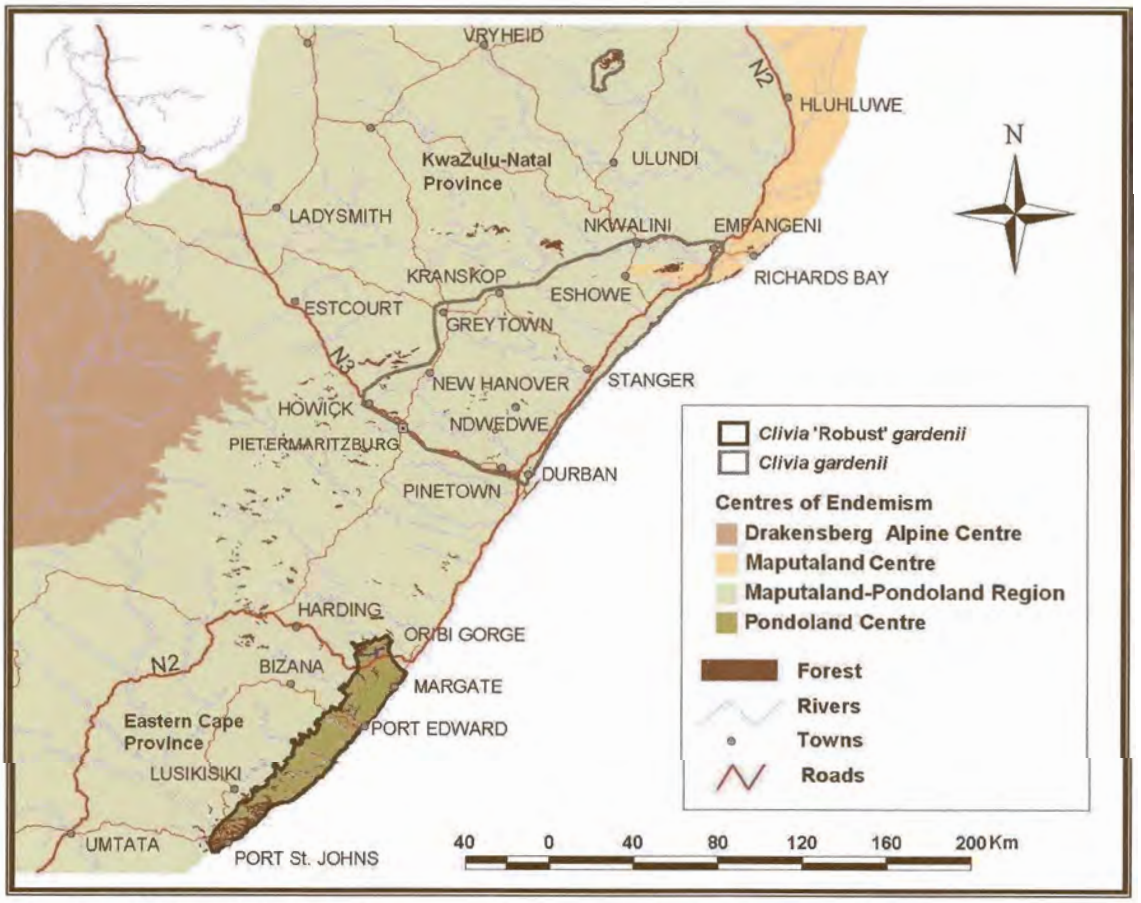

Figure 4.2 Natural distribution range of *Clivia gardenii* and *Clivia* 'Robust' *gardenii*.

Clivia ' Robust' *gardenii* has not yet been formally described as a separate species, therefore specimens of this taxon are filed together with *C. gardenii* in herbaria. In this section, both *Clivia gardenii* and *Clivia* ' Robust' *gardenii* records were used to produce a distribution map for *C. gardenii* in a broad sense. Sightings of *Clivia* 'Robust' *gardenii* were compared to the habitat descriptions on herbarium specimens and localities of *C. gardenii*. In doing this, it became obvious that the distribution area for *Clivia* 'Robust' *gardenii* is limited to the southernmost area on the *C. gardenii* distribution map (Figure 4.2). Furthermore, all sightings of *Clivia* 'Robust' *gardenii* are restricted to this southernmost distribution area. If this southern area is accepted as the distribution range for *Clivia* 'Robust' *gardenii*, then the distribution of *C. gardenii* in a strict sense, appears to extend only from the Durban area northwards.

Molecular evidence produced by Ran *et al.* (2001b) (Chapter 3) has already suggested that the *C.* 'Robust' *gardenii* is a new taxon. This is supported by the proposed distribution pattern, with *C.* 'Robust' *gardenii* geographically isolated (allopatric) from *C. gardenii*. Flowering times coincide during late autumn and early winter (Chapter 3), but genetic isolation is further enhanced when one takes into account that the pendulous species are essentially selfers (Rourke 2002a). Artificial attempts to hybridise these two taxa, also produce conflicting results (Chapter 3, Table 3.3). These, including some morphological differences (Chapter 3, Table 3.2), concur with the requirements of the biological species concept and supports the recognition of *C.* 'Robust' *gardenii* as a new species (Mayr 1992).

The distribution range of *C.* 'Robust' *gardenii* corresponds to that of the Pondoland Centre of endemism. The Pondoland Centre (PC) forms part of the larger Maputaland-Pondoland Region (Tongaland-Pondoland Region of Goldblatt (1978);(Van Wyk 1990, 1994; Van Wyk & Smith 2001). This centre encompasses a 1 880 km^2 large outcrop of Msikaba Formation sandstone, covering the area between the Mzimkulu River (southern KwaZulu-Natal) and the Ntsubane region (Egossa Fault) in the Eastern Cape, including smaller sandstone outcrops such as those at Port St Johns and Uvongo (Van Wyk 1994; Van Wyk & Smith 2001). Msikaba Formation sandstone was previously grouped together

with the Natal Group sandstone, but are now recognised to be unrelated in origin (Van Wyk & Smith 2001, and references therein).

Topographically the PC is characterised by rugged plateaus (100–500 m above sea level) that are deeply dissected by narrow river gorges. Isolated forest patches, containing mixed tropical and Afromontane elements, are confined to the protection of these deep gorges. Mean annual rainfall varies from 1 000–1 200 mm and occurs mainly in the summer months with the southern part receiving an expected 50 mm monthly. The mean annual temperature along the coast is around 20°C. Soils in this centre are usually sandy, acidic, highly leached and often shallow (Van Wyk 1994; Van Wyk & Smith 2001).

The distribution range of *Clivia gardenii* lies within the Maputaland-Pondoland Region of endemism, between the Pondoland Centre and the Maputaland Centre. The most northern locality at Ngome Forest is situated in the KwaZulu-Natal Midlands and is part of the mistbelt Afromontane vegetation. This forest annually receives approximately 1 530 mm of rain and is found on sandstone, dolerite and shale. Afromontane trees are responsible for a 20 m high canopy (Midgley *et al.* 1997). More detailed notes on this forest follow later in this section. Information on the MPR has already been supplied in the previous section (4.3.2, *Clivia nobilis* Lindl.).

The grouping of *Clivia gardenii* and *C.* 'Robust' *gardenii* in the literature makes it difficult to establish the exact habitat for these two taxa. It would, however, appear as if *C. gardenii* prefers in general a well drained habitat, though populations have been recorded in marshy environments. *C.* 'Robust' *gardenii* occurs commonly in a marshy environment, with some populations growing in seepage areas below/on cliffs (pers. comm. J.T. Truter[1] and A. Hardinge[2]). Various diagnostic characters support a distinction between these two taxa (Chapter 3). It is here proposed that *C.* 'Robust' *gardenii* is an endemic to the Pondoland Centre and its outliers, whereas the typical *C. gardenii* occurs

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in the Maputaland-Pondoland Region from Durban northwards, with an outlier distributed in the Ngome Forest.

In communications between Mr. A. Hardinge[2] and the author, various environmental features of *C.* 'Robust' *gardenii* were mentioned. Mr. Hardinge, familiar with many populations of this taxon, reported that though *C.* 'Robust' *gardenii* occurred quite often in natural wetlands/swamps, there are also examples of communities growing in humus-rich soils, sides of cliffs or on rocks. A personal communication with Mr. J.T. Truter[1], suggests that plants most likely occurred in the wetter drainage portions at the base of cliffs. Mr. Hardinge reported that plants occurred under natural forest cover, in or near water. Population densities vary from 5 or 6 plants per 10 m^2 up to 20 plants per square metre, with higher densities observed in wetter areas. In swampy places, plants occurred in big clumps with individuals as high as 1.8 m with buttress roots, but those in dryer, rocky habitat are noticeably 'stockier'. Seed production appears to vary between communities. Removal of plant material from natural habitat, probably for 'muti' purposes, has been reported (Mr. A. Hardinge[2]).

In the course of this study, Ngome State Forest was visited and the environment of a population of *C. gardenii* in this forest was investigated.

Ngome Forest, situated between Vryheid and Nongoma, is part of the Ntendeka Wilderness Area. A population of *C. gardenii* was located under a tall, closed canopy of evergreen trees in the forest, occurring in small colonies with a patchy distribution, and preferring eastern or western aspects, with southern aspects apparently too cool and wet and northern aspects too hot and sunny.

Most *C. gardenii* plants were recorded on steeper slopes, usually 45° and even in cracks of the cliff face/river embankment. Here they form colonies all along the steep crest and ledges of the cliff/embankment. Some of these colonies are so inaccessible that only experienced rock climbers with the necessary equipment could possibly reach them. In this population, some colonies tend to flower more profusely than others. The induction

of flowering was shown to be temperature related in *Clivia miniata* (De Smedt *et al.* 1996; Honiball 2000). No similar studies on any of the other species are currently known. Could flowering be triggered by light and/or day length in *C. gardenii*? This hypothesis stems from the observation that colonies that are exposed to light, flower more profusely in the Ngome location.

Different colour forms, ranging from pastel to darker orange, were seen. Average flower number per umbel varies from 20 to 25. The population is estimated to contain 1 000–2 000 or more mature individuals, of which an estimated 10–15% were in flower. Seed production was high (50–60% of population) with all the colonies that were in flower having fruit of the previous year. Usually one or two large seeds are found in each berry. With the slope being very steep and with some colonies seemingly without seedlings, it is proposed that colonization takes place lower down the embankment—if no other dispersal vector is present. Due to the inaccessibility of the terrain, this hypothesis could not be verified. An abundance of different size plants, ranging from seedlings through to mature flowering plants, were seen in some of the colonies.

Clivia gardenii has an extended flowering season, with some plants already setting seed while others are still in bud. The plants prefer well-drained loamy soil. Signs of leaf-miner activity were present in many plants, with white 'tunnels' visible on the leaf surfaces. Apical damage was also observed. Lichens were mainly found on plants growing in lighter, wetter parts of the forest. No fungal or viral diseased were noted.

The population in Ngome is healthy. Seed are produced in abundance and a number of seedlings were seen. A high percentage of the mature plants produce flowers. The inaccessibility of some colonies within the population ensures the survival of the population as it provides some protection against plant collectors. The size and extent of the forest ensure that there are many similar microhabitats within the forest system.

Herbarium records indicating *Clivia gardenii* localities in both Swaziland and in the Barberton District are wrong and were rejected in accordance to the criteria set out in the beginning of this chapter. These specimens belong to *C. caulescens*.

4.3.4 *Clivia caulescens* R.A.Dyer

Clivia caulescens's distribution is here accepted to be within the eastern part of the Mpumalanga and the Limpopo Provinces (formally the Northern Province)(Vorster 1994; Duncan 1999; Winter 2000). Winter (2000) recorded the most northern localities in the Soutpansberg, Limpopo Province and the most southern in the Sodenza Range on the border of Swaziland and Mpumalanga (Winter 2000).

Records employed in this study place the most southern community of *C. caulescens* in the Mbabane District, Swaziland, and confirmed the most northern locality in the Louis Trichardt (Makhado) area, Soutpansberg (Figure 4.3). This distribution range covers three Afromontane centres of endemism, namely the Barberton Centre (BC), Wolkberg Centre (WC) and the Soutpansberg Centre (SC). These three centres combine at higher hierarchical levels of phytochorology.

The Barberton Centre comprises the crescent-shaped mountain ranges northeast, east, southwest and south of Barberton. This rugged, mountainous region receives 800–1400 mm of precipitation during the summer months. A temperate climate, with mild to cool winters, is experienced with fog common at higher altitudes. Rock of the Barberton Supergroup forms the substrate for this centre (Van Wyk & Smith 2001).

Vegetation of the BC is broadly classified as North-Eastern Mountain Grassland, with scattered Afromontane Forest and Sour Lowveld Bushveld (Van Wyk & Smith 2001). Forests, occurring as pockets, are confined to sheltered ravines, moist valleys and incised valley heads. The Barberton Centre forms part of the Afromontane Region and has close floristic links to the Wolkberg Centre. It is proposed that the Barberton Mountain Land acted as a refuge for Afromontane flora, a hypothesis supported by the high levels of local endemism (Van Wyk & Smith 2001).

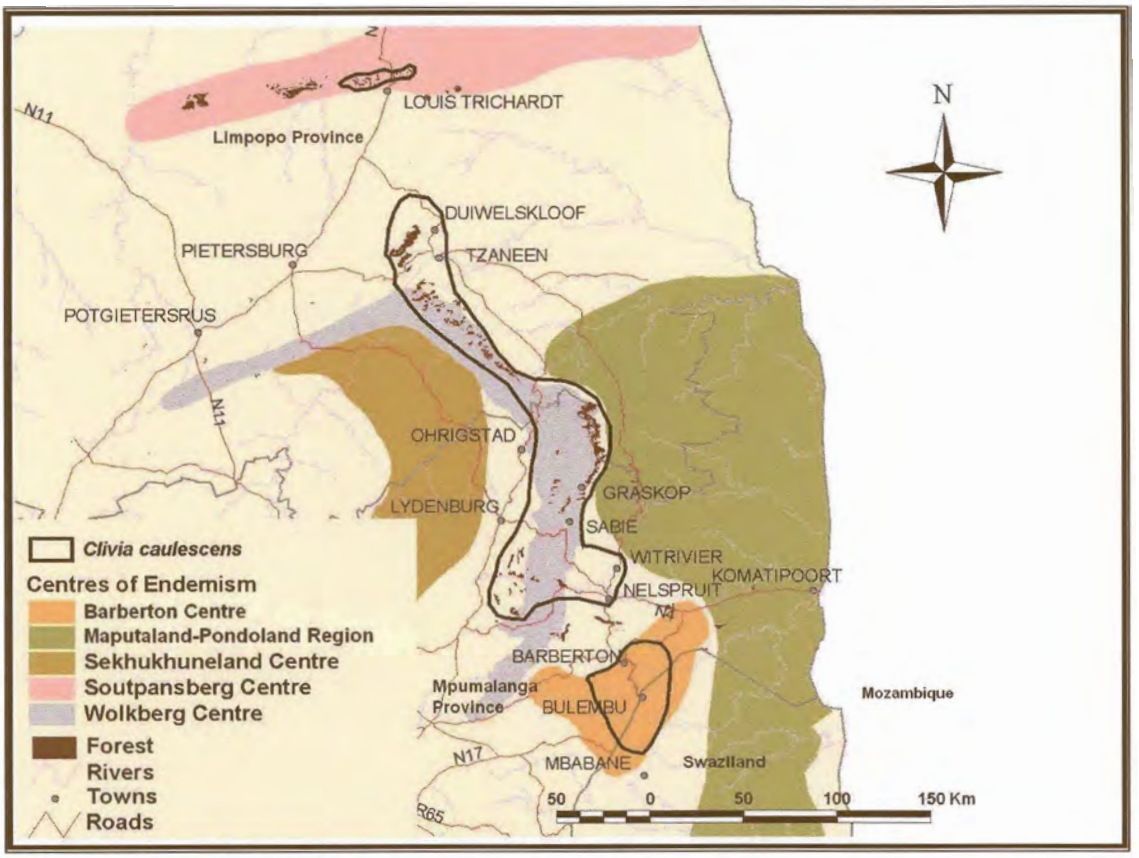

Figure 4.3 Natural distribution range of *Clivia caulescens*.

Situated in Limpopo and Mpumalanga Provinces, the Wolkberg Centre is defined by rocky outcrops of the Transvaal Supergroup. These outcrops extend from Kaapsehoop in the south to near Tzaneen in the north and westwards to the Strydpoort Mountains ending near Potgietersrus. Two subcentres have been proposed for the WC, the Blyde Subcentre (south of the Olifants River) and the Serala Subcentre (north of the Blyde subcentre) (Van Wyk & Smith 2001). Records indicate that *C. caulescens* grows in both these subcentres.

The WC is characterised by hot, wet summers (drier to the west) that is more temperate at higher altitudes and dry, cool winters with frost (higher altitudes). Mean annual precipitation is in excess of 1 000 mm and may reach 2 000 mm in some places. Various

soil types occur in this centre, for example sandy soils derived from quartzite (Van Wyk & Smith 2001).

Vegetation is predominantly montane grassland with Afromontane Forest confined to pockets along the slopes of the main escarpment, sheltered ravines and incised valley heads. These Afromontane Forests form part of the Afromontane Region and has floristic links with BC, SC and Chimanimani-Nyanga Centre. Links to the mountains of the Albany Centre and Cape floristic elements are conspicuous at higher altitudes (Van Wyk & Smith 2001).

The SC encompasses the Soutpansberg and the adjacent Blouberg Massif with rocks of the Soutpansberg Group as main substrate. Southern slopes of the mountains are wetter (up to 2 000 mm) than the northern ones (370 mm). A temperate climate is experienced at higher altitudes with a more subtropical climate in the lowlands. Fog is common at high altitudes and frost is usually absent. Sandstone and quartzite are the two main rock types (Van Wyk & Smith 2001 and references therein).

The vegetation of the SC consists largely out of bushveld and thicket, with well-developed pockets of Afromontane Forests on the wetter slopes and grassland at higher altitudes. Wetter parts form part of the Afromontane Region and link this centre to the WC. Weak floristic links also exist with the Eastern and Western Cape (Van Wyk & Smith 2001 and references therein)

Clivia caulescens grows like the other *Clivia* species in association with evergreen forest, where it occupies the forest floor, either on moss and lichen covered rocks or tree trunks. According to the literature, populations have been found in association with sandstone (Duncan 1999; Winter 2000). Herbarium records and literature references suggest that this species occurs at high altitudes, with some even above the snowline (Winter 2000). Specimens have been seen growing in full sun to light shade and on slopes of 5–45°. *Clivia caulescens* has been noted growing in sandy loam derived from Wolkberg quartzites (*F. Venter*, PRU 81136, 1986/4/8).

The sketchy available descriptions in the literature of *C. caulescens* ecology prompted various visits to different localities in an attempt to fill these gaps. Five different localities were visited and observations are summarised below.

Bearded Man Mountain is on the Swaziland border with Mpumalanga Province and 1 337 m above sea level. Plants grow on a WSW aspect, on a slope of 45–55°, under a close canopy in what can be described as a 'dry forest' (based on the scarcity of epiphytes). Many saplings were observed in this forest. At this locality, *C. caulescens* grows in close association with *C. miniata* (also see section 4.3.5 for more detailed description of habitat). Identification was hampered with only a single *C. caulescens* in flower. No competition was observed where both species are growing, with an increase in undergrowth resulting in fewer plants. The *C. caulescens* plants have distinct stems and no obvious hybrids were identified. Only a few plants were seen, therefore limited assumptions can be made about this *C. caulescens* population.

The grass-covered mountainous terrain of the Sodenza Mountains, Songimvelo Game Reserve, has many natural valleys and seepage areas. In these natural drainage areas woodland patches occur. Two *C. caulescens* communities associated with the elaborate drainage system of the mountain, were investigated, namely a forest patch around Malandweni Cottage and a forest-covered kloof, Lobodtlyana.

Lobodtlyana Kloof, a forest patch, consists of sheer cliff faces connected by moderate steep slopes, ensuring excellent drainage. The forest canopy is high and open, but the undergrowth increases as one moves towards the exposed edges of the forest. Again *Clivia miniata* and *C. caulescens* were found growing together.

Clivia caulescens plants are mainly found on rocky outcrops that form part of the higher, steeper parts of the slopes. These well-drained areas contain humus rich soil in which the plants grow and are also exposed to more light than surrounding areas. Inaccessibility confined the exploration to the valley, with the steeper slopes and lower areas not fully

investigated. This would clearly influence estimates, probably to a minimum value rather than an average. The *C. caulescens* population size is estimated to be around 100 plants of which 50% are seedlings. The number of positively identified individuals, based on the presence of inflorescence and leaf morphology, also influenced this estimate. Plants grow as small colonies or as individuals dispersed throughout the *C. miniata* community and had a dark red flower colour.

Lichens grow on both *C. caulescens* and *C. miniata*, but seem to prefer the *C. miniata* that grows in area with more light. No fruits were seen in both these species. Although human activities were noted, this site is well protected by its seclusion. This community is categorized as healthy and actively growing. Although no seed-set was observed, large numbers of seedlings were present.

Malandweni Cottage was built in one of the woodland patches of the natural drainage system and is surrounded on three sides by a closed-canopy forest that grows along a V-shaped drainage area. *Clivia caulescens*, in the forest patch that surrounds the cottage, has deep red flowers. Many of the *C. caulescens* plants formed multi-stems at their bases. This is attributed to abscission rot—older stems tend to break-up due to localized rot.

The valley floor was severely trampled by animals visiting the stream. *C. caulescens* plants were some of the casualties and many of the remaining plants are located on rocks within the stream (majority) and in other areas where the animals cannot disturb them (growing next to trees on the denser outskirts of the forest). This *C. caulescens* population contains to approximately 50 to 60 individuals. Seedlings account for around 10% of the population. Very few plants were in flower (5–10% of the population), but it was still very early in the flowering season.

Although only a limited number of individuals were found, we believe that *C. caulescens* is more abundant and would be found in similar interconnected forest patches that occur within the reserve. The Malandweni Cottage's valley is indeed connected to other similar

seepage areas and forms part of a larger "interconnecting seepage forest system" occurring along the mountain. Similar *C. caulescens* habitats are therefore common.

The last two communities are in the vicinity of God's Window and Kowyns Pass near Graskop. The God's Window population is on a hiking trail, with plants growing from open sunny spots at the top of the valley, to cool forest shade at the bottom. At the top, this area is located 1 730 m above sea level, with most plants growing on level ground.

The majority of the plants (almost 100%) were single-stemmed, very old plants, with no obvious suckers on them. Plants with aerial stems up to 3 m long (i.e. stems reclining on rocks/forest floor and against trees) were seen. Flower colour was mostly orange to deep red-orange, with up to 50 flowers per umbel. At least 40% of the plants were in flower/in bud/pushing inflorescences. Very few seedlings, in comparison to the mature plants, were seen. Seeds were present together with clear indications of seed-set in old inflorescences. Some of the plants in open settings show yellow, scorched leaves. Plants grow in the humus layer as well as epiphytically on rocks (lithophytes). This is an extremely large population (at least 2 000 mature plants) with very inaccessible communities. In general, the population consists of big, very old, mature plants. Indications of plant removal by humans were obvious, an action clearly facilitated by the presence of a hiking trail traversing the population.

In the Kowyns Pass area, plants grow on the top, sides and floor of the valley. Seedlings and younger plants were abundant, with older plants largely limited to the higher portions of the valley/boulders. Streaks of white/yellow on the foliage gave the plants a 'variegated' appearance, but it is possibly a viral infection. Approximately 30% of the adult plants were in flower with 20–35 flowers per umbel.

4.3.5 *Clivia miniata* (Lindl.) Regel

Currently *Clivia miniata* is accepted to occur from the Kei River in the south, through the Eastern Cape and KwaZulu-Natal Provinces, with the most northern localities in the Sondeza Mountains, between Swaziland and Mpumalanga Province (Vorster 1994; Duncan 1999; Winter 2000).

The present study also places the most northern limits of *C. miniata* in the Barberton area (Figure 4.4). Communities were also recorded in Swaziland, near the border with Moçambique and in the Lebombo Mountains, northern KwaZulu-Natal Province (Figure 4.4). The most extensive distribution area begins in the Hluhluwe District, KwaZulu-Natal Province, from where it extends southwards along the coast to the most southern locality at the Koek-Koek River, Eastern Cape Province. The far northern localities show a disjunct pattern (Figure 4.4).

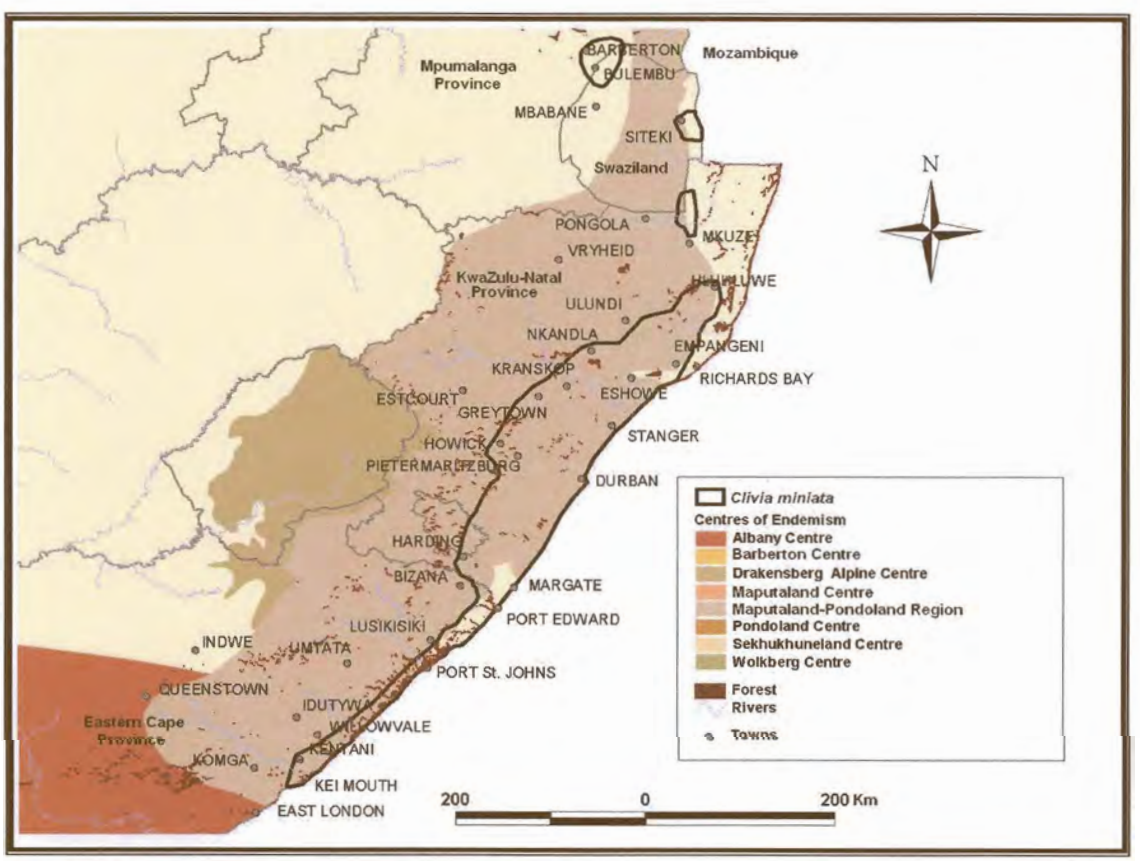

Figure 4.4 Natural distribution range of *Clivia miniata*.

This disjunct pattern is probably not real if one considers that the mountainous terrain limits botanical exploration and that it is very likely that favourable habitats exist between these localities.

The distribution range of *C. miniata* falls within four regions/centres of endemism, namely the Barberton Centre (see section 4.3.4), Maputaland-Pondoland Region (see section 4.3.2), Maputaland Centre and Pondoland Centre (see section 4.3.3). Natural hybrids have been reported in cases were *Clivia miniata* grows in close proximity to other members of the genus (Chapter 3).

Clivia miniata communities have been reported on sandstone and dolerite (Winter 2000). It usually occurs in large colonies in evergreen forest. Populations have been noted along shaded watercourses, ravines and ledges. *Clivia miniata* occasionally grows epiphytically on rocks and in tree forks (Duncan 1999; Winter 2000).

In the present study three different populations of this species were investigated, namely in the vicinity of Port St Johns, Bearded Man Mountain and Lobodtlyana Kloof, Songimvelo Game Reserve.

The Port St Johns locality, part of the Pondoland Centre, is midway up a mountainous slope. At this locality *C. miniata* plants form a green 'sea', with small boulders or trees appearing as enclosed islands therein. Closer observations revealed the plants were growing in the debris (humus) accumulated between the boulders of a rock scree. Plants grow on the south-facing aspect of the mountain at a slope of 30–40°. A high canopy, comprised out of larger than 20 m tall trees, allowed for a high light intensity environment. Holes in the canopy further increased the light intensity. *Clivia miniata* plants grow without any competition in this habitat, with the population covering a strip estimated to be at least 30 m in width and 50–80 m or more in length. The boundaries of the colony are formed by shadier undergrowth.

Plants formed both multi-stems, with on average 2 or 3 stems per plant, or occurred as single-stemmed individuals. Caterpillar damage was visible on some plants and other insect activity was also noted (beetles). Lichens, which covered almost the entire leaf surface exposed to the sun, were frequently observed. Plants in more shady areas had few or no lichens.

The plants grow shallowly/loosely on top/between rocks, with some roots running into cracks. Growth substrates are mainly rotting bark/detritus/leaves and other humus. Boulders were estimated to be ± 500 mm to 2 000 mm in diameter.

Population size is well over 200 mature plants (plant clusters were counted). Semi-broad to narrow-leaved plants (35–50 mm) made-up the colony, some leaves as wide as 70 mm. Many plants were markedly stemmed (old stems). The stems were sometimes rotten away, but with new suckers being produced. One plant grew epiphytically in the hollow trunk of a *Ficus* spp. This community seems to reproduce mainly asexually via suckers or 'rotting stems'. Some sexual reproduction was observed but this clearly is of minor significance.

Flowers were narrow-petalled, pastel pink to orange, with very faint colouring in the centre (which could be absent). Average number of flowers per umbel was estimated to be between 10 and 15. Little variation between flowers was noted. There is, however, some variation in general plant morphology and leaf width.

Humidity of the understorey was high (it had rained earlier) with damp undergrowth. However, water seeped away from the plants due to the high number of boulders—thus a well drained, though damp environment.

Very few seedlings (less than 3% of total individuals) were located. This is to be expected if the tendency is for only 15–20% of the mature plants to flower per season. Poor seed-set was noted.

The population seemed limited to the old disturbed area (rock fall). Though genetic recombination had occurred (very low), it is not clear if the population is healthy with only vegetative reproduction.

The second population investigated was at Bearded Man Mountain, on the border between Swaziland and Mpumalanga (see also section 4.3.4). Plants occurred along a strip of approximately 200 m in length, up the mountain on a slope of 30–45°. *Clivia caulescens* were growing alongside *C. miniata*.

Most of the *C. miniata* plants were in flower. Few flowers (4–8) per umbel were produced, but the flowers were usually large and hibiscus-shaped. Flower centres were either white or yellow with flower colour ranging from light orange and pink pastel to darker orange. Approximately 600 to a 1 000 individuals formed this community. Seedlings account for approximately 20% of the population. Most of the population looked like old, mature plants.

At this locality *C. miniata* grows in the humus layer, with shallow, surface roots. There were a few boulders/rocks with an average width of 0.5 m. Lichens covered some leaves and 'leaf-miners' were also present. Plants grow as individuals, with clumps seldom seen. Some individuals produced marked/aerial stems, as in *C. caulescens*, but not to the same extent.

The Lubodtlyana Kloof population (also see section 4.3.4) contains both *C. miniata* and *C. caulescens* communities, with *C. miniata* clearly dominant. The *C. miniata* community consisted of approximately 300 individuals, with an estimated 20% seedlings. *Clivia miniata* plants are found on both the steeper slopes and the valley floor, where they grow in well-drained humus-rich soil.

Plants grow as individuals or small colonies throughout the kloof. Individuals in flower were largely limited to the higher, lighter parts of the valley. An estimated 10% of the population flowered. Seed-set was noted, though no seeds were found. Flower colour and

shape varied from a closed reddish flower to an open pastel-pink flower. Flower number ranged from 4–15. Leaf shape also varied.

4.3.6 *Clivia mirabilis* Rourke

Clivia mirabilis is only known from the eastern part of Oorlogskloof Nature Reserve, Northern Cape Province (Rourke 2002a). There are no other records or confirmed reports of other populations outside the reserve (Figure 4.5).

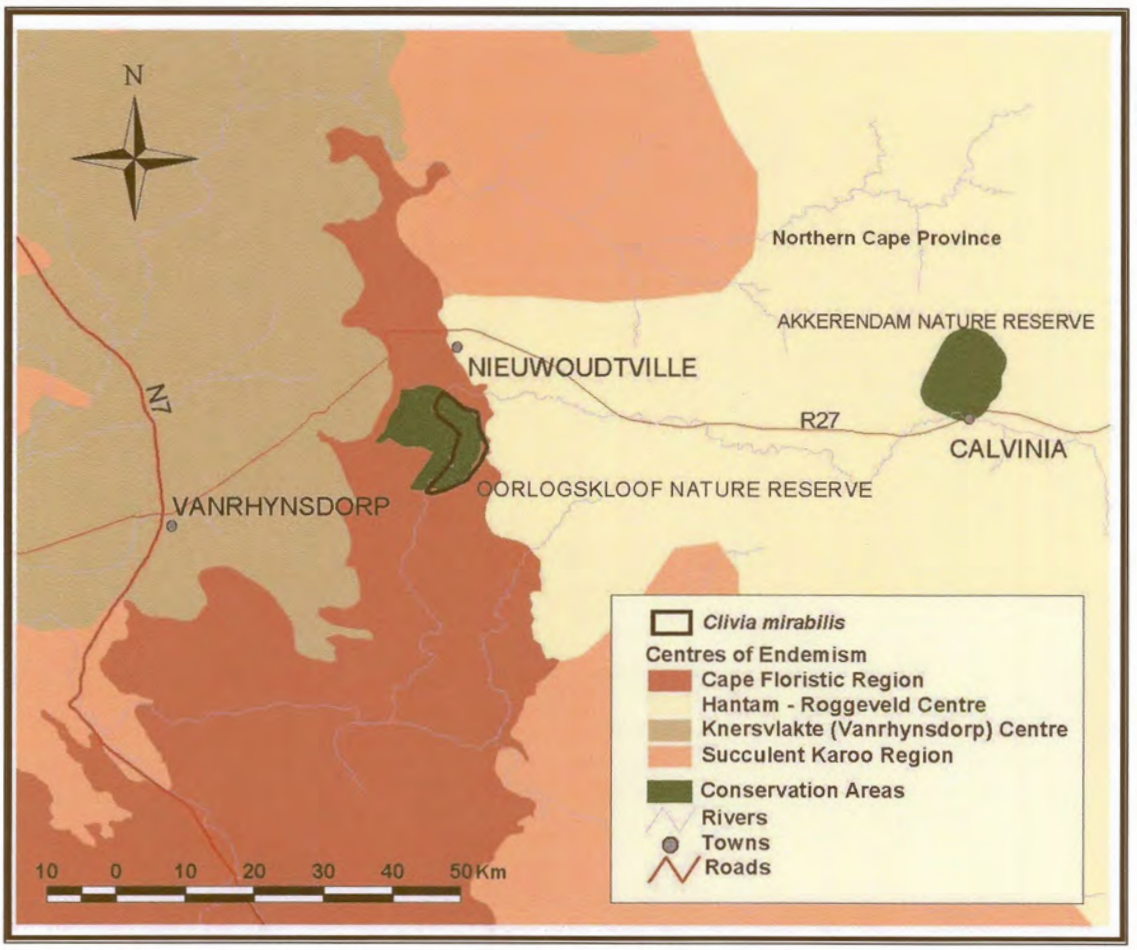

Figure 4.5 Natural distribution range of *Clivia mirabilis*.

The Oorlogskloof Nature Reserve falls within the Cape Floristic Region (CFR). Recognised by some as one of the six Floristic Kingdoms, this region covers approximately 90 000 km^2. In this relatively small area, the highest extratropical concentration of higher plant taxa in the world are contained, confirming its status as one

of the earth's 25 species diversity hotspots (Cowling & Hilton-Taylor 1997; Van Wyk & Smith 2001).

Average annual precipitation for the CFR is between (250–)300–2 000(–5 000) mm, with mean annual temperatures of 15–16°C (coast) to 17–18°C (inland) (Cowling & Hilton-Taylor 1997; Van Wyk & Smith 2001). The main geological formation the Cape Supergroup, with the quartzites of this group giving rise to acidic, low-nutrient, coase-grained soils (Van Wyk & Smith 2001). The Cape Floristic Region contains five broad vegetation types, namely Afromontane Forests, Subtropical Thicket, Fynbos, Renosterveld and Succulent Karoo (Van Wyk & Smith 2001, and references therein).

The Oorlogskloof Nature Reserve receives an average precipitation of 414 mm annually, strictly during the winter months. Characteristic of this area is its semi-arid Mediterranean climate. Light frost is probably experienced briefly during these months—a consequence of being 100 km inland and at an elevation of 850–900 m (Rourke 2002a, b). A more detailed ecological description of the habitat of *C. mirabilis* is given by Rourke (2002a).

In this harsh environment, *C. mirabilis* plants are found growing in humus, caught between cracks produced by the erosion of Nardouw Formation Sandstone. Coarse sandstone talus screes, the result of erosion, contain relictual Afromontane elements. *Clivia mirabilis* occurs as solitary or grouped/clumped specimens on these rock screes, usually under the relictual Afromontane woodlands, though occasional clumps have been noted to grow in full sun. The only known population is estimated to be well over 1 000 individuals, distributed over several hectares.

4.4 Biogeography of *Clivia*

4.4.1 Introduction

One can only speculate about the past distribution and migration of *Clivia* and its predecessors. This section focuses on the establishment of the current distribution patterns for the various *Clivia* species—taking into account environmental alterations brought about by both past climatic and topographic changes.

4.4.2 Development of Africa and its flora

The Cretaceous period featured the onset of dramatic floristic change, eventually resulting in the predominance of the angiosperms. During the early Cretaceous, angiosperms achieved importance in low palaeo-latitudes, mainly in northern Gondwana. At the time the climate was warm and moist (tropical). Separation of Africa and India from the super-continent, Gondwana, occurred through continental drift by mid-Cretaceous times. Angiosperms developed tolerance to cold and spread into middle and high palaeo-latitudes, resulting in an explosion of diversity towards the end of the Cretaceous period. Australia, New Zealand, Antarctica and South America separated during this latter part of the Cretaceous period. Worldwide, angiosperms were well established by the mid-Cretaceous (Bredenkamp *et al.* 2002, and references therein).

The late Cretaceous and Paleocene had Africa located approximately 15° south of its current position, placing northern Africa over the equator. Westerlies probably resulted in a cool, wet climate in the southern third of the continent, with warm, humid climate and low relief in the rest of the continent. This resulted in tropical forest extending from coast to coast over northern and eastern Africa (which was not elevated then) (Goldblatt 1978; Partridge 1997; Bredenkamp *et al.* 2002, and references therein). Bredenkamp *et al.* (2002) refer to pollen records suggesting extensive forest cover even as far south as southern Africa. The temperate forests located in the Cape today are possible relics of the forest flora that covered the southern third of the continent during this time (Goldblatt 1978).

The Cenozoic period is regarded as the time during which the current biomes developed. The Neogene coincided with considerable climatic changes within southern Africa. Tectonism elevated the southern African central Highveld and parts of the eastern Great Escarpment. These geographic changes brought about cooler and drier conditions. Older ecosystems became restricted, eventually being replaced by newer ones. These vegetation types progressively expanded to adapt to the drier climate (Bredenkamp *et al.* 2002).

The Eocene-Oligocene was marked by the separation of other continents, resulting in the development of a circum-Antartic current that led to a drop in temperature in the high southern latitudes. Towards the end of the Oligocene, Antarctica was torn from the remaining landmass and drifted southwards. The cold Benguela current, flowing along the west coast of Africa, originated in ice covered Antarctica. Factors such as the destruction of the Tethys sea (altering the major latitudinal circulation), the closure of the Panamanian portal, formation of the Mexican plateaux, tectonic uplifts producing the Alps and Andes, resulted in an increase in aridity to the former moist west coast of Africa. This seasonal dry climate promoted selection for drought resistance. Forests were pushed into sheltered ravines that contained sufficient moisture, forming discontinuous forest patches (Goldblatt 1978; Bredenkamp *et al.* 2002, and references therein).

Quaternary times encompassed major climate changes in response to glacial-interglacial periods. Pleistocene interglacial periods stimulated forest expansions and major plant migrations, with interglacial periods reducing these communities (Goldblatt 1978; Bredenkamp *et al.* 2002, and references therein).

4.4.3 Proposed migration of *Clivia* species

Biogeographically, *Clivia* evolved and moved as part of the family Amaryllidaceae. Meerow *et al.* (1999) support previous hypotheses that the Amaryllidaceae originated in western Gondwana, using plastid DNA phylogeny. They showed the deepest topological branches originated in Africa, some with considerable innovations as typified by the Afrocentric tribes (Amaryllideae, Haemantheceae and Cyrtantheae). They concluded that the family was African in origin. Today Amaryllidaceae has three centres of diversity, in

the Andean region (28 genera), Mediterranean (8 genera) and southern Africa (18 genera) (Meerow & Snijman 1998).

The greatest diversity of the African Amaryllidaceae is concentrated in South Africa (Meerow *et al.* 1999, and references therein). *Clivia*, as part of the sub-Saharan tribe Haemantheae, corroborates this pattern with all species situated in South Africa (though two occur also in Swaziland). The origin of an ancestral *Clivia* species is therefore likely to be south-southwest Africa.

Increasing aridity, the uplift of the continental mass and quaternary fluctuations in Africa's climate, pressurised the Amaryllidaceae to diversify and adapt to increasing drought. Meerow *et al.* (1999, and references therein) stated that the adaptations in the Afrocentric tribes could be the result of an increase in radiation that occurred in Africa within recent paleoclimatic and geological history. *Clivia* adapted by developing thick, fleshy, perennial roots (Duncan 1999; Winter 2000; Rourke 2002a). Instead of growing in soil, it circumvented competition with tree roots by adapting to grow in the top humus layer of the forest floor (personal observations). *Cryptostephanus*, *Scadoxus* and *Clivia*, all genera of the baccate-fruited Haemantheae, did not form bulbs like other Amaryllidaceae, but remained forest understorey taxa and closely connected to this forest element (Meerow *et al.* 1999).

Midgley *et al.* (1997) suggest that Afromontane forests had a southern, temperate origin. One can argue that *Clivia* either developed with these forests and therefore had a more southern origin or found refuge in them while migrating to escape the increasing arid western side of Africa. Midgley and co-workers further concluded that distribution patterns of Afromontane Forests were the result of biotic interactions, rather than the influence of the historical centre of origin. Though this might be true for Afromontane forests, *Clivia*'s centre of origin may have played a major part in the current geographic distribution of the genus. This statement is substantiated by the current phylogenetic analysis of the genus (Ran *et al.* 2001a; Ran *et al.* 2001b; Conrad & Reeves 2002). *Clivia mirabilis* was shown to be sister to the other species of *Clivia*. It does not imply that

C. mirabilis occupied the 'centre of origin' of the genus. Other taxa reflecting a different 'centre of origin' may have gone extinct.

The discovery of *C. mirabilis* in Oorlogskloof Nature Reserve in the Northern Cape, supports the hypothesis that the Cape region acted as a refuge for previous tropical vegetation types in this part of Africa (Meerow *et al.* 1999; Rourke 2002a). Increasing temperatures and lower precipitation experienced in western Africa following the break-up of Gondwana and the formation of the Great Escarpment, probably forced *Clivia* (in association with the Afromonatane element) towards the wetter southern and eastern coastal areas of South Africa.

Clivia mirabilis (or rather its predecessor) found refuge in the Oorlogskloof canyon during this migration. It adapted to the increasing aridness of the surrounding landscape, as the forest environment gradually deteriorated. This isolation and adaptation eventually give rise to *C. mirabilis*. *Clivia* migrated eastwards where it reached the east coast of South Africa. Either a sub-tropical or tropical environment existed along the coast or glacial periods provided connections between the Afromontane communities. In both cases, *Clivia* moved northwards along the coast, bordered by the sea and the Great Escarpment. Fragmentation, probably as a result of inter-glacial times, isolated communities at the southern part of the distribution first. These isolated communities of the Eastern Cape Province experienced alterations in the climate that favoured the adaptation to the region of *C. nobilis*.

Van Wyk (1990) suggested that the Pondoland Centre, with its sandstone substrate, acted as a barrier to plant migrations from the east coast (mainly during the Quaternary period). This would have 'trapped' species to the south of this region during the contraction or advancement of vegetation. He proposed a closer connection between the various sandstone formations and vegetation. The Msikaba Formation Sandstone (Pondoland) together with the Natal Group sandstone, have been shown to be related to the Table Mountain Group, with the latter closely related to rocks of the Cape Supergroup that is closely associated with the Cape Floristic Region. Various endemics connect these

regions and support this hypothesis (Van Wyk 1990; Van Wyk & Smith 2001, and references therein).

Van Wyk (1990) proposed that the sandstone outcrops of Pondoland and KwaZulu-Natal acted as edaphic islands to migratory vegetation (Van Wyk 1990). *Clivia* probably dispersed further northwards via the Natal Sandstone Group outcrops, to the Afromontane areas of Mpumalanga and the Limpopo Provinces. Van Wyk (1990) mentions various taxa in a similar pattern of distribution, probably during Plio-Pleistocene epoch. Subsequent fragmentation and isolation of the different communities, would explain how the current *Clivia* species originated, but this doesn't always correspond to hitherto proposed phylogenies (Ran *et al.* 2001a; Ran *et al.* 2001b; Conrad & Reeves 2002).

The proposed origin of the different species as explained in the previous paragraphs, correlates to the phylogeny of Conrad & Reeves (2002) but not to those proposed by Ran an co-workers (Ran *et al.* 2001a; Ran *et al.* 2001b) (Chapter 3). Conrad & Reeves (2002) utilised the plastid genome for their analysis, while Ran and co-workers used a random technique on total genomic DNA (RAPDs) as well as sequence information from the ITS region (Ran *et al.* 2001a; Ran *et al.* 2001b). According to both Ran *et al.* 2001a and 2001b, *C. caulescens* is basal to *C.* 'Robust' *gardenii*, the sister to both *C. miniata* and *C. gardenii*. These phylogenetic trees suggest that *C. nobilis* evolved first, followed by *C. caulescens*, *C.* 'Robust' *gardenii*, *C. gardenii* and *C. miniata*. If vicariance is assumed then this biogeographic pattern is possible. The ancestral species may have been more widespread in the past prior to the fragmentation of its range within the summer-rainfall region. The fragmentation may not have proceeded in a northerly direction but could have been at peripheral sites in the north and south.

The phylogenetic tree proposed by Conrad & Reeves (2002) suggests that the species started to differentiate as one moves up along the coast, with first *C. nobilis*, then *C. gardenii* and finally *C. caulescens* and *C. miniata*. Unfortunately they did not distinguish between *C. gardenii* and *C.* 'Robust' *gardenii* in their study.

Chloroplast DNA diversity in *C. miniata* (Chapter 6), suggests an origin for the species in the northern part of Pondoland or in southern KwaZulu-Natal. From this location, *C. miniata* migrated along the coast in both a northerly and southerly direction. This would fit in with the assumed phylogeny, with *C. miniata* developing from a *C.* 'Robust' *gardenii* or *C. gardenii* like ancestor.

4.5 Conservation status

The previous sections highlighted the limited geographic range of the various *Clivia* species, all more or less confined to certain regions/centres of endemism. This limited distribution, further restricted by microhabitats associated with patchy Afromontane forests, raises the question of the conservation status of the various infrageneric taxa.

At present, some *Clivia* species are mainly placed under Lower Risk-Least Concerned, Lower Risk-Near Threatened and Vulnerable headings (Table 4.1) (Golding 2002; Lötter & Krynauw 2002). This seems insufficient when one considers the data presented in the previous sections.

Both environmental and human-induced factors are threatening the survival of *Clivia*. The main environmental factor is the microhabitat to which *Clivia* is evolutionary connected, namely the Afromontane Forest of southern Africa.

Afromontane Forest occupies approximately 6 000 km^2 in South Africa and Swaziland, of which only 17.64% is conserved (Lubke & Mckenzie 1996). Geographical distribution of these forests are determined by a single key-limiting factor, namely water, restricting them to wetter kloofs and gullies. Fire is also important in maintaining the forest boundaries especially within the grassland and Fynbos vegetation—with forest mainly confined to fire safe refuges (Lubke & Mckenzie 1996).

Table 4.1 Current published conservation status of the genus *Clivia*.

Clivia taxon	Specific distribution area used for risk assessment	2001 IUCN Red List Category	Reference
C. nobilis	KwaZulu-Natal, Eastern Cape	Lower risk-least concerned	Golding 2002
C. miniata	KwaZulu-Natal, Eastern Cape	Lower risk-least concerned	Golding 2002
C. caulescens	Eastern Cape[1]	Lower risk-least concerned	Golding 2002
C. gardenii	KwaZulu-Natal	Lower risk-least concerned	Golding 2002
C. caulescens	Devils Bridge	Data deficient	Golding 2002
C. miniata var. *citrina*	?	Data deficient	Golding 2002
C. nobilis	Swaziland[1]	Data deficient	Golding 2002
C. miniata	Swaziland (Lebombo, Piggs Peak area)	Lower risk-near threatened	Golding 2002
C. miniata	Mpumalanga	Vulnerable (VU B2abii, iii, v)	Lötter & Krynauw 2002

[1] Distribution record incorrect

Clivia species, in conjunction with Afromontane Forest, show a patchy (disjunct) distribution pattern (Lubke & Mckenzie 1996; Cowling & Hilton-Taylor 1997). A restricted ecological niche within Afromontane Forest—usually a well drained area—limits the available microhabitat that *Clivia* species can successfully colonise. Microhabitats, though restricted in number, are not always exploited by *Clivia* species. This might indicate inadequate seed dispersal. In certain microhabitats, usually without competition from other taxa, *Clivia* communities are sometimes termed 'locally abundant' due to their high numbers. Unfortunately, the use of this term may lead to the conclusion that once a *Clivia* species has established itself in an area, it becomes the climax taxon. This might be true in some cases, but with low seed production and recalcitrant seed, such climax communities might take several generations (years) to establish. Any changes in environmental conditions, with an effect on microhabitat, will have a serious impact on the survival of this hardy genus.

Human factors threatening the survival of this genus are mainly habitat destruction and illegal removal of specimens from nature. Habitat destruction is the result of woody material being removed for fuel or agricultural purposes and urbanisation (Chubb 1996;

Duncan 1999). Plants collected for horticultural or medicinal properties are probably the most serious threat to natural *Clivia* populations (Chubb 1996; Duncan 1999; Lötter & Krynauw 2002). The high demand by traditional healers for *Clivia* plants was clearly evident when Mander (1998) identified *C. miniata* as the tenth most sought after medicinal plant to be traded in Durban, KwaZulu-Natal. Williams *et al.* (2001) found *Clivia* species in 70% of the Witwatersrand '*muti*' shops they surveyed.

Clivia populations are currently located in several nature/game reserves across the country. This can, in part, be attributed to the geographic range of the various species, most of which have communities in centres/regions of endemism that currently boast some areas that receive special protection. Will this be sufficient to save *Clivia* species, or will the inaccessibility of *Clivia* communities and their disjunct distribution pattern eventually prove to be the key to their survival?

Current conservation status classifications for *Clivia* seem insufficient when one considers the data presented, therefore we propose a new conservation classification for the different members of the genus (Table 4.2).

Clivia mirabilis is placed as Critically endangered (CR) on the basis of a single location with its extent of occurrence being less than 100 km^2 and area of occupancy is estimated to be less than 10 km^2. This species is currently receiving maximum protection, thanks to its confinement to a rather inaccessible part of the Oorlogskloof Nature Reserve.

Clivia 'Robust' *gardenii* is categorised Endangered (EN) on the grounds of the populations being highly fragmented within its limited extent of occurrence (estimated to be less than 5 000 km^2) and area of occupancy (less than 500 km^2).

Table 4.2 Proposed conservation status of *Clivia* species.

Clivia species	Distribution	Proposed Category[1]
C. miniata	Eastern Cape, KwaZulu-Natal, Mpumalanga Provinces, Swaziland	Vulnerable (VU), A3d
C. caulescens	Mpumalanga, Limpopo Provinces, Swaziland	Vulnerable (VU), A3d
C. gardenii	KwaZulu-Natal Province	Endangered (EN) B2a
C. nobilis	Eastern Cape Province	Endangered (EN) B2a
C.* 'Robust' *gardenii	Eastern Cape, southern KwaZulu-Natal (Pondoland) Provinces	Endangered (EN) B1a+2a
C. mirabilis	Northern Cape Province	Critically Endangered (CR) B1a+2a

[1] According to 2001 IUCN Red List Categories, Version 3.1, as in Golding 2002.

Clivia gardenii and *Clivia nobilis* are categorised as EN based on an area of occupancy that is estimated to be severely fragmented and less than 500 km^2. *Clivia miniata* and *C. caulescens* are labelled vulnerable (VU) with an estimated population size reduction of 30% or more, projected/suspected to be met in the following three generations as a result of actual/potential levels of exploitation. While the remaining *C. miniata* populations are usually rather inaccessible, some *C. caulescens* populations are more readily accessible, placing more pressure onto this taxon.

4.6 Conclusions

Distributions maps for the different *Clivia* species showed the geographical ranges of all to fall within various Centres/Regions of floristic endemism (Table 4.3 and Figure 4.6). The geographic isolation of *Clivia* 'Robust' *gardenii* supports molecular evidence produced by Ran *et al.* (2001b), in establishing this form as a new species. The biogeographical patterns displayed by the species, environmental information from the present and the past, as well as available molecular phylogenies, were used in the formulation of a hypothesis to explain the current distribution of the genus.

Table 4.3 *Clivia* species and the regions/centres of endemism to which they are mainly confined.

***Clivia* taxon**	**Centres of plant endemism**
Clivia caulescens	Barberton Centre Soutpansberg Centre Wolkberg Centre*
Clivia gardenii	Maputaland-Pondoland Region
Clivia miniata	Albany Centre* Barberton Centre Pondoland Centre* Maputaland-Pondoland Region
Clivia mirabilis	Cape Floristic Region*
Clivia nobilis	Albany Centre* Maputaland-Pondoland Region
Clivia 'Robust' *gardenii*	Pondoland Centre*

*Regarded global 'hot-spots' of biodiversity (Cowling & Hilton-Taylor 1997)

Forests are reported to constitute less than 1% of the total land cover of southern Africa (Cowling & Hilton-Taylor 1997). The relevant environmental factors, together with the biogeographical distribution, were used to propose IUCN conservation statuses for the different *Clivia* taxa.

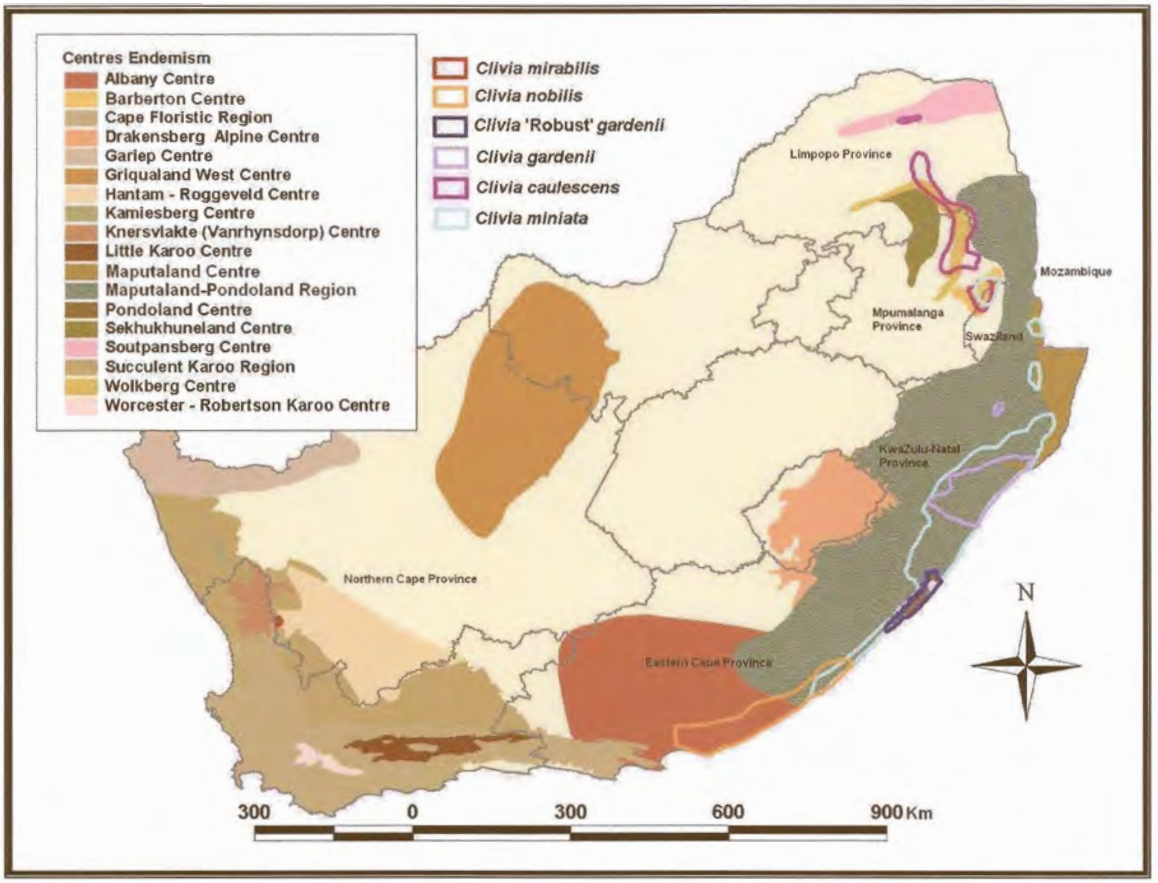

Figure 4.6 The distribution range of *Clivia* taxa and the regions/centres of endemism to which they are mainly confined.

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