Molecular phylogenetics of Alooideae (Asphodelaceae)

by

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DECLARATION

I declare that this dissertation has been composed by myself and the work contained within, unless otherwise stated, is my own.

Barnabas Haruna Daru (January 05, 2012)

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FOREWORD

This study is a contribution to the phylogenetic understanding of Alooideae (Asphodelaceae) and follows on the efforts of Reynolds (1966), Van Jaarsveld (1994), Bayer (1999), Treutlein *et al.* (2003a, b), and Vosa (2004). These taxonomic treatments provided the baseline for the evaluation of hypotheses presented in this study.

The dissertation is presented in five chapters. Chapter one presents the general synopses, motivation and objectives of the study. Chapter two provides details of the sampling, methodology and phylogenetic analyses used. In Chapter three, the results of the phylogenetic relationships within the subfamily Alooideae is presented. Appendices include abstracts of papers presented at conferences as well as paper submitted to *Taxon*. Preliminary findings of the study was presented at the 2011 Congress of the Southern African Society of Systematic Biology (SASSB) and earned me the award for the *Best MSc Presentation*. Appendix III resulted from collaborative research with John Manning and Stephen Boatwright and form part of the paper submitted to *Taxon* (Appendix II).

All analyses, tables, images and figures used in the dissertation are by the author, unless otherwise stated.

LIST OF ABBREVIATIONS

°C = Degrees Celsius

A.Berger = Alwin Berger (1871-1931)

ABI = Applied Biosystems, Inc

ACCTRAN = Accelerated transformation optimisation

ACDB = African Center for DNA Barcoding

AIC = Akaike Information Criterion

Aloes = *Aloe*

APG = Angiosperm Phylogeny Group

Baijnath = Baijnath, Himansu (1943-)

BI = Bayesian inference

Bp = base pair

BP = Bootstrap Percentage (support)

ca. = approximately

CBOL PG = Consortium for the Barcode of Life Plant Working Group

CI = Consistency Index

Corp. = Corporation

CTAB = Hexadecyltrimethylammonium bromide

DELTRAN = Delayed transformation optimisation.

DMSO = Dimethyl Sulfoxide

DNA = Deoxyribonucleic acid

EtOH = Ethanol

F = Forward primer

GenBank = National Center for Biotechnology Information

GTR+I+G = General Time Reversible + Gamma + Proportion Invariant

i.e. = id est (that is)

Inc. = Incorporated

iso. = Isotype

ITS = Internal Transcribed Spacer

JRAU = Herbarium of the University of Johannesburg (UJ), Johannesburg, South Africa

K = Herbarium of the Royal Botanic Gardens, Kew, Richmond, United Kingdom

Kunth = Kunth, Karl Sigismund (1788-1850)

L. = Linnaeus (von Linné), Carl (1707-1778)

M.Bieb. = Marschall von Bieberstein, Friedrich August (1768-1826)

m = meter

MCMC = (Bayesian) Markov Chain Monte Carlo

min. = Minimum

min = Minutes

mm = millimeter

Moench. = Moench, Conrad (1744-1805)

MP = Maximum Parsimony

MRCA = Most recent common ancestor

MulTrees = Multiple equally parsimonious trees

Mya = Millions years ago

NaCl = Sodium Chloride

No. = Number

nom. cons. = nomen conservandum

nrDNA = nuclear ribosomal deoxyribonucleic acid

NRF = National Research Foundation (South Africa)

PAUP = Phylogenetic Analysis Using Parsimony software program

PCR = Polymerase chain reaction

Poelln. = von Poellnitz, Karl (1896-1945)

PP = Posterior probabilities

PVP = Polyvinyl pyrolidone

R = Reverse primer

rbcL = ribulose-bisphosphate carboxylase gene

RBG Kew = the Royal Botanic Gardens Kew

RI = Retention Index

s.l. = sensu lato

s.s. = sensu stricto

SANBI = South African National Biodiversity Institute

sect. nov. = section novis

SEM = Scanning Electron Microscope

sp. nov. = species novis (new species)

Syn = Synonymous

TBR = Tree-bisection-reconnection

TIM + G = Transitional Model + Gamma

TL = Tree length

TReeBoL Africa = DNA Barcoding Africa Trees

trnH-psbA = spacer between *trnH* and *psbA* genes

Uitew. = Uitewaal, Antonius Josephus Adrianus (1899-1963)

UK = United Kingdom

USA = United States of America

UV = Ultraviolet

µl = microliter

ABSTRACT

Alooideae (Asparagales–Asphodelaceae) currently comprises five genera, four of which are endemic to southern Africa. Despite their importance in commercial horticulture, evolutionary relationships among the genera are still incompletely understood, and the generic taxonomy remains unresolved. This study addresses generic delimitation in subfamily Alooideae using an expanded molecular dataset from three plastid regions (*rbcLa*, *matK* and *trnH-psbA*) and the first subunit of the nuclear ribosomal internal transcribed spacer (ITS1). Sequence data were analysed using maximum parsimony and bayesian statistics, and selected morphological traits were mapped onto the molecular phylogeny. Among the polytypic genera, only *Astroloba* and *Gasteria* were retrieved as monophyletic. *Haworthia* is shown to be polyphyletic and to comprise three main clades largely correllated with current subgeneric circumscriptions. The taxonomic implications of these findings are discussed.

CHAPTER ONE

General Introduction and Objectives

Photo: O. Maurin

1. GENERAL INTRODUCTION AND OBJECTIVES

In this chapter, I present an overview of Alooideae taxonomy and phylogenetics and state the objectives of my study. The classification system of APG III (2009) placed members of Alooideae i.e. Asphodelaceae (sensu Mabberley, 2008) within the family Xanthorrhoeaceae yet, most molecular studies in Alooideae systematics retained Alooideae as a subfamily within Asphodelaceae (e.g. Chase *et al.*, 2000; Treutlein *et al.*, 2003a, b; Klopper *et al.*, 2010; Ramdhani *et al.*, 2011). I have adopted the latter classification system throughout the dissertation.

1.1 Family Asphodelaceae

Asphodelaceae is a monocotyledon family in the order Asparagales and comprises about 13 genera and ± 800 species (Klopper *et al.*, 2010). Members of this family are distributed in xeric and mesic regions of the temperate, subtropical and tropical zones of the Old World (including Africa, Eurasia, Australia, New Zealand) with the main centre of distribution in southern Africa (Van Wyk *et al.*, 1993; Smith & van Wyk, 1998; Treutlein *et al.*, 2003a, b; Klopper *et al.*, 2010).

Many species of Asphodelaceae are cultivated as ornamentals with some listed on the IUCN Redlist of species (IUCN, 2001). For example, *Aloe bowiea* Schult. & Schult.f., *Bulbine frutescens* (L.) Willd. var. *chalumnensis* Baijnath ined., *Gasteria baylissiana* Rauh, *G. ellaphieae* Van Jaarsv., *G. glomerata* Van Jaarsv., *Haworthia bryunsii* M.B.Bayer, *H. springbokvlakensis* C.L.Scott, *H. woolleyi* Poelln. and *Kniphofia acrea* Codd have been listed as threatened in the wild with extinction by version 3.1 of the IUCN categories and criteria (Victor & Dold, 2003).

Asphodelaceae is divided into two subfamilies, based on vegetative and reproductive characters: Asphodeloideae and Alooideae (Treutlein *et al.*, 2003a, b; Klopper *et al.*, 2010).

1.1.1 Subfamily Asphodeloideae

Subfamily Asphodeloideae consists of the genera *Trachyandra* Kunth., *Asphodelus* L., *Asphodeline* Rchb., *Eremurus* M. Bieb., *Bulbine* Wolf., *Jodrellia* Baijnath, *Bulbinella* Kunth and *Kniphofia* Moench (Smith & van Wyk, 1998; Klopper *et al.*, 2010). These genera are widespread throughout the temperate and subtropical regions of Africa, Saudi-Arabia, Madagascar and some of the Mascarene Islands off the east coast of Africa. The subfamily has varying proportions of small to large chromosomes with a basic set of six chromosomes (2*n* = 12).

1.1.2 Subfamily Alooideae

Subfamily Alooideae (Aloaceae *sensu* Smith & Steyn, 2004) of the family Asphodelaceae is an Old World group (Fig. 1.1a) often characterised by conspicuous leaf succulence, cresentiform or cymbiform leaf outline in cross-section, and a rarely altered diploid karyotype *n* = 7 (Taylor, 1925; Smith & van Wyk, 1998). Cladistically this character represents a synapomorphy for all taxa of Alooideae. It also represents a sharp discontinuity between the Asphodeloideae and Alooideae, and suggests a distinct barrier for gene interchange (Smith & van Wyk, 1991, 1998). Representatives of the subfamily also share some chemical characters, notably the presence of *anthrone-C-glycosides* in their leaves and of *1-methyl-8-hydroxyanthraquinones* in their roots (Smith & van Wyk, 1998).

Modern taxonomy of the Alooideae begins with Linnaeus (1753), whose concept of the genus *Aloe* L. was rather heterogeneous one. Of the 16 names that he included in this genus, four are not members of Alooideae (three are now in *Sansevieria* and one is in *Kniphofia*). The remainder, grouped by flower size and shape, are currently segregated among *Aloe* (four species), *Gasteria* (one species), *Astroloba* (one species) and *Haworthia* (five species). Linnaeus' preliminary groupings were subsequently formalised, first at sectional level within *Aloe* (Linnaeus, 1774) and later by the recognition of the segregate genera *Gasteria*, defined by the moderately large, curved and often gasteriform flowers, and *Haworthia*, with very much smaller, whitish flowers (Duval, 1809). *Haworthia* was later further split when those species with often actinomorphic flowers were removed from those with bilabiate flowers into the small genus *Astroloba* Uitewaal (Roberts, 1965). Three additional small genera have since been recognised: *Chortolirion, Lomatophyllum and Poellnitzia. Lomatophyllum* consists of about 14 species from Madagascar and the Mascarene islands and it is now included in the genus *Aloe* (Klopper *et al.*, 2010) and the monotypic *Poellnitzia rubriflora* (L.Bolus) Uitewaal was recently included in *Astroloba* as *A. rubriflora* (L.Bolus) Gideon F.Sm. & J.C.Manning, (Manning & Smith, 2000; Germishuizen *et al.*, 2006). Thus the current classification of the subfamily (reviewed in Klopper *et al*., 2010) recognises the following five genera: *Aloe, Astroloba*, *Chortolirion*, *Gasteria*, and *Haworthia* (Table 1.1).

Alternative classification systems are briefly discussed below.

Classification system suggested by Cronquist (1981) **—** The classification system employed by Cronquist (1981) for flowering plants is based solely on morphological features. He placed Alooideae genera in the order Liliales within families Aloeaceae and Liliaceae (Table 1.2). The systematic framework for Liliales laid down by Cronquist (1981) was based on the unifying synapomorphy of seed-coat micro-morphology (the characteristic black seeds caused by phytomelan incrustation of the seed coat in most capsular and berry-fruited taxa). Cronquist (1981) maintained families such as Alliaceae, Amaryllidaceae, Agavaceae, Iridaceae, and Orchidaceae within Liliales, but lumped most of the rest into a single family Liliaceae, which included taxa from both orders Asparagales and Liliales. Other authors over the years adopted this system but modified the spelling of the family name from "Aloeaceae" sensu Cronquist (1981) to "Aloaceae" (e.g. Glen & Hardy, 2000; Smith & Steyn, 2004).

Classification system suggested by Mabberley (2008) — The classification upheld by Mabberley (2008) recognised the family Asphodelaceae (Asparagales) to consist of 14 genera (Table 1.3) with 800 species. Mabberley (2008) noted a superficial resemblance between Asphodelaceae, Hyacinthaceae and Anthericaceae. In his system, two subfamilies are recognised: Alooideae (pachycaul trees with secondary growth to succulent rosettes) and Asphodeloideae (herbaceous plants).

Classification system by APG III (2009) and Chase et al. (2009) — The Angiosperm Phylogeny Group (APG III, 2009) reduced the numbers of families within Asparagales to 15 (Table 1.2). All members of Alooideae i.e. Asphodelaceae (sensu Mabberley, 2008) were placed within Xanthorrhoeaceae. Chase *et al.* (2009) however, in a subfamilial classification of Asparagales (Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae), recommended that Xanthorrhoeaceae be split into three subfamilies (Asphodeloideae, Hemerocallidoideae and Xanthorrhoeoideae) to simplify taxonomic delimitation in this family.

1.2 Review of genera within Alooideae

1.2.1 *Aloe* L. (1753)

The genus *Aloe*, with about 400 species native to Africa, the Arabian Peninsula, and Madagascar plus other islands in the western Indian Ocean (Fig. 1.1e), is the largest within the Alooideae (Reynolds, 1966; Viljoen, 1999; Glen & Hardy, 2000; Klopper & Smith, 2007). Its species are characterised by tough, spiked/toothed leaves with astringent/unpalatable juice, sunken pores, vivid (red, white, or sometimes bicoloured) flowers, and wind-dispersed seeds. There are however numerous exceptions. Some *Aloe* species, have tall inflorescences with reddish-toned flowers with erect to spreading pedicels. Polystichous leaf arrangements in spiral rosettes with spiny margins are typical of most *Aloe* species (Fig. 1.2a) others have leaves arranged distichously in two vertical rows on opposite sides of an axis. *Aloe plicatilis* (L.) Mill. (Fig. 1.2b) for example, often called "fan aloe", is characterised by its leaf clusters resembling an open fan with margins almost smooth, except for some small teeth in the upper part and an arborescent growth habit. Other exceptions within the genus include a wide variation in growth form (trees, shrubs and stemless, sometimes geophytic perennials), margin almost entire to toothed) and surface (smooth to verrucose), flower size, shape, symmetry and colour (small to large, campanulate to tubular or gasteriform, actinomorphic to bilabiate, and pallid to brightly coloured), and fruit characteristics (small to large and dry to fleshy).

Aloe is one of the genera within Alooideae that has been studied the most comprehensively. Recent studies include phytochemical analysis (Viljoen *et al.*, 1998; Viljoen, 1999; Grace *et al.*, 2010), leaf morphology (Grace *et al.*, 2009), molecular evidence (Adams *et al.,* 2000a, b) and ecology (Botes *et al.*, 2008) all of which are based on the work done by Reynolds (1966).

Ecologically, due to the ease of hybridisation between *Aloe* species, often flowering simultaneously and mostly ornithophilous, Botes *et al.* (2008) indicated that co-flowering *Aloe* species partitioned flower-visiting birds through differences in floral morphology and nectar traits. Species that share bird pollinators tend to flower sequentially or utilise different pollen placement sites on the same birds. *Aloe inconspicua* Plowes is however pollinated exclusively by insects (*Amegilla fallax*, Apidae) that visit its flowers for nectar and pollen (Hargreaves *et al.*, 2008). It has white flowers, strongly zygomorphic with perianth tubes curved upwards. Recent molecular evidence suggests that insect-pollination is the ancestral state for Alooidea, with the ancestral *Aloe* species postulated to originate in the highlands of south-east Africa and spreading during the Tertiary era (Holland, 1978). These differences in pollination system, flowering phenology, and biogeography may suggest taxonomic affinities within the *Aloe* genus and also enable a greater number of *Aloe* species to coexist. However, detailed pollination and biogeographic studies of *Aloe* species, just like other Alooideae genera, is lacking.

1.2.2 *Astroloba* Uitew. (1947)

Astroloba is a clump forming, *Haworthia*-like perennial succulent endemic to the Western and Eastern Cape regions of South Africa (Mabberley, 2008; Fig. 1.1d). This genus contains six species plus *Poellnitzia rubriflora* L.Bolus from Western Cape recently transferred to *Astroloba* (as *A. rubriflora* (L.Bolus) Gideon F.Sm. & J.C.Manning) by Manning & Smith (2000) due to its close vegetative similarity to some species of *Astroloba*, as well as similarities in lipophilic anthronoid aglycones. It was essentially distinguished from *Astroloba* by its inclined racemes of secund, orange-red flowers with connivent tepals, apparently an adaptation to sunbird pollination (Manning & Smith, 2000). The six species of *Astroloba* are typically multi-stemmed at ground level with stems totally covered by hard, spirally arranged, stiff, pungent leaves (Fig. 1.3). The abaxial side of the leaf is tuberculate or smooth. The inflorescences are axillary racemes with simple or branched peduncles. The flowers are pedicellate with a straight tubular perianth (Smith, 1995b; Manning & Smith 2000).

Astroloba is vegetatively very similar to some species of *Haworthia* and the two are distinguished solely by floral symmetry. Flowers of *Astroloba* are actinomorphic with tepals spreading at the tips (star-shape) whereas the perianth in *Haworthia* is irregular and nearly always bilabiate.

1.2.3 *Chortolirion* A.Berger (1908)

Chortolirion comprises a single species, *C. angolense* (Baker) A.Berger (Mabberley, 2008). This monotypic genus has an underground bulb (Fig. 1.4a) and narrow grass-like leaves weakly armed with small, white marginal teeth (Smith & Van Wyk, 1993; Smith, 1995b) which die back to ground level during fire or in winter, a character unique to this genus. Its flowers closely resemble those of *Haworthia* species (Fig. 1.4b) and it has been included in that genus in the past (Obermeyer, 1973) but has generally been retained as distinct from it on the basis of the distinct, bulb-like swelling of the leaf bases (Smith, 1995a). *Chortolirion* is also characterised by spirally twisted, graminoid, deciduous leaves (Smith & van Wyk, 1993). Furthermore, *Chortolirion* has a widespread northern distribution in Angola, Botswana, Namibia and the summer rainfall areas in South Africa (Fig. 1.1f), whereas *Haworthia* is predominantly restricted to the winter rainfall regions in the Western and Northern Cape provinces (Fig. 1.1c).

Chortolirion, just like most Alooideae genera, have witnessed some taxonomic controversy over the years so that a considerable number of synonyms have been attributed to it. Smith & van Wyk (1993) using leaf anatomy found that the distribution of tissues in the leaves of *C. angolense* is similar to that described for grass-like *Aloe* species in sect. *Leptoaloe* Berger (e.g. *Aloe verecunda* Pole-Evans, *A. boylei* Baker, and the graminifoliate *Haworthia blackburniae* Baker). This relationship was confirmed by a recent molecular study (Treutlein *et al.*, 2003b). Remarkably, it shares a xerophytic character with other xerophytes such as sunken and protected stomata. A distinctive palisade layer immediately inside the epidermis is conspicuous, although this has not been accepted as a generic marker to delimit this monotypic genus (Smith *et al.*, 1996). It however shares the presence of inner bundle sheath-caps consisting of thin-walled parenchymatous cells with other alooid genera.

1.2.4 *Gasteria* Duval (1809)

Linnaeus (1753) in *Species Plantarum* included *Gasteria* under the name *Aloe disticha* (*Gasteria disticha* sensu Van Jaarsveld, 1994). In 1809 Duval formally described *Gasteria*, recognising 43 species. Since then many new species have been described. Finally in 2007, Van Jaarsveld arranged *Gasteria* into two sections, *Longiflorae* and *Gasteria*, based on the shape and epidermal texture of the leaf and recognised 23 species. This classification system is widely accepted and the most comprehensive work on the genus to date. *Gasteria* is endemic to South Africa with the greatest concentration occurring in the Eastern Cape Province (Fig. 1.1b). Only one species, *Gasteria pillansii* Kensit, extends beyond South Africa's borders and crosses the Orange River into southern Namibia (Van Jaarsveld, 2007).

Gasteria is defined as a monophyletic group within Alooideae based on floral morphology, vegetative characters and molecular data (Zonneveld & Van Jaarsveld, 2005; Treutlein *et al*., 2003b). It is distinguished from most, but not all aloes by its inclined racemes of pendulous, curved flowers (Fig. 1.5b) sometimes swollen at the base (gasteriform). Sunbirds (Nectariniidae) and insects pollinate the flowers of *Gasteria*. Bimodal karyotype is uniform in all *Gasteria* species with eight large and six small chromosomes (Vosa & Bennett, 1990). The leaves of *Gasteria* are triangular in section and firm-textured and mostly dark green with bands of whitish spots (Fig. 1.5a), and the margins are horny but never spiny (Van Jaarsveld, 2007). Brandham (1977) evaluated the inheritance of leaf pigmentation in *Gasteria* and showed that despite their wide morphological diversity the 'spotted' *Gasteria* species have identical genetic systems controlling the distribution of leaf pigmentation, which underlines their basic genetic similarities. Today *Gasteria* species are threatened in the wild due to succulent collectors and agricultural practices.

1.2.5 *Haworthia* Duval (1809)

Haworthia includes approximately 61 species and numerous infra-specific taxa (Bayer, 1999; 2002; Mabberley, 2008). Most species are highly localised and are largely restricted to the southern regions of South Africa, with outliers in the Mpumalanga, KwaZulu-Natal and Free State provinces of South Africa, Swaziland, Mozambique and Namibia (Fig. 1.1c). *Haworthia* species are small succulent herbs (Fig. 1.6a) with their leaves arranged in a rosette. The size and shape of the leaves are variable, even within species. The flowers are small, tubular, bilabiate and white.

Haworthia has a long history of taxonomic changes (Table 1.4) since Linnaeus (1753) broadly placed it within “aloes” and Duval formally described it in 1809. In 1999, Bayer subdivided *Haworthia* into three subgenera namely *H*. subgenus *Haworthia*, *H.* subgenus *Hexangulares*, and *H.* subgenus *Robustipendunculares* (see also Vosa, 2004). This was based on morphological characters as well as continuity of characters in relation to their geographical distribution. Subgenus *Haworthia* has a wide range of distribution from the Western to the Eastern Cape Provinces including the Free State. Flowers with elongated triangles petals as well as perianths that are triangular in cross-section (e.g. *Haworthia mirabilis*; Fig. 1.6b) unified members of this subgenus. *Haworthia* subgenus *Hexangulares* occurs in the Eastern Cape Province with a disjunct distribution of *H. limifolia* Marloth in the Swaziland-Mpumalanga area. Flowers are six-sided at the base. Only four species (*Haworthia marginata* (Lam.) Stearn, *H. pumila* (L.) Duval, *H. minor* (Aiton) Duval and *H. kingiana* Poelln.) are recognised within *H.* subgenus *Robustipendunculares.* They are distributed exclusively in the Western Cape Province and it is characterised by robust tubular perianths. Two recent molecular studies (Treutlein *et al.*, 2003a; Ramdhani *et al.*, 2011) recovered the above-mentioned subgenera but demonstrated that *Haworthia* was paraphyletic. Sampling in both studies were however limited.

1.3 Objectives of the study

From the above it is evident that evolutionary relationships among the Alooideae genera are still incompletely understood and the generic taxonomy remains uncertain. As yet, no adequately sampled and well-supported phylogenetic analysis exists on which to base a classification for Alooideae. Treutlein *et al*. (2003b) offered three alternative scenarios for resolving this problem but however refrained from implementing any of them. This study was thus aimed at addressing previous morphological and molecular hypotheses on generic circumscriptions and affinities among the five genera of Alooideae using molecular sequence data.

The specific objectives of the study were to:

1) Reconstruct a phylogeny for Alooideae (154 species representing all five genera and 20 sections within *Aloe*, incorporating a wide range of vegetative and floral diversity in the subfamily) using DNA sequences from the internal transcribed spacers (ITS1) of nuclear ribosomal DNA and three plastid regions (*rbcLa*, *trnH-psbA* and *matK*).
2) Mapped morphological trait data onto the phylogeny to determine the distribution of these characters in order to investigate whether these traits are phylogenetically structured.
3) Data from 1 and 2 were then used to: a) clarify phylogenetic relationships among the genera, b) assess the monophyly of various groups within Alooideae, and c) reconcile the current taxonomy with the molecular data.

Table 1.1 Alooideae genera (Van Wyk *et al.*, 1993; Smith & Steyn, 2004; Klopper *et al.*, 2010)

Currently recognised Alooideae genera	**References**	**Names not upheld**
Aloe Linnaeus: 319 (1753)	Reynolds (1966, 1969), Glen & Hardy (2000), Germishuizen *et al.* (2006)	*Catevala* Medikus: 67 (1786) *pro parte*; *Kumara* Medikus: 69 (1786); *Rhipidodendrum* Willdenow 164 (1811); *Pachidendron* Haworth: 35 (1821); *Bowiea* Haworth: 299 (1824) *non* J.D. Hooker: t. 5619 (1867); *Agriodendron* Endlicher: 144 (1836); *Succosaria* Rafinesque: 137 (1840); *Busipho* Salisbury: 76 (1866); *Ptyas* Salisbury: 76 (1866); *Chamaealoe* A.Berger: 43 (1905); *Leptaloe* Stapf: t. 9300 (1933) *Aloinella* Lemee: 27 (1939) *non* Cardot: 76 (1909); *Guillauminia* Bertrand: 41 (1956); P.V.Heath: 153 (1993); *Lomatophyllum* Willdenow: 5 (1811)
Gasteria Duval: 6 (1809)	Van Jaarsveld (1992, 1994, 2007)	*Atevala* Rafinesque: 136 (1840); *Papilista* Rafinesque: 137 (1840)
Haworthia Duval: 7 (1809) *nom. cons*.	Bayer (1999)	*Catevala* Medikus: 67 (1786) *pro parte*; *Apicra* Willdenow: 167 (1811) *non* Haworth: 61 (1819); *Kumaria* Rafinesque: 137 (1840); *Tulista* Rafinesque: 137 (1840)
Chortolirion A.Berger: 72 (1908)	Smith (1995a)	—
Astroloba Uitewaal: 53 (1947)	Roberts (1965), Smith (1995b), Manning & Smith (2000)	*Apicra* Haworth: 61 (1819) *non* Willdenow: 167 (1811); *Poellnitzia* Uitewaal Succulenta 22:61 (1940)

Table 1.2 Proposed families within Asparagales from 1981 to 2009. Names in brackets are the combination of ‘groups’ belonging to the family.

Cronquist (1981)	**Mabberley (2008)**	**APG II (2003)**	**APG III (2009)**
Order: Liliales	Order: Asparagales	Order: Asparagales	Order: Asparagales
—	Alliaceae	—	—
—	Agapanthaceae	—	—
Agavaceae	Amaryllidaceae	Agavaceae (Anemarrhenaceae, Anthericaeae, Behniaceae, Herreriaceae)	Amaryllidaceae (Agapanthaceae)
Aloeaceae	Asparagaceae (Agavaceae, Anthericaceae, Aphyllanthaceae, Behniaceae, Convallariaceae, Dracaenaceae, Eriospermaceae, Herriaceae, Hyacinthaceae)	Asparagaceae	Asparagaceae (Agavaceae, Aphyllanthaceae, Hesperocallidaceae, Hyacinthaceae, Laxmanniaceae, Ruscaceae, Themidaceae)
Cyanastraceae	Asphodelaceae	—	—
Dioscoreaceae	Asteliaceae	—	Asteliaceae
Haemodoraceae	Blandfordiaceae	—	Blandfordiaceae
Hanguanaceae	Boryaceae	—	Boryaceae
Liliaceae	Doryanthaceae	—	Doryanthaceae
—	Hemerocallidaceae	—	—
—	Hypoxidaceae	—	Hypoxidaceae
Iridaceae	Iridaceae	—	Iridaceae
Philydraceae	Ixioliriaceae	—	Ixioliriaceae
Pontederiaceae	Lanariaceae	—	Lanariaceae
Smilacaceae	Orchidaceae	Ruscaceae (Convallariaceae, Dracaenaceae, Eriospermaceae, Nolinaceae)	Orchidaceae
Stemonaceae	—	—	Ruscaceae
Taccaceae	Tecophilaeceae	—	Tecophilaeceae
Velloziaceae	—	—	—
Xanthorrhoeaceae	Xanthorrhoaceae	Xanthorrhoeaceae (Asphodelaceae, Hemerocallidaceae, Phormiaceae).	Xanthorrhoaceae (Asphodelaceae, Hemerocallidaceae)
—	Xeronemataceae	—	Xeronemataceae

Table 1.3 History of genera recognised within Asphodelaceae from 1981 to 2010.

Cronquist (1981)	**Smith & van Wyk (1991)**	**Mabberley (2008)**	**Klopper *et al.* (2010)**
Family: Aloeaceae	**Family: Asphodelaceae**	**Family: Asphodelaceae**	**Family: Asphodelaceae**
Aloe L.	*Aloe* L.	*Aloe* L.	*Aloe* L.
—	—	*Asphodeline* Reichb.	*Asphodeline* Reichb.
—	—	*Asphodelus* L.	*Asphodelus* L.
—	*Astroloba* Uitew.	*Astroloba* Uitew.	*Astroloba* Uitew.
—	—	*Bulbine* Wolf.	*Bulbine* Wolf.
—	—	*Bulbinella* Kunth.	*Bulbinella* Kunth.
—	*Chortolirion* A.Berger	*Chortolirion* A.Berger	*Chortolirion* A.Berger
—	—	*Eremurus* M.Bieb.	*Eremurus* M.Bieb.
Gasteria	*Gasteria*	*Gasteria* Duval	*Gasteria* Duval
Haworthia (including *Astroloba, Chortolirion,* and *Poellnitzia*)	*Haworthia* Duval	*Haworthia* Duval	*Haworthia* Duval
—	—	*Jodrellia* Baijnath	*Jodrellia* Baijnath
*Kniphofia*Moench.	*Kniphofia*Moench.	*Kniphofia*Moench.	*Kniphofia*Moench.
*Lomatophyllum*Willd.	*Lomatophyllum*Willd.	*Lomatophyllum*Willd.	—
—	*Poellnitzia*Uitew.	—	—
—	—	*Trachyandra* Kunth.	*Trachyandra* Kunth.

Table 1.4 Historical bibliographical notes on genus *Haworthia* (Author dates are based on Scott (1985) and Bayer (1999).

Author (Date)	**Placement of *Haworthia***	**Reference**
Linnaeus (1753)	"Aloes"	In *Species Plantarum* Part I (first edition) (1753)
Linnaeus (1762)	"Aloes"	In *Species Plantarum* Part I (second edition) (1762)
Burman (1768)	"Aloes"	In Appendix: *Florae Capensis Prodromus* in *Flora Indica* (1768)
Miller (1768)	"Aloes"	In *Gardeners' Dictionary*, ed. 8 (1768)
Aiton (1789)	"Aloes"	*Hortus Kewensis* Vol. 1, pp. 466-471 (1789).
Willdenov (1799)	"Aloes"	*Species Plantarum* 2: 184-192 (1799)
De Candolle (1799-1801)	"Aloes"	"Aloes" illustrations: *Plantarum historia succulentarum (Histoire des plantes grasses)*
Jacquin (1804)	"Aloe"	"Aloes" illustrations (1804)
Thunberg (1785)	"Aloe"	*Dissertation* on the genus "Aloe" (1785)
Friedrich Medicus (1786)	"Aloe" genus *Catevala*	First to subdivide and separate genus *Aloe* (today's *Aloe + Haworthia*) from genus *Catevala*
Haworth (1804)	"Aloe" section *Paviflorae*	"A new arrangement of the genus *Aloe*" in *Trans. Linn. Soc.* 7:1-28 (1804).
Duval (1809)	*Haworthia*	*Plantae succulentae in Horto Alenconio*, separated genera *Haworthia* and *Gasteria* from *Aloe* (1809).
Willdenov (1811)	Generic name: *Apicra* for *Paviflorae*	*Ges. Naturf. Fr. Berl. Mag*. 5: 167 (1811)
Aiton (1811)	"Aloes"	*Hortus Kewensis* ed. 2, 2:292-301 (1811)
Haworth (1812-27)	*Haworthia*	In *Synopsis plantarum succulentarum* pp. 90-99, accepted Duval's genera *Haworthia* and *Gasteria*
Schultes & Schultes (1829)	Reverted to "Aloe" in broad Linnaean sense	Roemer and Schultes, *Systema Vegetabilium* 7: 682-715 (1829)
Salm-Dyck (1836-63)	"Aloe" in the broad sense	*Monographiae Generum Aloes et Mesembryantheni*
Kunth (1843)	"Aloe" in a broad sense	*Enumeratio Plantarum*, Vol 4, gave *Apicra* and *Haworthia* separate status as sections (1843).
Baker (1870-71; 1896)	*Haworthia*	Saunders, *Refugium Botanicum* Vol. 4; *Flora Capensis* Vol.6: 332-355 (edited by Sir W. T. Thiselton-Dyer) (1896)
Baker (1880)	Aloineae	"A synopsis of Aloineae and Yuccoideae" in *J. Linn. Soc. Bot.* Vol. 18 (1880);
Berger (1908)	*Haworthia*	Engler's *Pflanzenreich* 4, 38: 74-114 (1908).
Smith (1942)	*Haworthia*	*J. S. Afr. Bot.* 8: 247. Collections donated to Kirstenbosch in 1957
Uitewaal (1938-1951)	*Haworthia*	Possibly the greatest European student of *Haworthia*. Published widely in *Succulenta.*
Bayer (1962, 1976; 1982; 1999, 2002)	*Haworthia*	*Haworthia Handbook* (1976), and *The New Haworthia Handbook* (1982, 1999, 2002); *Haworthia revisited (1999); Haworthia update. (1999)*

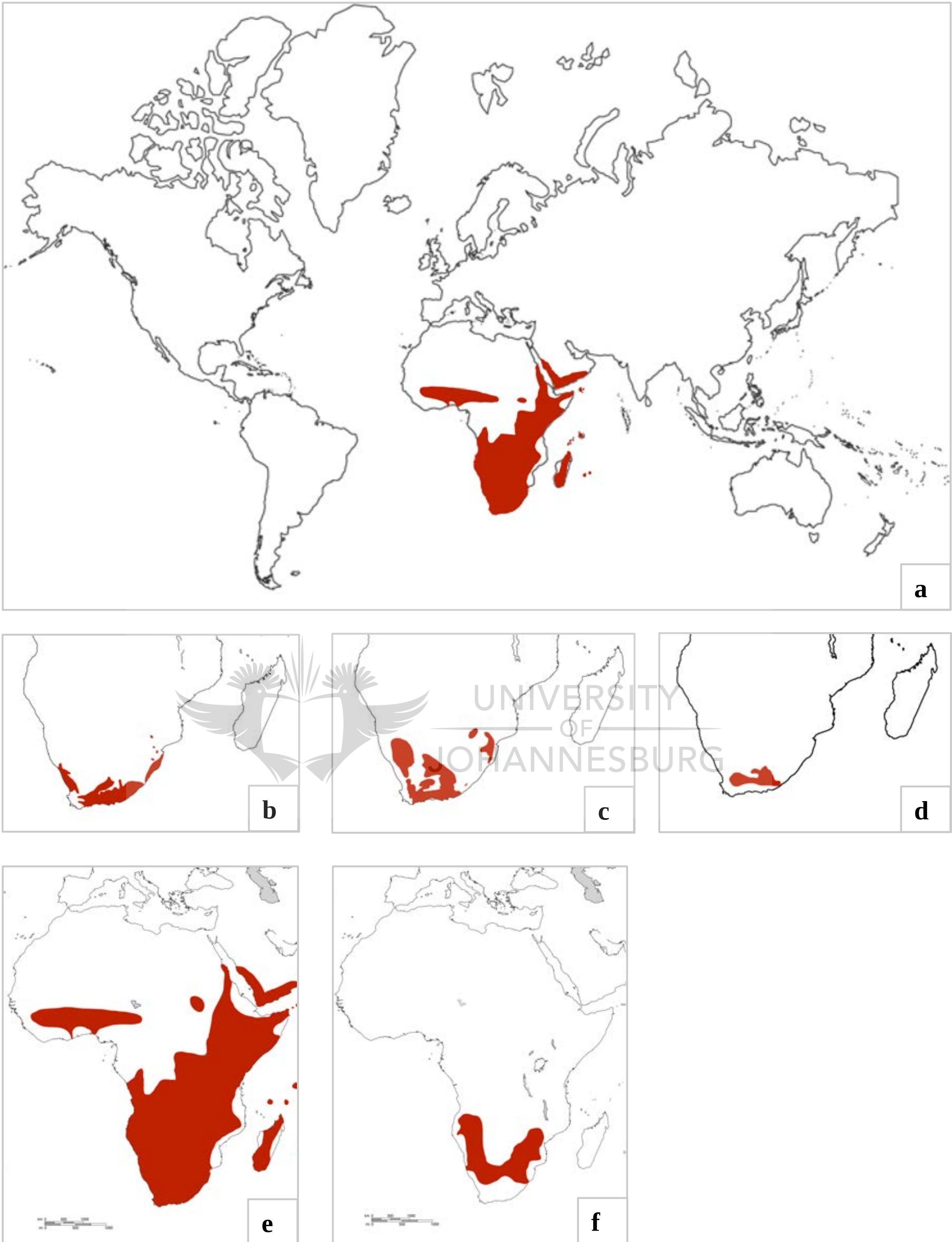


Figure 1.1 (a) Global distributions of all Alooideae genera highlighted in red. Distributions of (b) *Gasteria*; (c) *Haworthia*; (d) *Astroloba*; (e) *Aloe*; (f) *Chortolirion*. Adapted from Smith & van Wyk (1991), Bayer (1999) and Viljoen (1999).

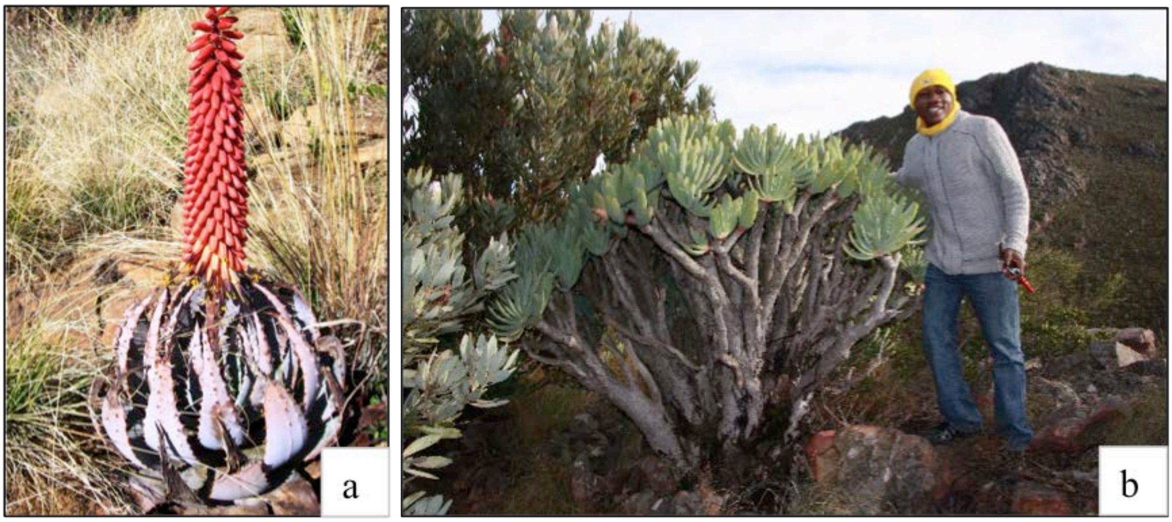


Figure 1.2 (a) *Aloe peglarae* Schönland and (b) *A. plicatilis* with distichous leaf arrangement. Photo: O. Maurin

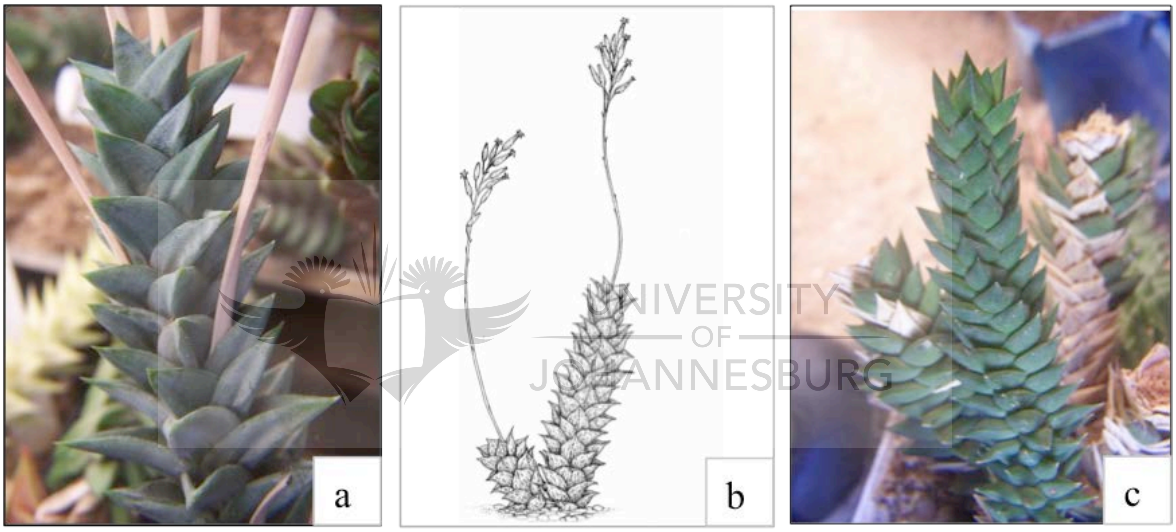
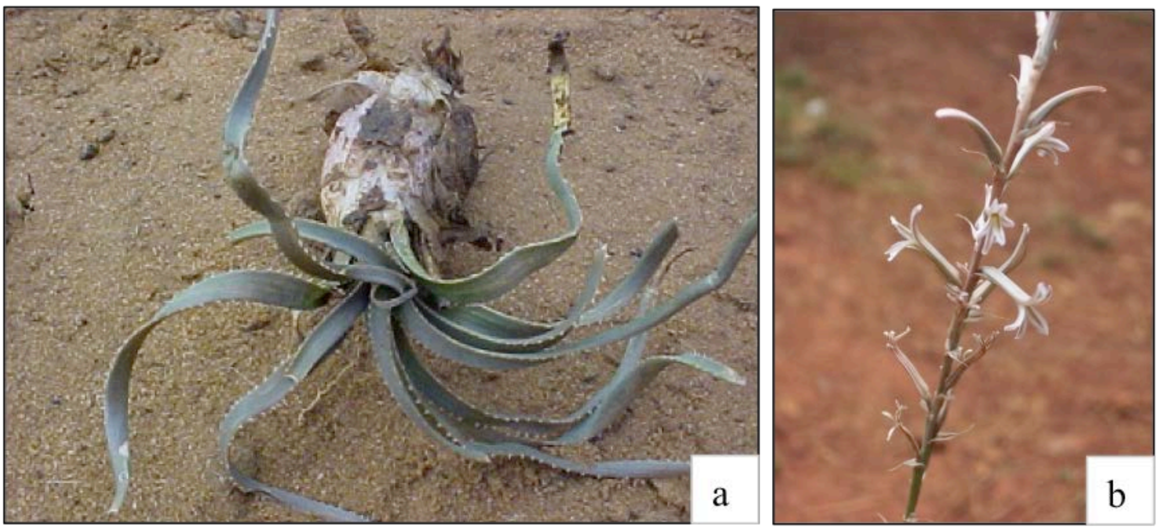


Figure 1.3 (a) *Astroloba rubriflora* (L.Bolus) G.F.Sm. & J.C.Manning, (b) *Astroloba corrugata* N.L.Mey. & Gideon F.Sm. and (c) *A. spiralis* (L.) Uitewaal showing the densely packed triangular leaves (Photo: M.B.Bayer).



www.lifestyleseeds.co.za

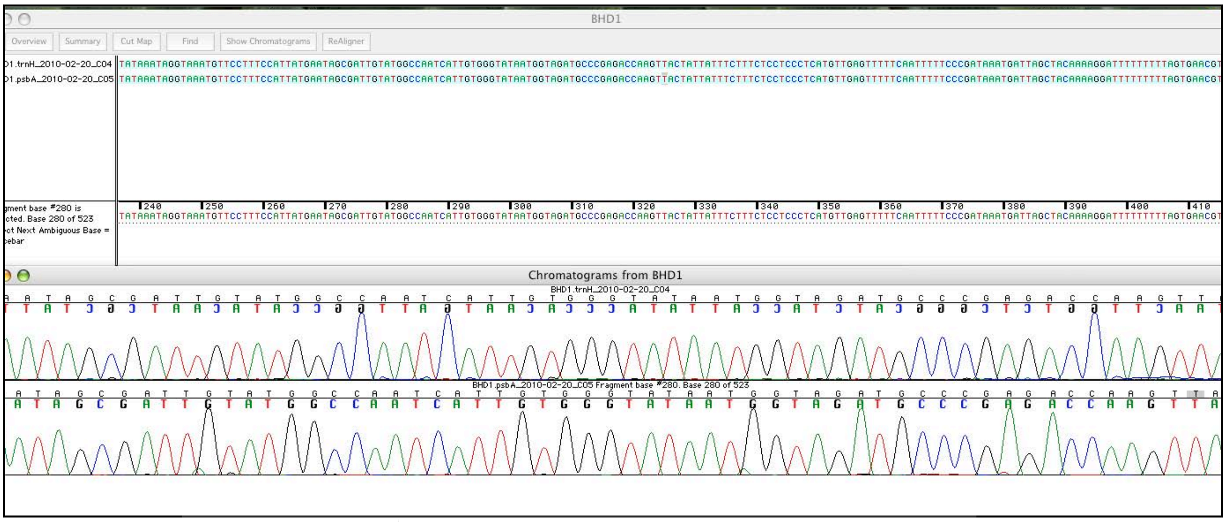
Figure 1.4 (a) *Chortolirion angolense* showing leaves and underground bulb and (b) zygomorphic flowers. Photo: Sean Gildenhuys (a) and www.lifestyleseeds.co.za (b)

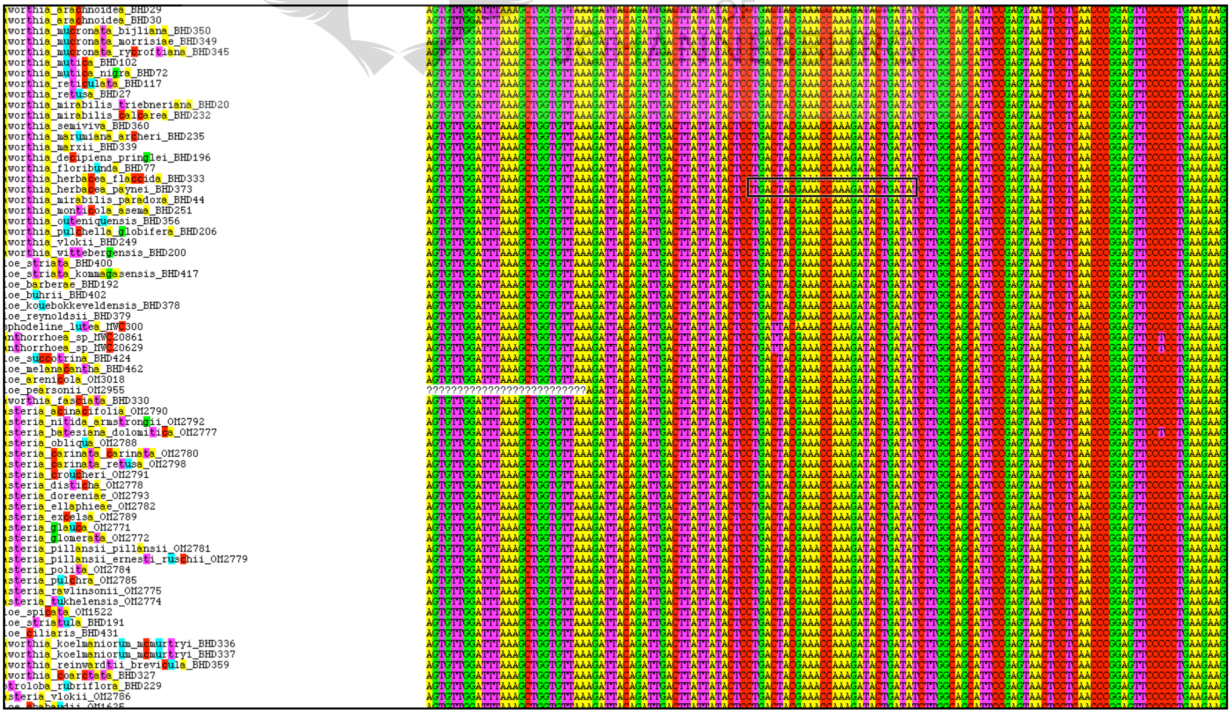
Figure 1.5 (a) *Gasteria croucheri* (Hook.f) leaves with bands of whitish spots and (b) the secund inflorescence of *G. carinata* var. *retusa* van Jaarsv. Photo: O. Maurin

Figure 1.6 (a) *Haworthia mirabilis* (Haw.) Haw. Showing succulent leaves and (b) bilabiate flower. Photo: O. Maurin

CHAPTER TWO

Material and Methods





2. MATERIAL AND METHODS

2.1 Choice of gene regions

The first subunit of the nuclear ribosomal internal transcribed spacer (ITS1; Fig. 2.1) and three plastid regions (*matK*, *rbcLa* and *trnH-psbA*) were chosen to reconstruct a phylogeny of Alooideae in order to add to the already existing phylogenies of Chase *et al.* (2000) and Treutlein *et al.* (2003b). Also, these regions have been shown to be useful in resolving phylogenetic relationships at various taxonomic levels in other plant groups (e.g. Hillis & Dixon, 1991; Baldwin *et al.*, 1995; Small *et al*., 2004).

2.2 Taxon sampling

Representatives of all five genera accepted in subfamily Alooideae (including 154 species and subspecies) were analysed for the four gene regions mentioned above. I included 20 species of *Gasteria*, 69 *Haworthia*, 60 *Aloe* from 20 sections (including *Lomatophyllum*), four *Astroloba* (including *Poellnitzia*) and one *Chortolirion* species in the analyses. Samples were collected from living material in private and national collections in South Africa (Sheilam Nursery, Robertson; Gariep Nursery, Pretoria; University of Johannesburg (JRAU); Kirstenbosch Botanical Gardens, Cape Town). Most of these accessions were originally wild-collected (Figure 2.2). Representatives of Anthericaceae (*Anthericum liliago* L.), Asphodelaceae: Asphodeloideae (*Asphodeline lutea* (L.) Reichb., *Bulbine frutescens* (L.) Willd., *Bulbine semibarbata* (R.Br.) Haw., *Eremurus spectabilis* M.Bieb., *Bulbine fistulosa* (Chiov.) Baijnath, *Kniphofia galpinii* Baker, and *Kniphofia uvaria* (L.) Oken), Tecophilaeaceae (*Tecophilaea cyanocrocus* Leyb. and *Zephyra elegans* D.Don), and Xanthorrhoeaceae (*Xanthorrhoea resinosa* Pers. and *Xanthorrhoea* sp.) were selected as outgroups based on previous molecular and morphological studies within Asparagales (Smith & van Wyk, 1991; Chase *et al*., 2000; Treutlein *et al*., 2003a, b; Devey *et al*., 2006). These samples were obtained from the DNA

Bank at the Royal Botanic Gardens, Kew (UK). Voucher specimen information and GenBank accession numbers are listed in Table 2.1. Taxonomic concepts in *Gasteria* and *Haworthia* follow Van Jaarsveld (2007) and Bayer (1999), respectively.

2.3 DNA extraction, amplification and sequencing

Total genomic DNA was extracted from either fresh or silica gel dried leaf material using the 10× CTAB method described by Doyle & Doyle (1987). Polyvinyl pyrolidone (2% PVP) was added to reduce the effect of high polysaccharide concentrations in the samples. All samples were purified using QIAquick purification columns (QIAgen, Inc., Hilden, Germany) according to the manufacturer’s protocol.

Primers used for the polymerase chain reaction (PCR) amplification of the cpDNA *rbcLa*, *matK*, and *trnH-psbA*, were *rbcLa*-F: *rbcLa*-R, Kim *K*i-Joong-3F: Kim *K*i-Joong-1-R (CBOL Plant Working Group, 2009), and *psbA*F: *trnH*-R (Sang *et al*., 1997), respectively. The ITS1 region was amplified using the primer combination ITS18: ITS5 (Treutlein *et al*., 2003a). The PCR amplification primers were also used as cycle sequencing primers (Table 2.2).

PCR amplification for *rbcLa* and *matK* was carried out at the Canadian Centre for DNA Barcoding (CCDB), Biodiversity Institute of Ontario, University of Guelph, Canada. Details of the project including voucher information, GPS coordinates, pictures and DNA barcodes are available on BOLD (http://www.boldsystems.org) (Ratnasingham & Herbert, 2007) within the project file ‘Alooideae of Africa’ (ALOAF). Sequencing of ITS1 and *trnH-psbA* as well as some additional *matK* and *rbcLa* was carried out at the African Centre for DNA Barcoding (ACDB) of the University of Johannesburg, South Africa. All PCR amplifications were performed using ReadyMix Master mix (Advanced Biotechnologies, Epsom, Surrey, UK). Bovine serum albumin (3.2%) was added to both nuclear and plastid reactions, whereas 4.5% dimethyl sulfoxide (DMSO) was added only to *matK* and ITS1 amplifications. These additives serve as stabilisers for enzymes, reduce problems with secondary structure and improve annealing (Palumbi, 1996). PCR reaction amplification was performed using the following programs: for *rbcLa* and *trnH-psbA*, pre-melt at 94°C for 3 min, denaturation at 94°C for 1 min, annealing at 48°C for 1 min, extension at 72°C for 1 min (for 28 cycles), followed by a final extension at 72°C for 7 min; for *matK*, the protocol consisted of pre-melt at 94°C for 1 min, denaturation at 94°C for 30 s, annealing at 50°C for 40 s, extension at 72°C for 40 s (for 35 cycles), and a final extension at 72°C for 5 min. The ITS1 protocol consisted of pre-melt at 94°C for 3 min, denaturation at 94°C for 1 min, annealing at 48°C for 1 min, extension at 72°C for 3 min (for 26 cycles), followed by a final extension at 72°C for 7 min. Prior to cycle sequencing, PCR products were visualised on a 1.5% agarose gel and subsequently purified using QIAquick (Qiagen Inc.) silica columns according to the manufacturer's protocol. The PCR products were purified using QIAquick (Qiagen Inc.) silica columns according to the manufacturer's protocol.

Cycle sequencing reactions for all genes used in this study were performed using ABI PRISM® BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., California, USA). Cycle sequenced products were precipitated in ethanol and sodium acetate to remove excess dye terminators before sequencing on an ABI 3130*x1* genetic analyser.

2.4 Phylogenetic analyses and tree construction

Complementary strands were assembled and edited using Sequencher 4.8 (Gene Codes, Ann Arbor, Michigan, USA). The sequences were aligned using Multiple Sequence Comparison by Log-Expectation (MUSCLE v. 3.8.31; Edgar, 2004) and the alignment finally adjusted manually in PAUP* (v. 4.0b.10; Swofford, 2002) without difficulties because of low levels of insertions/deletions except for the *trnH-psbA* region of which 15.5% regions were excluded from analyses due to alignment difficulties at positions 1-24, 123-202; 272-290; 830-839 of the aligned matrix. The aligned matrices are available from the author (darunabas@gmail.com).

The separate datasets were assessed for congruence by means of visual inspection of the individual bootstrap consensus trees. The bootstrap trees were declared incongruent in cases where they exhibit "hard" (high bootstrap support) instead of "soft" (low bootstrap support; Seelanan *et al.*, 1997) incongruence. Maximum parsimony analyses (MP) were performed on the ITS1, combined plastid, and total combined datasets whereas Bayesian inference (BI) analysis was employed only on the combined data set. Maximum parsimony analyses were performed using PAUP* version 4.0b.10 (Swofford, 2002). Tree searches were conducted using 1 000 random sequence additions, retaining 10 trees at each step, with tree-bisection-reconnection (TBR) branch swapping and MulTrees in effect. Resulting trees were then used in a second search with the same parameters, however, without a limit for the number of trees per replicate (swopping to completion). Delayed transformation (DELTRAN) character optimisation was used instead of acceleration of transformation (ACCTRAN) for calculating branch lengths, because of reported errors with version 4.0b.10 of PAUP* (http://paup.csit.fsu.edu/problems.html). Branch support was estimated using bootstrap analysis (Felsenstein, 1985) with 1 000 replicates, simple sequence addition, no swapping, with MulTrees in effect saving 10 trees per replicate. The following scale for evaluating bootstrap support (BP) was applied: weak (50-74%), moderate (75-84%) or high (85-100%).

Bayesian inference analysis (BI; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) was performed using MRBAYES v. 3.1.2. For each matrix (ITS1, *rbcLa*, *matK*, *trnH-psbA*) the most appropriate model was selected based on Akaike Information Criterion (AIC) implemented in MODELTEST v. 3.06 (Posada & Crandall, 1998). The model

TRN+G was selected for ITS1, TVM+G for *matK*, HKY+G for *rbcLa* and TVM+1+ G for *trnH-psbA*. I used 2 000 000 generations with a sample frequency of 200. Partition analysis was run for the combined dataset. The log-likelihood scores were plotted to determine the point of stationarity, and all trees prior to stationarity were discarded as the “burn-in” phase (1000 trees). All remaining trees were imported into PAUP* 4.0b.10 and a majority-rule consensus tree was produced showing the frequencies (posterior probabilities or PP) of all observed bi-partitions. The following scale was used to evaluate the PPs: below 0.95, weakly supported; 0.95-1.0, strongly supported. To map the BP and PP values (MrBayes tree) onto the tree, the nexus tree file from the Bayesian analysis was rescaled using the *ape* vs. 2.0-1 (Paradis *et al.,* 2004) and *adephylo* vs. 1.1 (Jombart & Dray, 2010) packages implemented in R (R Development Core Team, 2011).

2.5 Coding of morphological characters

A matrix of 20 morphological characters was prepared for the 154 species of Alooideae included in the analyses. Most morphological studies in Alooideae systematics employed these diagnostic characters (often chosen at species level) to infer relationships within the subfamily (Germishuizen *et al.,* 2006; Gildenhuys, 2007; Glen & Hardy, 2000; Glen & Smith, 1995; Jeppe, 1969; Bayer, 1982, 1999, 2002, 2009; Klopper & Smith, 2007; Meyer & Smith, 1998, 2001; Mössmer *et al*., 1995; Reynolds, 1966, 1969; Smith *et al.*, 1995; Smith & Steyn, 2005; Smith, 1995a, b; Van Jaarsveld *et al.*, 1994; Van Jaarsveld & van Wyk, 2004, 2005, 2006; Van Jaarsveld, 1992, 1994, 1998, 2001; Van Wyk & Smith, 2003). To test for phylogenetic structure in morphological characters, the R function ‘table.phylo4d’ implemented in the R package *adephylo* vs. 1.1 (Jombart & Dray, 2010) was used. By plotting phylogeny and traits simultaneously, this function allows a direct visualisation of phylogenetic structure in morphological traits.

The patterns of evolution of these characters were examined by reconstructing them onto the majority-rule consensus tree produced by the BI analysis using Mesquite vs 2.75 (Maddison & Maddison, 2011). Morphological characters and character-states are defined in Table 2.3 and the data matrix used for character reconstructions is shown in Table 2.4. All trait values are centred and scaled into binary values. These values are represented using coloured symbols, with the different colours proportional to the absolute value of each trait.

Table 2.1 Table of taxa, voucher information, and accession numbers from GenBank. DNA sequences obtained from GenBank are indicated by [1]Treutlein *et al*., 2003a, [2]Treutlein *et al.*, 2003b, and [3]Kim *et al.*, 2010. Original publication dates and synonyms were obtained from The Plant List Version 1 (2010).

	Original Publication Details	**Synonym(s)**	**Distribution**	**Voucher (herbarium)**	**GenBank Accession Number**			
					trnH-psbA	**ITS**	***rbcLa***	***matK***
***Aloe* L.**								
Section Aloe								
Aloe arenicola Reynolds	*J. S. African Bot.* 4: 21 (1938)	—	Western Cape	*OM3018* (JRAU)	JQ024863	JQ025268	JQ024487	JQ024111
Aloe pearsonii Schönland	*Rec. Albany Mus.* 2: 229 (1911)	—	Northern Cape, Namibia	*OM2955* (JRAU)	JQ024868	JQ025269	JQ024526	JQ024154
Aloe perfoliata L. **(TYPE)**	*Sp. Pl.* 319 (1753)	*Aloe albispina* Haw., *Aloe brevifolia* Haw. [nom. illegit.], *Aloe commelyni* Willd., *Aloe comptonii* Reynolds, *Aloe depressa* Salm-Dyck ex Steud. [nom. inva.], *Aloe distans* Haw., *Aloe flavispina* Haw., *Aloe mitriformis* Mill., *Aloe mitriformis* DC. [nom. illegit.], *Aloe mitriformis* Willd. [nom. illegit.], *Aloe mitriformis* var. *albispina* (Haw.) A.Berger, *Aloe mitriformis* var. *angustior* Lam., *Aloe mitriformis* var. *commelyni* (Willd.) Baker, *Aloe mitriformis* var. *comptonii* (Reynolds) Zonn., *Aloe mitriformis* subsp. *distans* (Haw.) Zonn., *Aloe mitriformis* var. *elatior* Haw., *Aloe mitriformis* var. *flavispina* (Haw.) Baker, *Aloe mitriformis* var. *humilior* Haw.,*Aloe mitriformis* var. *humilior* Willd., *Aloe mitriformis* var. *pachyphylla* Baker, *Aloe mitriformis* var. *spinosior* Haw., *Aloe mitriformis* var. *spinulosa* (Salm-Dyck) Baker, *Aloe mitriformis* var. *xanthacantha* (Willd.) Baker, *Aloe nobilis* Haw., *Aloe parvispina* Schönland, *Aloe perfoliata* var. *brevifolia* Aiton, *Aloe perfoliata* var. *mitriformis* (Mill.) Aiton, *Aloe reflexa* Marum ex Steud., *Aloe spinulosa* Salm-Dyck, *Aloe xanthacantha* Willd.	Eastern and Western Cape	*BHD391* (JRAU)*BHD461* (JRAU) *OM2819* (JRAU)	—	JQ025315 JQ024528	JQ024527	JQ024155 JQ024156
Section Aloidendron								
Aloe barberae Dyer	*Gard. Chron. n.s.,* 1874(1): 566 (1874)	*Aloe bainesii* Dyer, *Aloe bainesii* Dyer var. *barberae* (Dyer) Baker, *Aloe zeyheri* Baker [nom. illegit.]	Eastern Cape, Kwazulu-Natal, Mpumalanga, Swaziland	*BHD192* (JRAU)	JQ024864	JQ025262	JQ024489	JQ024113
Aloe eminens Reynolds and P.R.O.Bally	*J. S. African Bot.* 24: 187 (1958)	—	Northern Somalia	*BHD474* (JRAU)	JQ039258	JQ025369	—	—
Section Anguialoe								
Aloe alooides (Bolus) Druten	*Bothalia* 6: 544 (1956)	*Aloe recurvifolia* Groenew., *Notosceptrum alooides* (Bolus) Benth., *Urginea alooides* Bolus	Mpumalanga	*BHD476* (JRAU)	JQ039243	JQ025325	—	—
Aloe spicata L.f.	*Suppl. Pl.* 205 (1782)	*Aloe sessiliflora* Pole-Evans	Kwazulu-Natal, Limpopo, Mpumalanga, Swaziland	*OM1522* (JRAU)	JQ039282	JQ025290	—	—
Aloe vryheidensis Groenew.	*Tijdschr. Natuurk. Wetensch. Kunsten* 38 (1935)	*Aloe dolomitica* Groenew.	Kwazulu-Natal, Limpopo, Mpumalanga	*BHD467* (JRAU)	JQ039288	JQ025308	—	—
Section Arborescentes								

Aloe arborescens Mill.	*Gard. Dict. ed.* 8 3 (1768)	*Aloe perfoliata* var. *arborescens* (Mill.) Aiton, *Catevala arborescens* (Mill.) Medik.	Eastern and Western Cape, Gauteng, Kwazulu-Natal, Limpopo, Mpumalanga, North-West, Botswana, Swaziland	*BHD420* (JRAU)	JQ039246	JQ025326	JQ024486	JQ024110
Section Aristatae								
Aloe aristata Haw.	*Philos. Mag. J.* 67: 280 (1825)	*Aloe aristata* Haw. var. *leiophylla* Baker, *Aloe aristata* Haw. var. *parvifolia* Baker, *Aloe ellenbergeri* Guillaumin, *Aloe longiaristata* Schult. and Schult.f.	Eastern and Western Cape, Free State, Kwazulu-Natal, Limpopo	*BHD459* (JRAU) *BG HeidelbergBG Jena*	JQ039247	JQ025312 AY323651[1] AY323652[1]	AJ512319[1] AY323634[1]	AJ511407[1] AY323713[1]
Section Asperifoliae								
Aloe dewinteri Giess ex Borman and Hardy	*Aloes S. Afr. Veld* 287 (1971)	—	Namibia	*BHD386* (JRAU)	JQ039254	JQ025303	JQ024500	JQ024125
Aloe hereroensis Engl.	*Bot. Jahrb. Syst.* 10: 2 (1889)	—	Free State, Northern Cape, Namibia	*BHD388* (JRAU)	JQ039266	JQ025305	JQ024514	JQ024140
Section Chabaudia								
Aloe chabaudii Schönland	*Gard. Chron.* III, 1905(2): 102 (1905)	—	Kwazulu-Natal, Limpopo, Mpumalanga, Botswana, Swaziland	*OM1625* (JRAU)	JQ039249	JQ025299	—	—
Section Dracoaloe								
Aloe dichotoma Masson	*Philos. Trans.* 66: 310 (1776)	*Rhipidodendrum dichotomum* (Masson) Willd.	Northern Cape, Namibia	*OM2953* (JRAU)	—	JQ025368	JQ024501	JQ024126
Aloe dichotoma Masson subsp. *pillansii* (L.Guthrie) Zonn.	*Bradleya* 20: 10 (2002)	*Aloe pillansii* L.Guthrie	Northern Cape, Namibia	*BHD390* (JRAU)	JQ039255	JQ025372	JQ024502	JQ024127
Aloe dichotoma Masson subsp. *ramosissima* (Pillans) Zonn.	*Bradleya* 20: 10 (2002)	*Aloe dichotoma* Masson var. *ramosissima* (Pillans) Glen and D.S.Hardy, *Aloe ramosissima* Pillans	Namibia	*OM2954* (JRAU)	JQ039256	JQ025367	JQ024503	JQ024128
Section Echinatae								
Aloe melanacantha A.Berger	*Bot. Jahrb. Syst.* 36: 63 (1905)	—	Northern and Western Cape	*BHD462* (JRAU)	JQ039271	JQ025267	—	—
Aloe pictifolia Hardy	*Bothalia* 12: 62 (1976)	—	Eastern Cape	*BHD381* (JRAU)	JQ039277	JQ025324	JQ024530	JQ024158
Section Kumara								
Aloe plicatilis (L.) Mill.	*Gard. Dict. ed.* 8 7 (1768)	*Aloe disticha* L. var. *plicatilis* L., *Aloe flabelliformis* Salisb., *Aloe lingua* Thunb., *Aloe linguaeformis* L.f. [nom. illegit.], *Aloe tripetala* Medik., *Kumara disticha* Medick., *Rhipidodendron distichum* (Medick.) Willd., *Rhipidodendron plicatile* (L.) Haw.	Western Cape	*BHD193* (JRAU) *BG Heidelberg, Germany 9286*	JQ039278	JQ025373 AY323662[1]	JQ024531 AY323613[1]	JQ024159 AY323693[1]
Section Latebracteatae								
Aloe lutescens Groenew.	*Fl. Pl. South Africa* 18: 707 (1938)	—	Limpopo	*RL1193* (JRAU)	JQ039270	JQ025348	—	—
Section Leptoaloe								—
Aloe albida (Stapf) Reynolds	*J. S. African Bot.* 13: 101 (1947)	*Aloe kraussii* Baker var. *minor* Baker, *Aloe myriacantha* (Haw.) Schult. and Schult.f. var. *minor* (Baker) A.Berger, *Leptaloe albida* Stapf	Mpumalanga, Swaziland	*BHD475* (JRAU)	JQ039242	JQ025366	—	—
Aloe challisii van Jaarsv. and A.E.van Wyk	*Aloe* 43: 36 (2006)	—	Mpumalanga	*BHD471* (JRAU)	JQ039250	JQ025355	—	—
Aloe chortolirioides A.Berger	*Pflanzenr.* IV, 38: 171 (1908)	—	Limpopo, Mpumalanga, Swaziland	*BHD477* (JRAU)	JQ039251	JQ025374	—	—
Aloe ecklonis Salm-Dyck	*Aloes Mesembr.* 2: 21 (1849)	*Aloe agrophila* Reynolds	Eastern Cape, Free State, Kwazulu-Natal, Limpopo, Mpumalanga	*BHD465* (JRAU)	JQ039257	JQ025307	—	—
Aloe fouriei D.S.Hardy and Glen	*Fl. Pl. Africa* 49: 1941 (1987)	—	Limpopo, Mpumalanga	*BHD469* (JRAU)	JQ039261	JQ025358	—	—

Aloe nubigena Groenew.	*Tijdschr. Natuurk. Wetensch. Kunsten* 14: 3 (1936)	—	Limpopo, Mpumalanga	*BHD353* (JRAU)	JQ039274	JQ025356	—	—
Aloe saundersiae (Reynolds) Reynolds	*J. S. African Bot.* 13: 103 (1947)	*Leptaloe saundersiae* Reynolds	Kwazulu-Natal	*BHD463* (JRAU)	JQ039281	JQ025345	—	—
Aloe verecunda Pole-Evans	*Trans. Roy. Soc. South Africa* 5: 703 (1917)	—	Gauteng, Limpopo, Mpumalanga	*BHD444* (JRAU)	JQ039286	JQ025346	—	—
Aloe vossii Reynolds	*J. S. African Bot.* 2: 65 (1936)	—	Limpopo	*BHD0464* (JRAU)	JQ039287	JQ025347	—	—
Section Macrifoliae								—
Aloe ciliaris Haw.	*Philos. Mag. J.* 67: 281 (1825)	—	Eastern and Western Cape	*BHD431* (JRAU)	JQ024866	JQ025292	JQ024496	JQ024121
Aloe commixta A.Berger	*Pflanzenr.* IV, 38: 260 (1908)	—	Western Cape	*BHD405* (JRAU)	JQ039252	JQ025329	JQ024497	JQ024122
Aloe gracilis Haw.	*Philos. Mag. J.* 67: 280 (1825)	*Aloe laxiflora* N.E.Br.	Eastern and Western Cape	*BHD399* (JRAU)	JQ039263	JQ025330	JQ024510	JQ024136
Aloe striatula Haw.	*Philos. Mag. J.* 67: 281 (1825)	—	Eastern Cape, Limpopo	*BHD191* (JRAU)	JQ024872	JQ025291	JQ024538	JQ024166
Aloe tenuior Haw.	*Philos. Mag. J.* 67: 281 (1825)	*Aloe tenuior* Haw. var. *decidua* Reynolds, *Aloe tenuior* Haw. var. *densiflora* Reynolds, *Aloe tenuior* Haw. var. *glaucescens* Zahlbr., *Aloe tenuior* Haw. var. *rubriflora* Reynolds, *Aloe tenuior* Haw. var. *viridifolia* van Jaarsv.	Eastern and Western Cape, Kwazulu-Natal, Mpumalanga	*BHD393* (JRAU) *BHD415* (JRAU)	JQ039284	JQ025331	JQ024541	JQ024169
Section Pachydendron								
Aloe angelica Pole-Evans	*Fl. Pl. South Africa* 14: 554 (1934)	—	Limpopo	*OM2960* (JRAU)	JQ039244	JQ025310	—	JQ024109
Aloe excelsa A.Berger	*Notizbl. Königl. Bot. Gart. Berlin* 4: 247 (1906)	—	Limpopo, Botswana	*OM1621* (JRAU)	JQ039259	JQ025301	—	—
Aloe ferox Mill.	*Gard. Dict. ed.* 8 22 (1768)	*Aloe candelabrum* A.Berger [nom. illegit.], *Aloe ferox* var. *galpinii* (Baker) Reynolds, *Aloe ferox* var. *incurva* Baker, *Aloe ferox* var. *subferox* (Spreng.) Baker, *Aloe galpinii* Baker, *Aloe horrida* Haw., *Aloe muricata* Haw., *Aloe pallancae* Guillaumin [nom. inva.], *Aloe perfoliata* var. *ferox* (Mill.) Aiton, *Aloe pseudoferox* Salm-Dyck, *Aloe subferox* Spreng., *Aloe supralaevis* var. *erythrocarpa* Baker, *Busipho ferox* (Mill.) Salisb. [nom. inva.], *Pachidendron ferox* (Mill.) Haw., *Pachidendron pseudoferox* (Salm-Dyck) Haw., *Pachidendron supralaeve* (Haw.) Haw.	Eastern and Western Cape, Kwazulu-Natal, Limpopo	*BHD407* (JRAU)	JQ039260	JQ025327	—	—
Aloe petricola Pole Evans	*Trans. Roy. Soc. South Africa* 5: 707 (1917)	—	Limpopo, Mpumalanga	*OM2959* (JRAU)	JQ039276	JQ025300	JQ024529	JQ024157
Aloe rupestris Baker	*Fl. Cap.* 6: 327 (1896)	*Aloe nitens* Baker [nom. illegit.]	Kwazulu-Natal, Swaziland	*BHD468* (JRAU)	JQ039280	JQ025317	—	—
Aloe thraskii Baker	*J. Linn. Soc., Bot.* 18: 180 (1880)	—	Kwazulu-Natal	*BHD411* (JRAU)	JQ039285	JQ025319	JQ024542	JQ024170
Section Paniculatae								
Aloe buhrii Lavranos	*J. S. African Bot.* 37: 37 (1971)	—	Northern Cape	*BHD402* (JRAU)	JQ024865	JQ025263	JQ024494	JQ024118
Aloe kouebokkeveldensis van Jaarsv. and A.B.Low	*Aloe* 41: 36 (2004)	—	Western Cape	*BHD378* (JRAU)	JQ024867	JQ025264	JQ024518	JQ024144
Aloe reynoldsii Letty	*Fl. Pl. South Africa* 14: 558 (1934)	—	Eastern Cape	*BHD379* (JRAU)	JQ024869	JQ025265	JQ024532	JQ024160
Aloe striata Haw.	*Trans. Linn. Soc. London* 7: 18 (1804)	—	Eastern and Western Cape	*BHD400* (JRAU)	JQ024870	JQ025260	JQ024534	JQ024162
Aloe striata Haw. subsp. *karasbergensis* (Pillans) Glen and D.S.Hardy	*S. African J. Bot.* 53: 491 (1987)	*Aloe karasbergensis* Pillans	Northern Cape	*BHD408* (JRAU)	JQ039283	JQ025306	JQ024536	JQ024164

Aloe striata Haw. subsp. *komaggasensis* (Kritz. and van Jaarsv.) Glen and D.S.Hardy	*S. African J. Bot.* 53: 491 (1987)	*Aloe komaggasensis* Kritz. and van Jaarsv.	Northern Cape	*BHD417* (JRAU)	JQ024871	JQ025261	JQ024537	JQ024165
Section Pictae								
Aloe greatheadii var. *davyana* (Schönland) Glen and D.S.Hardy	*S. African J. Bot.* 53: 490 (1987)	*Aloe barbertoniae* Pole-Evans, *Aloe comosibracteata* Reynolds, *Aloe davyana* Schönland, *Aloe davyana* var. *subolifera* Groenew., *Aloe graciliflora* Groenew., *Aloe labiaflava* Groenew., *Aloe longibracteata* Pole-Evans, *Aloe mutans* Reynolds, *Aloe verdoorniae* Reynolds	Free State, Gauteng, Kwazulu-Natal, Limpopo, Mpumalanga, North-West, Swaziland	*BHD380* (JRAU)	JQ039264	JQ025304	JQ024512	JQ024138
Section Proliferae								
Aloe brevifolia Mill.	*Gard. Dict. Abr. ed.* 6 8 (1771)	*Aloe brevifolia* Mill. var. *postgenita* (Schult. and Schult.f.) Baker	Western Cape	*BHD460* (JRAU)	JQ039248	JQ025314	—	—
Section Purpurascentes								
Aloe microstigma Salm-Dyck	*Aloes Mesembr.* 2: 26 (1849)	—	Northern and Western Cape, Namibia	*BHD392* (JRAU)	JQ039272	JQ025323	JQ024525	JQ024152
Aloe succotrina Weston	*Encycl.* 1: 85 (1783)	*Aloe perfoliata* L. var. *purpurascens* Aiton, *Aloe perfoliata* L. var. *succotrina* (Lam.) Aiton, *Aloe purpurascens* (Aiton) Haw., *Aloe sinuata* Thunb., *Aloe sinuata* Willd. [nom. illegit.], *Aloe soccotorina* Schult. and Schult.f., *Aloe soccotrina* Garsault, *Aloe soccotrina* DC. var. *purpurascens* (Aiton) Ker Gawl., *Aloe succotrina* Lam. var. *saxigena* A.Berger, *Aloe vera* Mill. [nom. illegit.]	Western Cape	*BHD424* (JRAU)	JQ024873	JQ025266	JQ024539	JQ024167
Section Rhodacanthae								
Aloe comosa Marloth and A.Berger	*Bot. Jahrb. Syst.* 38: 86 (1905)	—	Western Cape	*BHD385* (JRAU)	JQ039253	JQ025328	JQ024499	JQ024124
Aloe glauca Mill.	*Gard. Dict. ed.* 8 16 (1768)	*Aloe glauca* Mill. var. *elatior* Salm-Dyck, *Aloe glauca* Mill. var. *humilior* Salm-Dyck, *Aloe glauca* Mill. var. *major* Haw., *Aloe glauca* Mill. var. *minor* Haw., *Aloe glauca* Mill. var. *muricata* (Schult.) Baker, *Aloe glauca* Mill. var. *spinosior* Haw., *Aloe muricata Schult.* [nom. illegit.], *Aloe perfoliata* var. *glauca* (Mill.) Aiton, *Aloe rhodacantha* DC.	Northern and Western Cape	*BHD419* (JRAU)	JQ039262	JQ025313	JQ024508	JQ024134
Aloe lineata (Aiton) Haw.	*Trans. Linn. Soc. London* 7: 18 (1804)	*Aloe perfoliata* var. *lineata* Aiton	Eastern and Western Cape	*BHD416* (JRAU)	JQ039267	JQ025320	JQ024520	JQ024147
Aloe lineata (Aiton) Haw.*var. muirii*(Marloth) Reynolds	*Aloes S. Afr.* 205 (1950)	*Aloe muirii* Marloth	Eastern and Western Cape	*BHD412* (JRAU) *BHD387* (JRAU)	JQ039269	JQ025322 JQ025321	JQ024521 JQ024522	JQ024148 JQ024149
Madagascan Aloes								
Aloe anivoranoensis (Rauh and Hebding) L.E.Newton and G.D.Rowley	*Bradleya* 16: 114 (1998)	*Lomatophyllum anivoranoense* Rauh and Hebding	North-Eastern Madagascar	*BHD473* (JRAU)	JQ039245	JQ025371	—	—
Aloe haworthioides Baker	*J. Linn. Soc., Bot.* 22: 529 (1887)	*Aloinella haworthioides* (Baker) Lemée, *Leemea haworthioides* (Baker) P.V.Heath	Central Madagascar	*BHD190* (JRAU)	JQ039265	JQ025357	JQ024513	JQ024139
Aloe propagulifera (Rauh and Razaf.) L.E.Newton and G.D.Rowley	*Bradleya* 16: 114 (1998)	*Lomatophyllum propaguliferum* Rauh and Razaf.	Central to Eastern Madagascar	*BHD472* (JRAU)	JQ039279	JQ025359	—	—
Taxonomic Status Unknown								
Aloe munchii Christian	*Fl. Pl. Africa* 28: 1091 (1951)	—	Zimbabwe, Mozambique (Chimanimani Mountains)	*BHD470* (JRAU)	JQ039273	JQ025302	—	—
Astroloba **Uitewaal**								

Astroloba corrugata N.L.Mey. and Gideon F.Sm.	*Bothalia* 28: 61 (1998)	*Apicra aspera* var. *major* Haw., *Haworthia aspera* var. *major* (Haw.) Parr, *Haworthia corrugata* (N.L.Mey. and Gideon F.Sm.) M.Hayashi	Western Cape	*BHD219* (JRAU)	JQ039290	JQ025350	JQ024545	JQ024173
Astroloba foliolosa (Haw.) Uitewaal	*Succulenta (Netherlands)* 28: 54 (1947)	*Aloe foliolosa* Haw., *Apicra foliolosa* (Haw.) Willd., *Astroloba smutsiana* nom. prov. Reinecke, *Astroloba spiralis* subsp. *foliolosa* (Haw.) L.E.Groen, *Haworthia foliolosa* (Haw.) Haw.	Eastern Cape	*BHD228* (JRAU)	JQ039291	JQ025351	JQ024547	JQ024175
Astroloba herrei Uitewaal	*Desert Pl. Life* 20: 37 (1948)	*Astroloba dodsoniana* Uitewaal, *Haworthia dodsoniana* (Uitewaal) Parr, *Haworthia harlandiana* Parr	Western Cape	*BHD225* (JRAU)	JQ039292	JQ025349	JQ024548	JQ024176
Astroloba rubriflora (L.Bolus) Gideon F.Sm. and J.C.Manning	*Bothalia* 30: 53 (2000)	*Aloe rubriflora* (L.Bolus) G.D.Rowley, *Apicra jacobseniana* Poelln., *Apicra rubriflora* L.Bolus, *Haworthia rubriflora* (L.Bolus) Parr, *Poellnitzia rubriflora* (L.Bolus) Uitewaal, *Poelnitzia rubriflora* var. *jacobseniana* (Poelln.) Uitewaal	Western Cape	*BHD229* (JRAU)	JQ039293	JQ025297	JQ024549	JQ024177
***Chortolirion* A.Berger**								
Chortolirion angolense (Baker) A.Berger	*Pflanzenr.* IV, 38: 73 (1908)	*Catevala angolensis* (Baker) Kuntze, *Chortolirion bergerianum* Dinter, *Chortolirion stenophyllum* Berger, *Chortolirion subspicatum* (Baker) A.Berger, *Chortolirion tenuifolium* (Engl.) A.Berger, *Chortolirion tenuifolium* Berger, *Haworthia angolensis* Baker, *Haworthia stenophylla* Baker, *Haworthia subspicata* Baker, *Haworthia tenuifolia* Engl.	Eastern and Northern Cape, Free State, Gauteng, Kwazulu-Natal, Limpopo, Mpumalanga, North West, Botswana, Namibia, Swaziland	*BHD466* (JRAU)	JQ039295	JQ025344	—	—
***Gasteria* Duval**								
Section Gasteria								
Gasteria disticha (L.) Haw.	*Philos. Mag. Ann. Chem.* 2: 351 (1827)	*Aloe disticha* L., *Ptys disticha* (L.) Salisb. [nom. inva.]	Western Cape	*OM2778* (JRAU)	JQ024879	JQ025278	JQ024560	JQ024187
Gasteria doreeniae van Jaarsv. and A.E.van Wyk	*Aloe* 41: 81 (2004)	—	Eastern Cape	*OM2793* (JRAU)	JQ024880	JQ025279	JQ024561	JQ024188
Gasteria glomerata van Jaarsv.	*Bradleya* 9: 100 (1991)	—	Eastern Cape	*OM2772* (JRAU)	JQ024884	JQ025283	JQ024566	JQ024193
Gasteria obliqua (Aiton) Duval	*Pl. Succ. Horto Alencon.* 6 (1809)	*Aloe bicolor* (Haw.) Schult. and Schult.f., *Aloe boureana* Schult. and Schult.f., *Aloe dictyodes* Schult. and Schult.f., *Aloe formosa* (Haw.) Schult. and Schult.f., *Aloe guttata* Salm-Dyck, *Aloe lingua* Ker Gawl. [nom. illegit.], *Aloe maculata* Thunb. [nom. illegit.], *Aloe maculata* var. *obliqua* Aiton, *Aloe marmorata* Steud., *Aloe nigricans* var. *fasciata* Salm-Dyck, *Aloe obliqua* (Aiton) Haw. [nom. illegit.], *Aloe planifolia* Baker [nom. illegit.], *Aloe vittata* Schult. and Schult.f., *Aloe zeyheri* Salm-Dyck, *Gasteria bicolor* Haw., *Gasteria bicolor* var. *fallax* (Haw.) van Jaarsv., *Gasteria bicolor* var. *liliputana* (Poelln.) van Jaarsv., *Gasteria biformis* Poelln., *Gasteria caespitosa* Poelln., *Gasteria chamaegigas* Poelln., *Gasteria colubrina* N.E.Br., *Gasteria fasciata* (Salm-Dyck) Haw., *Gasteria formosa* Haw., *Gasteria herreana* Poelln., *Gasteria kirsteana* Poelln., *Gasteria liliputana* Poelln., *Gasteria lingua* (Ker Gawl.) A.Berger [nom. illegit.], *Gasteria loeriensis* Poelln., *Gasteria longiana* Poelln., *Gasteria longibracteata* Poelln., *Gasteria maculata* Haw., *Gasteria maculata* var. *dregeana* A.Berger, *Gasteria maculata* var. *fallax* Haw., *Gasteria marmorata* Baker, *Gasteria multiplex* Poelln., *Gasteria nigricans* var.	Eastern Cape	*OM2788* (JRAU)	JQ024886	JQ025274	JQ024569	JQ024196

		fasciata (Salm-Dyck) Haw., *Gasteria picta* Haw., *Gasteria planifolia* (Baker) Baker, *Gasteria retata* Haw., *Gasteria multiplex* Poelln., *Gasteria nigricans* var. *fasciata* (Salm-Dyck) Haw., *Gasteria picta* Haw., *Gasteria planifolia* (Baker) Baker, *Gasteria retata* Haw., *Gasteria salmdyckiana* Poelln., *Gasteria spiralis* Baker, *Gasteria spiralis* var. *tortulata* Baker, *Gasteria variolosa* Baker, *Gasteria zeyheri* (Salm-Dyck) Baker						
Gasteria pillansii Kensit var. *ernesti-ruschii* (Dinter and Poelln.) van Jaarsv.	*Aloe* 29: 17 (1992)	*Gasteria ernesti-ruschii* Dinter and Poelln.	Northern Cape, Namibia	*OM2779* (JRAU)	—	JQ025285	JQ024570	JQ024197
Gasteria pillansii Kensit var. *pillansii*	Unknown	*Gasteria neliana* Poelln.	Northern Cape	*OM2781* (JRAU)	JQ024874	JQ025284	JQ024553	JQ024180
Gasteria rawlinsonii Oberm.	*Fl. Pl. Africa* 43: t. 1701 (1976)	—	Eastern Cape	*OM2775* (JRAU)	JQ024889	JQ025288	JQ024573	JQ024200
Section Longiflorae Haw.								
Gasteria acinacifolia (J.Jacq.) Haw.	*Suppl. Pl. Succ.* 49 (1819)	*Aloe acinacifolia* J.Jacq., *Aloe acinacifolia* var. *minor* Salm-Dyck, *Aloe candicans* (Haw.) Schult. and Schult.f., *Aloe ensifolia* (Haw.) Schult. and Schult.f., *Aloe nitens* Schult. and Schult.f., *Aloe pluripunctata* Schult. and Schult.f., *Aloe venusta* Schult. and Schult.f., *Gasteria acinacifolia* var. *ensifolia* (Haw.) Baker, *Gasteria acinacifolia* var. *nitens* (Haw.) Baker, *Gasteria acinacifolia* var. *pluripunctata* (Haw.) Baker, *Gasteria acinacifolia* var. *venusta* (Haw.) Baker, *Gasteria candicans* Haw., *Gasteria ensifolia* Haw., *Gasteria fuscopunctata* Baker, *Gasteria huttoniae* N.E.Br., *Gasteria inexpectata* Poelln., *Gasteria linita* Haw., *Gasteria lutzii* Poelln., *Gasteria nitens* Haw., *Gasteria pluripunctata* Haw., *Gasteria venusta* Haw.	Eastern Cape	*OM2790* (JRAU)	JQ024875	JQ025271	JQ024554	JQ024181
Gasteria batesiana var. *dolomitica* van Jaarsv. and Van Wyk	*Aloe* 36(4) 74 (1999)	—	Mpumalanga	*OM2777* (JRAU)	JQ024876	JQ025273	JQ024555	JQ024182

Gasteria carinata (Mill.) Duval var. *carinata*	Unknown	*Aloe angulata* Willd., *Aloe angulata* var. *truncata* Willd., *Aloe carinata* Ker Gawl., *Aloe carinata* var. *subglabra* Haw., *Aloe excavata* Willd., *Aloe glabra* (Haw.) Salm-Dyck, *Aloe laetepunctata* (Haw.) Schult. and Schult.f., *Aloe laevis* Salm-Dyck, *Aloe lingua* var. *angulata* Haw., *Aloe lingua* var. *multifaria* Haw., *Aloe linguiformis* DC. [nom. illegit.], *Aloe pseudoangulata* Salm-Dyck, *Aloe pusilla* Schult. and Schult.f., *Aloe subcarinata* Salm-Dyck, *Aloe sulcata* Salm-Dyck, *Aloe tristicha* Medik., *Aloe undata* Schult. and Schult.f., *Gasteria angulata* (Haw.) Duval, *Gasteria angulata* (Willd.) Haw. [nom. illegit.], *Gasteria angulata* var. *truncata* (Willd.) A.Berger, *Gasteria bijliae* Poelln., *Gasteria carinata* var. *falcata* A.Berger, *Gasteria carinata* var. *glabra* (Salm-Dyck) van Jaarsv., *Gasteria carinata* var. *latifolia* A.Berger, *Gasteria carinata* var. *parva* (Haw.) Baker, *Gasteria carinata* var. *strigata* (Haw.) Baker, *Gasteria disticha* var. *angulata* (Willd.) Baker, *Gasteria excavata* (Willd.) Haw., *Gasteria glabra* Haw., *Gasteria humilis* Poelln., *Gasteria laetepunctata* Haw., *Gasteria laevis* (Salm-Dyck) Haw., *Gasteria pallescens* Baker, *Gasteria parva* Haw., *Gasteria parvifolia* Baker, *Gasteria patentissima* Poelln., *Gasteria porphyrophylla* Baker, *Gasteria schweickerdtiana* Poelln., *Gasteria strigata* Haw., *Gasteria subcarinata* (Salm-Dyck) Haw., *Gasteria sulcata* (Salm-Dyck) Haw., *Gasteria trigona* var. *kewensis* A.Berger, *Gasteria undata* Haw.	Western Cape	*OM2780* (JRAU)	JQ039297	JQ025275	—	—
Gasteria carinata var. *retusa* van Jaarsv.	*Aloe* 29: 15 (1992)	*Gasteria retusa* (van Jaarsv.) van Jaarsv.	Western Cape	*OM2798* (JRAU)	JQ024877	JQ025276	JQ024556	JQ024183
Gasteria croucheri (Hook.f.) Baker	*J. Linn. Soc., Bot.* 18: 196 (1880)	*Aloe croucheri* Hook.f., *Gasteria disticha* var. *natalensis* Baker	Eastern Cape, Kwazulu-Natal	*OM2773* (JRAU) *OM2791* (JRAU)	JQ024878	JQ025277	JQ024559 JQ024558	JQ024186
Gasteria ellaphieae van Jaarsv.	*Cact. Succ. J. (Los Angeles)* 63: 3 (1991)	—	Eastern Cape	*OM2782* (JRAU)	JQ024881	JQ025280	JQ024562	JQ024189
Gasteria excelsa Baker	*J. Linn. Soc., Bot.* 18: 195 (1880)	—	Eastern Cape	*OM2789* (JRAU)	JQ024882	JQ025281	JQ024564	JQ024191
Gasteria glauca van Jaarsv.	*Cact. Succ. J. (Los Angeles)* 70: 65 (1998)	—	Eastern Cape	*OM2771* (JRAU)	JQ024883	JQ025282	JQ024565	JQ024192
Gasteria nitida var. *armstrongii* (Schönland) van Jaarsv.	*Aloe* 29: 12 (1992)	*Gasteria armstrongii* Schönland, *Gasteria armstrongii* Schoenland	Eastern Cape	*OM2792* (JRAU)	JQ024885	JQ025272	JQ024567	JQ024194
Gasteria polita van Jaarsv.**(TYPE)**	*Cact. Succ. J. (Los Angeles)* 73: 127 (2001)	—	Western Cape	*OM2784* (JRAU)	JQ024887	JQ025286	JQ024571	JQ024198
Gasteria pulchra (Aiton) Haw.	*Syn. Pl. Succ.* 86 (1812)	*Aloe maculata* var. *pulchra* Aiton, *Aloe obliqua* DC., *Aloe pulchra* (Aiton) Jacq., *Gasteria poellnitziana* H.Jacobsen [nom. inva.]	Eastern Cape	*OM2785* (JRAU)	JQ024888	JQ025287	JQ024572	JQ024199
Gasteria tukhelensis van Jaarsv.	*Bothalia* 35: 164 (2005)	—	Kwazulu-Natal	*OM2774* (JRAU)	JQ024890	JQ025289	JQ024574	JQ024201
Gasteria vlokii van Jaarsv.	*Cact. Succ. J. (Los Angeles)* 59: 170 (1987)	—	Western Cape	*OM2786* (JRAU)	JQ039298	JQ025298	JQ024575	JQ024202
Haworthia* Duval subgenus *Haworthia								

Haworthia angustifolia Haw.	*Philos. Mag. J.* 67:283 (1825)	*Catevala angustifolia* (Haw.) Kuntze, *Haworthia chloracantha* var. *angustifolia* (Haw.) Halda, *Haworthia chloracantha* subsp. *angustifolia* (Haw.) Halda	Eastern Cape	*BHD234* (JRAU)	JQ039299	—	JQ024593	JQ024219
Haworthia arachnoidea (L.) Duval **(TYPE)**	*Pl. Succ. Horto Alencon.* 7 (1809)	*Aloe arachnoidea* (L.) Burm.f., *Aloe pumila* var. *arachnoidea* L., *Apicra arachnoides* (L.) Willd., *Catevala arachnoidea* (L.) Medik., *Haworthia pallida* var. *paynei* L. Bolus.	Western Cape	*BHD029* (JRAU) *BHD030* (JRAU)	JQ024891 JQ024892	—	JQ024601 JQ024602	JQ024226
Haworthia bayeri J.D.Venter and S.A.Hammer	*Cact. Succ. J. (Los Angeles)* 69: 75 (1997)	—	Eastern and Western Cape	*BHD110* (JRAU)	JQ039301	JQ025360	JQ024615	JQ024239
Haworthia blackburniae W.F.Barker	*J. S. African Bot*. 3: 93 (1937)	*Haworthia blackburniae* Poelln. [nom. illegit.]	Western Cape	*BHD198* (JRAU)	JQ024893	JQ025226	JQ024616	JQ024240
Haworthia blackburniae W.F.Barker var. *derustensis* M.B.Bayer	*Haworthia Revisited* 41 (1999)	*Haworthia derustensis* (M.B.Bayer) M.Hayashi	Western Cape	*BHD243* (JRAU)	JQ039302	JQ025361	JQ024617	JQ024241
Haworthia blackburniae W.F.Barker var. *graminifolia* (G.G.Sm.) M.B.Bayer	*Haworthia Revisited* 42 (1999)	*Haworthia blackburniae* subsp. *graminifolia* (G.G.Sm.) Halda, *Haworthia graminifolia* G.G.Sm.	Western Cape	*BHD201* (JRAU)	JQ039303	JQ025362	JQ024618	JQ024242
Haworthia chloracantha Haw.	*Saxifrag. Enum.* 2: 57 (1821)	*Aloe chloracantha* (Haw.) Schult. and Schult.f., *Catevala chloracantha* (Haw.) Kuntze	Western Cape	*BHD075* (JRAU)	JQ039305	JQ025363	JQ024625	JQ024249
Haworthia cooperi Baker	*Refug. Bot.* 4: t. 233 (1870)	*Catevala cooperi* (Baker) Kuntze	Eastern Cape	*BHD133* (JRAU)	JQ024895	JQ025227	JQ024631	JQ024255
Haworthia cooperi Baker var. *doldii* M.B.Bayer	*Haworthiad* 16: 65 (2002)	*Haworthia doldii* (M.B.Bayer) M.Hayashi	Eastern Cape	*BHD209* (JRAU)	JQ024896	JQ025228	JQ024634	JQ024258
Haworthia cymbiformis (Haw.) Duval	*Pl. Succ. Horto Alencon.* 7 (1809)	*Aloe cymbiformis* Haw., *Catevala cymbiformis* (Haw.) Kuntze, *Haworthia planifolia*	Eastern Cape	*BHD322* (JRAU)	JQ024898	JQ025229	JQ024645	JQ024269
Haworthia cymbiformis (Haw.) Duval var. *ramosa* (G.G.Sm.) M.B.Bayer	*Haworthia Revisited* 60 (1999)	*Haworthia cymbiformis* f. *ramosa* (G.G.Sm.) M.B.Bayer, *Haworthia ramosa* G.G.Sm.	Eastern Cape	*BHD321* (JRAU)	JQ024897	JQ025231	JQ024644	JQ024268
Haworthia cymbiformis (Haw.) Duval var. *setulifera* (Poelln.) M.B.Bayer	*Haworthia Revisited* 62 (1999)	*Haworthia cymbiformis* f. *obesa* (Poelln.) Pilbeam, *Haworthia cymbiformis* var. *obesa* Poelln., *Haworthia planifolia* var. *setulifera* Poelln.	Eastern Cape	*BHD320* (JRAU) *BHD325* (JRAU)	JQ024899 JQ024900	JQ025230 JQ025232	JQ024648 JQ024649	JQ024272 JQ024273
Haworthia decipiens Poelln.	*Repert. Spec. Nov. Regni Veg.* 28: 103 (1930)	—	Eastern and Western Cape	*BHD132* (JRAU)	JQ024901	JQ025233	JQ024652	JQ024276
Haworthia decipiens var. *pringlei* (C.L.Scott) M.B.Bayer	*Haworthia Revisited* 67(1999)	*Haworthia bolusii* var. *pringlei* (C.L. Scott) M.B.Bayer, *Haworthia pringlei* C.L.Scott	Eastern Cape	*BHD196* (JRAU)	JQ024902	JQ025250	JQ024654	JQ024278
Haworthia decipiens Poelln. var. *virella* M.B.Bayer	*Haworthiad* 16: 63 (2002)	*Haworthia virella* (M.B.Bayer) M.Hayashi		*BHD328* (JRAU)	JQ024903	JQ025234	JQ024656	JQ024280
Haworthia emelyae Poelln.	*Repert. Spec. Nov. Regni Veg.* 42: 271 (1937)	*Haworthia retusa* subsp. *emelyae* (Poelln.) Halda, *Haworthia retusa* var. *emelyae* (Poelln.) Halda	Western Cape	*BHD105* (JRAU)	JQ024904	JQ025236	JQ024661	JQ024285
Haworthia emelyae Poelln. var. *comptoniana* (G.G.Sm.) J.D.Venter and S.A.Hammer	*Cact. Succ. J. (Los Angeles)* 69: 77 (1997)	*Haworthia comptoniana* G.G.Sm., *Haworthia retusa* var. *comptoniana* (G.G.Sm.) Halda	Eastern Cape	*BHD220* (JRAU)	JQ039307	JQ025364	JQ024663	JQ024287
Haworthia floribunda Poelln.	*Repert. Spec. Nov. Regni Veg.* 40: 149 (1936)	*Haworthia chloracantha* var. *floribunda* (Poelln.) Halda	Western Cape	*BHD077* (JRAU)	JQ024906	JQ025251	JQ024666	JQ024290
Haworthia herbacea (Mill.) Stearn	*Cact. Succ. J. Gr. Brit.* 7: 40 (1938)	*Aloe herbacea* Mill.	Western Cape	*BHD237* (JRAU)	—	—	JQ024685	JQ024307
Haworthia herbacea (Mill.) Stearn var. *flaccida* M.B.Bayer	*Haworthia Revisited* 86 (1999)	*Haworthia pallida* var. *flaccida* (M.B.Bayer) M.Hayashi	Western Cape	*BHD333* (JRAU)	JQ024907	JQ025252	JQ024686	JQ024308
Haworthia herbacea (Mill.) Stearn var. *paynei* (Poelln.)	*Haworthia Revisited* 87 (1999)	*Haworthia pallida* var. *paynei* (Poelln.) Poelln., *Haworthia paynei* Poelln.	Western Cape	*BHD373* (JRAU)	JQ024908	JQ025254	JQ024687	JQ024309

M.B.Bayer								
Haworthia lockwoodii Archibald	*Fl. Pl. South Africa* 20: t. 792 (1940)	*Haworthia mucronata* subsp. *lockwoodii* (Archibald) Halda	Western Cape	*BHD213* (JRAU)	—	JQ025378	JQ024711	JQ024336
Haworthia maculata (Poelln.) M.B.Bayer	*Haworthia Handb.* 130 (1976)	*Haworthia intermedia* var. *maculata* (Poelln.) Esterhuizen, *Haworthia schuldtiana* var. *maculata* Poelln.	Western Cape	*BHD203* (JRAU)	JQ024911	JQ025237	JQ024715	JQ024340
Haworthia magnifica Poelln. var. *dekenahii* (G.G.Sm.) M.B.Bayer	*Aloe* 34: 6 (1997)	*Haworthia dekenahii* G.G.Sm., *Haworthia retusa* var. *dekenahii* (G.G.Sm.) M.B.Bayer	Western Cape	*BHD092* (JRAU)	JQ024912	JQ025238	JQ024716	JQ024341
Haworthia marumiana Uitewaal var. *archeri* (W.F.Barker ex M.B.Bayer) M.B.Bayer	*Haworthia Revisited* 104 (1999)	*Haworthia archeri* W.F.Barker ex M.B.Bayer	Northern and Western Cape	*BHD235* (JRAU)	JQ024913	JQ025248	JQ024727	JQ024352
Haworthia marxii Gildenh.	*Aloe* 44: 4 (2007)	—	Western Cape	*BHD339* (JRAU)	JQ024914	JQ025249	JQ024728	JQ024353
Haworthia mirabilis (Haw.) Haw.	*Syn. Pl. Succ.* 95 (1812)	*Aloe mirabilis* Haw., *Apicra mirabilis* (Haw.) Willd., *Catevala mirabilis* (Haw.) Kuntze, *Haworthia beukmanii*, *Haworthia retusa var. mirabilis* (Haw.) Halda, *Haworthia willowmorensis Poelln.*	Western Cape	*BHD032* (JRAU)	JQ039317	JQ025365	JQ024650	JQ024274
Haworthia mirabilis (Haw.) Haw. var. *paradoxa* (Poelln.) M.B.Bayer	*Aloe* 34: 6 (1997)	*Haworthia magnifica* var. *paradoxa* (Poelln.) M.B.Bayer, *Haworthia paradoxa* Poelln.	Western Cape	*BHD044* (JRAU)	JQ024915	JQ025254	JQ024749	JQ024373
Haworthia mirabilis var. *calcarea* M.B.Bayer	*Haworthia Revisited* 110 (1999)	*Haworthia calcarea* (M.B.Bayer) M.Hayashi, *Haworthia rossouwii* var. *calcarea* (M.B.Bayer) M.B.Bayer	Western Cape	*BHD232* (JRAU)	JQ024916	JQ025246	JQ024771	JQ024397
Haworthia mirabilis var. *triebneriana* (Poelln.) M.B.Bayer	*Haworthia Revisited* 113 (1999)	*Haworthia mirabilis* f. *rubrodentata* (Triebner and Poelln.) Pilbeam, *Haworthia nitidula* Poelln., *Haworthia rossouwii* Poelln., *Haworthia triebneriana* Poelln., *Haworthia triebneriana* var. *depauperata* Poelln., *Haworthia triebneriana* var. *multituberculata* Poelln., *Haworthia triebneriana* var. *napierensis* Triebner and Poelln., *Haworthia triebneriana* var. *pulchra* Poelln., *Haworthia triebneriana* var. *rubrodentata* Triebner and Poelln., *Haworthia triebneriana* var. *subtuberculata* Poelln., *Haworthia triebneriana* var. *turgida* Triebner, *Haworthia willowmorensis* Poelln.	Western Cape	*BHD020* (JRAU)	JQ024917	—	JQ024773	JQ024399
Haworthia monticola Fourc. var. *asema* M.B.Bayer	*Haworthia Revisited* 117 (1999)	*Haworthia asema* (M.B.Bayer) M.Hayashi	Western Cape	*BHD251* (JRAU)	JQ024918	JQ025255	JQ024780	JQ024405
Haworthia mucronata Haw. var. *habdomadis* (Poelln.) M.B.Bayer	*Haworthia Revisited* 120 (1999)	*Haworthia habdomadis* Poelln., *Haworthia inconfluens* var. *habdomadis* (Poelln.) M.B.Bayer	Western Cape	*BHD347* (JRAU)	—	JQ025379	JQ024784	JQ024409
Haworthia mucronata Haw. var. *morrisiae* (Poelln.) M.B.Bayer	*Repert. Spec. Nov. Regni Veg.* 49: 29 (1940)	*Haworthia altilinea* var. *morrisiae* Poelln., *Haworthia altilinea* f. *subglauca* Poelln., *Haworthia habdomadis* var. *morrisiae* (Poelln.) M.B.Bayer, *Haworthia inconfluens* var. *morrisiae* (Poelln.) M.B.Bayer, *Haworthia mucronata* f. *subglauca* (Poelln.) Poelln., *Haworthia sakaii* M.Hayashi	Western Cape	*BHD349* (JRAU)	JQ024920	JQ025240	JQ024785	JQ024410

Haworthia mucronata var. *bijliana* (Poelln.) ined.	Unknown	*Haworthia altilinea* f. *inconfluens* Poelln., *Haworthia bijliana* Poelln., *Haworthia bijliana* var. *joubertii* Poelln., *Haworthia fergusoniae* Poelln., *Haworthia habdomadis* var. *inconfluens* (Poelln.) M.B.Bayer, *Haworthia inconfluens* (Poelln.) M.B.Bayer, *Haworthia mucronata* var. *inconfluens* (Poelln.) M.B.Bayer, *Haworthia mucronata* f. *inconfluens* (Poelln.) Poelln.), *Haworthia setata* var. *bijliana* (Poelln.) Poelln.	Western Cape	*BHD350* (JRAU)	JQ024919	JQ025239	JQ024782	JQ024407
Haworthia mucronata var. *rycroftiana* (M. B. Bayer) M.B. Bayer	*Haworthia Revisited* 124 (1999)	*Haworthia rycroftiana* M.B.Bayer	Western Cape	*BHD345* (JRAU)	JQ024921	JQ025241	JQ024787	JQ024412
Haworthia mutica Haw.	*Saxifrag. Enum.* 2: 55 (1821)	*Aloe mutica* (Haw.) Schult. and Schult.f., *Haworthia retusa* var. *mutica* (Haw.) Halda	Western Cape	*BHD102* (JRAU)	JQ024922	JQ025242	JQ024797	JQ024421
Haworthia mutica Haw. var. *nigra* M.B.Bayer	*Haworthia Revisited* 126 (1999)	*Haworthia silviae* var. *nigra* (M.B.Bayer) M.Hayashi	Western Cape	*BHD072* (JRAU)	JQ024923	JQ025243	JQ024798	JQ024422
Haworthia outeniquensis M.B.Bayer	*Haworthia Revisited* 130 (1999)	—	Western Cape	*BHD356* (JRAU)	JQ024924	JQ025256	JQ024807	JQ024431
Haworthia pulchella M.B.Bayer var. *globifera* M.B.Bayer	*Haworthia Revisited* 136 (1999)	*Haworthia globifera* (M.B.Bayer) M.Hayashi	Western Cape	*BHD206* (JRAU)	JQ024925	JQ025257	JQ024813	JQ024437
Haworthia pygmaea Poelln.	*Repert. Spec. Nov. Regni Veg.* 27: 132 (1929)	—	Western Cape	*BHD358* (JRAU)	JQ039320	JQ025333	JQ024816	JQ024440
Haworthia reticulata (Haw.) Haw.	*Syn. Pl. Succ.* 94 (1812)	*Aloe reticulata* Haw., *Apicra reticulata* (Haw.) Willd., *Catevala reticulata* (Haw.) Kuntze, *Haworthia haageana*	Western Cape	*BHD117* (JRAU)	JQ024927	JQ025244	JQ024819	JQ024443
Haworthia retusa (L.) Duval	*Pl. Succ. Horto Alencon.* 7 (1809)	*Aloe retusa* L., *Apicra retusa* (L.) Willd., *Catevala retusa* (L.) Medik		*BHD027* (JRAU) *BHD120* (JRAU)	JQ024928	JQ025245	JQ024832	JQ024456 JQ024455
Haworthia semiviva (Poelln.) M.B.Bayer	*Haworthia Handb.* 153 (1976)	*Haworthia arachnoideae* var. *semiviva* (Poelln.) Halda, *Haworthia bolusii* var. *semiviva* Poelln.	Northern and Western Cape	*BHD360* (JRAU)	JQ024929	JQ025247	JQ024844	JQ024467
Haworthia springbokvlakensis C.L.Scott	*J. S. African Bot.* 36: 288 (1970)	*Haworthia retusa* var. *springbokvlakensis* (C.L.Scott) Halda	Eastern Cape	*BHD362* (JRAU)	—	—	JQ024847	JQ024470
Haworthia truncata Schönland	*Trans. Roy. Soc. South Africa* 1: 291 (1910)	—	Western Cape	*BHD210* (JRAU)	JQ039323	JQ025375	JQ024848	JQ024471
Haworthia variegata L.Bolus	*J. Bot.* 67: 137 (1929)	*Haworthia chloracantha* var. *variegata* (L.Bolus) Halda, *Haworthia chloracantha* subsp. *variegata* (L.Bolus) Halda	Western Cape	*BHD367* (JRAU)	JQ039324	JQ025376	JQ024850	JQ024473
Haworthia vlokii M.B.Bayer	*Haworthia Revisited* 160 (1999)	—	Western Cape	*BHD249* (JRAU)	JQ024930	JQ025258	JQ024858	JQ024481
Haworthia wittebergensis W.F.Barker	*J. S. African Bot.* 8: 245 (1942)	—	Western Cape	*BHD200* (JRAU)	JQ024931	JQ025259	JQ024859	JQ024482
Haworthia zantneriana Poelln.	*Repert. Spec. Nov. Regni Veg.* 41: 217 (1937)	*Haworthia chloracantha* var. *zantheriana* (Poelln.) Halda	Eastern Cape	*BHD230* (JRAU)	JQ039326	JQ025370	JQ024860	JQ024483
***Haworthia* subgenus *Hexangulares* Uitewaal ex M.B.Bayer**								
Haworthia attenuata Haw.	*Syn. Pl. Succ. 92 (1812)*	*Aloe attenuata* Haw., *Apicra attenuata* (Haw.) Willd., *Catevala attenuata* (Haw.) Kuntze, *Haworthia attenuata* var. *britteniae* Poelln., *Haworthia attenuata* var. *clariperla* (Haw.) M.B.Bayer, *Haworthia pumila* subsp. *attenuata* (Haw.) Halda	Eastern Cape	*BHD253* (JRAU) *BHD264* (JRAU) *BHD265* (JRAU)	JQ039300	JQ025311	JQ024610 JQ024609 JQ024608	JQ024234 JQ024233 JQ024232
Haworthia bruynsii M.B.Bayer	*J. S. African Bot.* 47: 789 (1981)	*Haworthia retusa* var. *bruynsii* (M.B.Bayer) Halda	Eastern Cape	*BHD374* (JRAU)	JQ039304	JQ025334	JQ024622	JQ024246

Haworthia coarctata Haw.	*Philos. Mag. J.* 66: 301 (1824)	*Aloe coarctata* (Haw.) Schult. and Schult.f., *Catevala coarctata* (Haw.) Kuntze, *Haworthia reinwardtii* var. *coarctata* (Haw.) Halda, *Haworthia reinwardtii* subsp. *coarctata* (Haw.) Halda	Eastern Cape	*BHD327* (JRAU)	JQ024894	JQ025296	JQ024629	JQ024253
Haworthia coarctata var. *adelaidensis* (Poelln.) M.B.Bayer	*Haworthia Revisited* 172 (1999)	*Haworthia coarctata* subsp. *adelaidensis* (Poelln.) M.B.Bayer, *Haworthia coarctata* f. *bellula* (G.G.Sm.) Pilbeam, *Haworthia reinwardtii* var. *adelaidensis* Poelln., *Haworthia reinwardtii* var. *bellula* G.G.Sm., *Haworthia reinwardtii* var. *riebeekensis* G.G.Sm.	Eastern Cape	*BHD326* (JRAU)	JQ039306	JQ025335	JQ024630	JQ024254
Haworthia fasciata (Willd.) Haw.	*Saxifrag. Enum.* 2: 54 (1821)	*Aloe fasciata* (Willd.) Salm-Dyck ex Schult. and Schult.f., *Aloe fasciata* var. *major* Salm-Dyck, *Aloe subfasciata* Salm-Dyck ex Schult. and Schult.f., *Apicra fasciata* Willd., *Catevala fasciata* (Willd.) Kuntze, *Catevala subfasciata* (Salm-Dyck ex Schult. and Schult.f.) Kuntze, *Haworthia browniana* Poelln., *Haworthia fasciata* f. *browniana* (Poelln.) M.B.Bayer, *Haworthia fasciata* var. *major* (Salm-Dyck) Haw., *Haworthia fasciata* f. *ovatolanceolata* Poelln., *Haworthia fasciata* f. *sparsa* Poelln., *Haworthia fasciata* f. *subconfluens* (Poelln.) Poelln., *Haworthia fasciata* var. *subconfluens* Poelln., *Haworthia fasciata* f. *vanstaadensis* Poelln., *Haworthia fasciata* f. *variabilis* Poelln., *Haworthia pumila* subsp. *fasciata* (Willd.) Halda, *Haworthia subfasciata* (Salm-Dyck ex Schult. and Schult.f) Baker	Eastern Cape	*BHD330* (JRAU) *BHD331* (JRAU)	JQ024905	JQ025270	JQ024664 JQ024665	JQ024288 JQ024289
Haworthia glauca (Salm-Dyck) Baker	*J. Linn. Soc., Bot.* 18: 203 (1880)	*Catevala glauca* (Baker)Kuntze, *Haworthia reinwardtii* subsp. *glauca* (Baker) Halda, *Haworthia reinwardtii* var. *glauca* (Baker) Halda	Eastern Cape	*BHD061* (JRAU) *BHD062* (JRAU) *BHD370* (JRAU)	JQ039308	JQ025336	JQ024673 JQ024674 JQ024676	JQ024295 JQ024296 JQ024298
Haworthia koelmaniorum Oberm. and D.S.Hardy var. *mcmurtryi* (C.L.Scott) M.B.Bayer	*Haworthia Revisited* 181 (1999)	*Haworthia mcmurtryi* C.L.Scott	Mpumalanga	*BHD336* (JRAU) *BHD337* (JRAU)	JQ024909 JQ024910	JQ025293 JQ025294	JQ024690 JQ024689	JQ024312 JQ024311
Haworthia limifolia Marloth	*Trans. Roy. Soc. South Africa* 1: 409 (1910)	—	Kwazulu-Natal, Mpumalanga	*BHD135* (JRAU) *BHD159* (JRAU) *BHD173* (JRAU) *BHD175* (JRAU) *BHD183* (JRAU)	JQ039311 JQ039312	JQ025341 JQ025342	JQ024702 JQ024697 JQ024694 JQ024693 JQ024707	JQ024326 JQ024321 JQ024317 JQ024316 JQ024331
Haworthia limifolia Marloth var. *ubomboensis* (I.Verd.) G.G.Sm.	*J. S. African Bot.* 16: 3 (1950)	*Haworthia ubomboensis* I.Verd., *Haworthia ubomboensis* Verdoorn	Swaziland	*BHD221* (JRAU)	JQ039313	JQ025343	JQ024710	JQ024335
Haworthia longiana Poelln.	*Repert. Spec. Nov. Regni Veg.* 41: 203 (1937)	*Haworthia pumila* subsp. *longiana* (Poelln.) Halda	Eastern Cape	*BHD048* (JRAU) *BHD049* (JRAU)	JQ039314	JQ025316	JQ024714 JQ024712	JQ024339 JQ024337
Haworthia nigra (Haw.) Baker	*J. Linn. Soc., Bot.* 18: 203 (1880)	*Aloe nigra* (Haw.) Schult. and Schult.f., *Apicra nigra* Haw., *Catevala nigra* (Haw.) Kuntze	Eastern and Western Cape	*BHD224* (JRAU)	JQ039318	JQ025352	JQ024799	JQ024423
Haworthia reinwardtii (Salm-Dyck) Haw.	*Saxifrag. Enum.* 2: 53 (1821)	*Aloe reinwardtii* Salm-Dyck, *Catevala reinwardtii* (Salm-Dyck) Kuntze	Eastern Cape	*BHD254* (JRAU)	JQ039321	JQ025332	JQ024817	JQ024441
Haworthia reinwardtii (Salm-Dyck) Haw. var. *brevicula* G.G.Sm.	*J. S. African Bot.* 10: 11 (1944)	*Haworthia reinwardtii* var. *diminuta* G.G.Sm.	Eastern Cape	*BHD359* (JRAU)	JQ024926	JQ025295	JQ024818	JQ024442
Haworthia sordida Haw.	*Saxifrag. Enum.* 2: 51 (1821)	*Aloe sordida* (Haw.) Schult. and Schult.f., *Catevala sordida* (Haw.) Kuntze, *Haworthia scabra* subsp. *sordida* (Haw.) Halda, *Haworthia scabra* var. *sordida* (Haw.) Halda	Eastern Cape	*BHD205* (JRAU)	JQ039322	JQ025354	JQ024845	JQ024468

Haworthia venosa (Lam.) Haw.	*Saxifrag. Enum.* 2: 44 (1821)	*Aloe venosa* Lam., *Catevala venosa* (Lam.) Kuntze	Western Cape	*BHD246* (JRAU)	JQ039325	JQ025309	JQ024852	JQ024474
Haworthia venosa Haw. subsp. *granulata* (Marloth) M.B.Bayer	*Haworthia Handb.* 120 (1976)	*Haworthia granulata* Marloth, *Haworthia scabra* subsp. *granulata* (Marloth) Halda	Northern and Western Cape	*BHD088* (JRAU)	—	JQ025377	JQ024853	JQ024475
***Haworthia* subgenus *Robustipedunculares* M.B.Bayer**								
Haworthia kingiana Poelln.	*Repert. Spec. Nov. Regni Veg.* 41: 203 (1937)	*Haworthia pumila* var. *kingiana* (Poelln.) Halda, *Haworthia subfasciata* var. *kingiana* (Poelln.) Poelln.	Western Cape	*BHD335* (JRAU) *BHD435* (JRAU)	JQ039309 JQ039310	JQ025339 JQ025340	JQ024688	JQ024310
Haworthia marginata (Lam.) Stearn	*Cact. Succ. J. Gr. Brit.* 7: 39 (1938)	*Aloe albicans* Haw., *Aloe laevigata* Schult. and Schult.f., *Aloe marginata* Lam., *Aloe ramifera* Schult. and Schult.f., *Aloe virescens* (Haw.) Schult. and Schult.f., *Apicra albicans* (Haw.) Willd., *Catevala marginata* (Lam.) Kuntze, *Haworthia albicans* (Haw.) Haw., *Haworthia albicans* var. *virescens* (Haw.) Baker, *Haworthia laevis* Haw., *Haworthia marginata* var. *laevis* (Haw.) H.Jacobsen, *Haworthia marginata* var. *ramifera* (Haw.) H.Jacobsen, *Haworthia marginata* var. *virescens* (Haw.) Uitewaal, *Haworthia ramifera* Haw., *Haworthia virescens* Haw., *Haworthia virescens* var. *minor* Haw.	Western Cape	*BHD439* (JRAU) *BHD026* (JRAU)	JQ039316 JQ039315	JQ025337 JQ025338	JQ024719	JQ024344
Haworthia minor (Aiton) Duval	*Pl. Succ. Horto Alencon.* 7 (1809)	*Aloe brevis* Schult. and Schult.f., *Aloe erecta* (Haw.) Schult. and Schult.f., *Aloe erecta* var. *laetivirens* Salm-Dyck, *Aloe granata* Schult. and Schult.f., *Aloe margaritifera* var. *major* Aiton, *Aloe margaritifera* var. *minima* Aiton, *Aloe margaritifera* var. *minor* Aiton, *Aloe minor* (Aiton) Schult. and Schult.f., *Apicra granata* Willd., *Apicra maxima* (Haw.) Steud., *Apicra minor* (Aiton) Steud., *Catevala minima* (Aiton) Kuntze, *Haworthia brevis* Haw., *Haworthia erecta* Haw., *Haworthia granata* (Willd.) Haw., *Haworthia granata* var. *polyphylla* Haw., *Haworthia major* (Aiton) Duval, *Haworthia margaritifera* var. *corallina* Baker, *Haworthia margaritifera* var. *erecta* (Haw.) Baker, *Haworthia margaritifera* var. *granata* (Willd.) Baker, *Haworthia minima* (Aiton) Haw., *Haworthia minima* var. *poellnitziana* (Uitewaal) M.B.Bayer, *Haworthia mutabilis* Poelln., *Haworthia poellnitziana* Uitewaal, *Haworthia pumila* subsp. *minima* (Aiton) Halda, *Haworthia uitewaaliana* Poelln.	Western Cape	*BHD341* (JRAU)	—	—	JQ024733	JQ024358

Haworthia pumila (L.) Duval	*Pl. Succ. Horto Alencon.* 7 (1809)	*Aloe arachnoides* var. *pumila* (L.) Aiton, *Aloe granata* Salm-Dyck [nom. illegit.], *Aloe margaritifera* (L.) Burm.f., *Aloe margaritifera* var. *maxima* Haw., *Aloe papillosa* Salm-Dyck, *Aloe pumila* L., *Aloe pumila* var. *margaritifera* L., *Aloe semiglabrata* (Haw.) Schult. and Schult.f., *Aloe semiglabrata* Salm-Dyck, *Aloe semiglabrata* var. *major* Salm-Dyck, *Aloe semiglabrata* var. *maxima* (Haw.) Salm-Dyck, *Aloe semiglabrata* var. *minor* Salm-Dyck, *Aloe semiglabrata* var. *multipapillosa* Salm-Dyck, *Aloe subalbicans* Salm-Dyck, *Aloe subalbicans* var. *acuminata* Salm-Dyck, *Aloe subalbicans* var. *laevior* Salm-Dyck, *Apicra margaritifera* (L.) Willd., *Catevala margaritifera* (L.) Kuntze, *Catevala semiglabrata* (Haw.) Kuntze, *Haworthia corallina* Baker, *Haworthia margaritifera* (L.) Haw., *Haworthia margaritifera* var. *laevior* (Salm-Dyck) Uitewaal, *Haworthia margaritifera* var. *semimargaritifera* (Salm-Dyck) Baker, *Haworthia margaritifera* var. *subalbicans* (Salm-Dyck) A.Berger, *Haworthia maxima* (Haw.) Duval, *Haworthia semimargaritifera* var. *major* (Salm-Dyck) Haw., *Haworthia semimargaritifera* var. *maxima* (Haw.) Haw., *Haworthia semimargaritifera* var. *multiperla* Haw., *Tulista margaritifera* (L.) Raf.	Western Cape	*BHD222* (JRAU)	JQ039319	JQ025353	JQ024815	JQ024439
OUTGROUP TAXA								
Asparagaceae								
Anthericum liliago L.	*Sp. Pl.* 310 (1753)	*Anthericum amoenum* Salisb. [nom. illegit.], *Anthericum intermedium* Willk. [nom. inva.], *Anthericum liliago* var. *australe* Willk., *Anthericum liliago* subsp. *macrocarpum* Boros, *Anthericum liliago* f. *macrocarpum* (Boros) Soó, *Anthericum liliago* var. *multiflorum* P.Küpfer, *Anthericum liliago* var. *sphaerocarpum* P.Küpfer, *Anthericum macrocarpum* Boros, *Anthericum non-ramosum* Gilib. [nom. inva.], *Liliago vulgaris* C.Presl., *Ornithogalum gramineum* Lam. [nom. illegit.], *Phalangites liliago* (L.) Bubani, *Phalangites acuminatum* Dulac, *Phalangites lilaceum* St.-Lag., *Phalangites liliaginoides* Schltdl., *Phalangites liliago* (L.) Schreb., *Phalangites renarnii* Booth ex Schltdl.	Europe, Turkey	*Chase515* (K)	—	—	—	—
Tecophilaeaceae								
Tecophilaea cyanocrocus Leyb.	*Bonplandia (Hannover)* 10: 370 (1862)	*Tecophilaea cyanocrocus* var. *leichtlinii* Regel, *Tecophilaea cyanocrocus* var. *regelii* Baker, *Tecophilaea cyanocrocus* var. *violacea*, *Zephyra cyanocrocus* (Leyb.) Ravenna	Chile	*Chase447* (K)	—	—	—	—
Zephyra elegans D.Don	*Edinburgh New Philos. J.* 1832(Oct.): 236 (1832)	*Dicolus caerulescens* Phil., *Zephyra amoena* Miers	Chile	*Chase1575* (K)	—	—	—	—
Xanthorrhoeaceae								
Asphodeline lutea (L.) Reichb.	*Fl. Germ. Excurs.* 116 (1830)	*Asphodelus luteus* L., *Asphodelus sibiricus* Schult. and Schult.f. [nom. illegit.], *Dorydium luteum* (L.) Salisb. [nom. inva.]	South-eastern Europe to Turkey	*UCI Arb.3440*	JQ039289	—	—	—

Bulbine frutescens (L.) Willd.	*Enum. Pl.* 372 (1809)	*Anthericum frutescens* L., *Anthericum fruticosum* Salisb. [nom. illegit.], *Anthericum incurvum* Thunb., *Anthericum multiceps* Poelln., *Anthericum rostratum* Jacq., *Bulbine caulescens* L., *Bulbine frutescens* var. *incurva* (Thunb.) Rowley, *Bulbine frutescens* var. *rostrata* (Jacq.) Rowley, *Bulbine incurva* (Thunb.) Spreng., *Bulbine rostrata* (Jacq.) Willd., *Phalangium frutescens* (L.) Kuntze, *Phalangium rostratum* (Jacq.) Kuntze	Eastern, Northern and Western Cape, Free State, Gauteng, Kwazulu-Natal, Limpopo, Namibia, Swaziland	*Van Wyk4115* (JRAU)	JQ039294	—	AJ512323.1[2]	AJ511414.1[2]
Bulbine semibarbata (R.Br.) Haw.	*Saxifrag. Enum.* 2: 33 (1821)	*Amaryllis semibarbata* (R.Br) Steud., *Anthericum semibarbatum* R.Br., *Bulbine floribunda* Schrad. ex Benth., *Bulbine semibarbata* f. *gracilescens* Domin, *Bulbinopsis semibarbata* (R.Br.) Borzì, *Phalangium semibarbatum* (R.Br.) Kuntze, *Triglochin racemosa* Endl.	Australia, except Northern Territory	*K.Dixon s.n.* (KPBG)	—	—	—	—
Eremurus spectabilis M.Bieb.	*Cent. Pl. Ross. Merid.* 2 (1810)	*Ashodelus regius* Heynh., *Eremurus bachtiaricus* Boiss., *Eremurus caucasicus* Steven, *Eremurus libanoticus* Boiss., *Eremurus sibiricus* Weinm., *Eremurus tauricus* Weinm.	Israel, Lebanon, North Caucasus, South European Russia, Transcaucasia, Armenia, Georgia, Asiatic Turkey, Ukraine	*Chase490* (K)	JQ039296	—	—	—
Jodrellia fistulosa (Chiov.) Baijnath	*Kew Bull.* 32: 576 (1978)	*Bulbine breviracemosa* Poelln., *Bulbine fistulosa* Chiov.	Eritrea	*Chase3941* (K)	JQ039327	—	—	—
Kniphofia galpinii Baker	*Fl. Cap.* 6: 281 (1896)	—	Kwazulu-Natal, Mpumalanga, Swaziland	*IPMB040340* (HEID)	—	—	AJ512329.1[2]	AJ511423.1[2]
Kniphofia uvaria (L.) Oken	*Allg. Naturgesch.* 3(1): 566 (1841)	*Aletris uvaria* (L.) L., *Aloe longifolia* Lam., *Aloe rigida* Salisb. [nom. illegit.], *Aloe uvaria* L., *Kniphofia aloöides* Moench, *Kniphofia bachmannii* Baker, *Kniphofia burchellii* (Sweet ex Lindl.) Kunth, *Kniphofia occidentalis* A.Berger, *Kniphofia odorata* Heynh. [nom. illegit.], *Kniphofia uvaria* (L.) Hook., *Kniphofia uvaria* var. *glaucescens* G.Nicholson, *Kniphofia uvaria* var. *nobilis* (Guillon) Baker, *Kniphofia uvaria* var. *serotina* Baker, *Triclissa uvaria* (L.) Salisb. [nom. illegit.], *Tritoma burchellii* Sweet ex Lindl., *Tritoma canari* Carrière, *Tritoma glauca* H.Vilm., *Tritoma nobilis* Guillon,*Tritoma recurva* H.Vilm., *Tritoma saundersii* Carrière, *Tritoma uvaria* (L.) Ker Gawl., *Tritomanthe uvaria* (L.) Link, *Tritomium uvaria* (L.) Link, *Veltheimia speciosa* Roth, *Veltheimia uvaria* (L.) Willd.	Northern and Western Cape	*IPMB040342* (HEID)	—	—	AJ512330.1[2]	AJ511425.1[2]
Xanthorrhoea resinosa Pers.	*Syn. Pl.* 1: 370 (1805)	*Acoroides resinifera* Sol. ex Kite [nom. illegit.], *Xanthorrhoea hastilis* R.Br., *Xanthorrhoea hastilis* Sm. [nom. illegit.], *Xanthorrhoea resinifera* (Sol. ex Kite) E.C.Nelson and D.J.Bedford [nom. illegit.]	Australia	*Chase192* (NCU)	—	—	HM640546.1[3]	HM640663[3]
Xanthorrhoea sp. (stemless)	—	—	Western Australia	*Brummitt21391*	JQ039328	—	—	—
Xanthorrhoea sp. (with.trunk)	—	—	Western Australia	*Brummitt, George and Oliver21376*	JQ039329	—	—	—

Table 2.2 Gene regions and primers used for amplifying the cpDNA and nrDNA in the current study.

Region	**Size (bp)**	**Primer sets (Sequence 5'-3')**	**Reference**
cpDNA regions			
trnH-psbA	812	trnH Rev: CGC GCA TGG TGG ATT CAC AAT CC psbA Fwd: GTT ATG CAT GAA CGT AAT GCT C	Sang *et al*., 1997
matK	945	3F KIM: CGT ACA GTA CTT TTG TGT TTA CGA G 1R KIM: ACC CAG TCC ATC TGG AAA TCT TGG TTC	CBOL Plant Working Group, 2009
rbcLa	552	rbcL-barcode-F: ATG TCA CCA CAA ACA GAG ACT AAA GC rbcL-barcode-R: GTA AAA TCA AGT CCA CCY CG	CBOL Plant Working Group, 2009
Nuclear region			
ITS1	482	ITS 18-F: GTC CAC TGA ACC TTA TCA TTT AGA GG ITS S5: TTC GGG CGC AAC TTG CGT TC	Treutlein *et al*., 2003a, b

Table 2.3 Characters, character states, and explanatory notes on characters used in the cladistic analyses of the morphological data matrix.

1. **Habit**: stemless = 0; caulescent = 1; arborescent (tree-like, with leafless, dichotomously branched stems) = 2

2. **Leaf insertion**: spiral or polystichous = 0; distichous = 1

3. **Leaf margins**: smooth or entire = 0; toothed or bristled = 1

4. **Leaf maculation (spots):** immaculate = 0; maculate = 1

5. **Leaf tubercles:** concolorous or absent = 0; white = 1

6. **Leaf apex:** acute = 0; truncate = 1

7. **Inflorescence**: unbranched = 0; branched = 1

8. **Inflorescence**: raceme erect/decumbent and symmetrical = 0; raceme oblique and secund = 1

9. **Inflorescence**: flowers less than pedicel length apart or equal to = 0; flowers more than pedicel length apart = 1

10. **Inflorescence**: raceme unicoloured = 0; raceme bicoloured = 1

11. **Flower orientation at anthesis**: spreading = 0; pendulous = 1

12. **Flower arrangement**: raceme elongate = 0; raceme capitate = 1

13. **Flower shape**: rotate = 0; campanulate = 1; tubular = 2

14. **Perianth symmetry**: actinomorphic = 0; weakly bilabiate = 1; strongly bilabiate = 2

15. **Pedicel length**: ¼ or more longer than perianth = 0; less than ¼ length of the perianth = 1 (perhaps consider using 1/3)

16. **Perianth colour**: white/cream/greenish = 0; yellow/orange/red = 1

17. **Perianth tube**: straight = 0; curved upwards = 1

18. **Perianth**: cylindrical and tapering into pedicel = 0; inflated basally or truncated = 1

19. **Tepals**: both whorls connate basally = 0; both whorls connate ½ or more of length = 1; only outer whorls connate for half or more of length = 2

20. **Stamens**: as long as/longer than perianth = 0; shorter than perianth = 1

Table 2.4 Character states for the 20 morphological characters scored for accessions included in the molecular analyses

Taxon	**Morphological characters**																			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Aloe albida	0	0	1	0	0	0	0	0	1	0	1	1	2	2	1	0	1	0	0	0
Aloe alooides	2	0	1	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0	0
Aloe angelica	2	0	1	0	0	0	1	0	0/1	1	0/1	1	2	1	0/1	1	1	0	0	0
Aloe anivoranoensis	1	0	1	0	0	0	0	0	0	0	1	0	2	0	0	1	0	0	0	0
Aloe arborescens	2	0	1	0	0	0	0	0	1	0	1	0	2	0	1	1	0	0	0	0
Aloe arenicola	1	0	1	1	0	0	1	0	0	0	1	1	2	0	0	1	0	0	2	0
Aloe aristata	0	0	1	1	1	0	1	1	1	0	1	0	2	0	1	1	0	0	2	0
Aloe barberae	2	0	1	0	0	0	1	0	1	0	0	0	2	1	1	1	0	0	0	0
Aloe brevifolia	0	0	1	0/1	0	0	0	0	1	0	1	0	2	1	1	1	0/1	0/1	0	0
Aloe buhrii	0	0	0/1	1	0	0	1	0	1	0	1	0/1	2	0	0/1	1	0	1	0	0
Aloe chabaudii	0	0	1	0/1	0	0	1	0	1	0	1	0/1	2	0	0	1	1	1	2	0
Aloe challisii	0	0	1	1	0	0	0	0	1	0	1	1	2	0	1	1	0	0	0	1
Aloe chortolirioides	1	0	1	1	0	0	0	0	1	0	1	1	2	0	1	1	0	0	0	0
Aloe ciliaris	1	0	1	0	0	0	0	0	1	0	1	0	2	0	1	1	0	0	0	0
Aloe commixta	1	0	1	0	0	0	0	0	1	0/1	1	1	2	0	1	1	0	0	0	0
Aloe comosa	2	0	1	0	0	0	0/1	0	1	1	1	0	2	0	1	0/1	0	0	0	0
Aloe dewinteri	0	0	1	0	0	0	1	0	1	1	1	0	2	0	1	1	0	0	0	0
Aloe dichotoma	2	0	1	0	0	0	1	0	1	0	0	0	2	1	1	1	0	0	0	0
Aloe dichotoma subsp. *pillansii*	2	0	1	0	0	0	1	1	1	0	1	0	2	1	1	1	0	0	0	0
Aloe dichotoma subsp. *ramosissima*	1	0	1	0	0	0	1	0	1	0	1	0	2	1	1	1	0	0	0	0
Aloe ecklonis	0	0	1	1	0	0	0	0	0	0	1	1	2	1	0	1	0/1	0	0	0
Aloe eminens	2	0	1	0	0	0	1	0	1	0	1	0/1	2	1	1	0	0	0	0	0
Aloe excelsa	2	0	1	0	0	0	1	0/1	1	0	1	0	2	1	1	1	0	0	0	0
Aloe ferox	2	0	1	0	0	0	1	0	1	0	1	0	2	1	1	1	0/1	0	0	0
Aloe fouriei	1	0	1	1	0	0	0	0	0	0	1	1	2	0	0	1	0	0	0	1
Aloe glauca	1	0	1	0	0	0	0	0	1	0	1	0	2	0	1	1	0/1	0/1	0	0

Aloe gracilis	1	0	1	0	0	0	0/1	0	1	0	1	0	2	0	1	1	0	0	2	0
Aloe greatheadii subsp. *davyana*	0	0	1	1	0	0	1	0	1	0	1	0	2	0	1	1	1	1	2	0
Aloe haworthioides	0	0	1	0	1	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0
Aloe hereroensis	0	0	1	1	0	0	1	0	0	0	1	1	2	1	0	1	1	0	2	0
Aloe kouebokkeveldensis	0	0	1	1	0	0	1	0	1	0	1	1	2	0	1	1	0	1	1	0
Aloe lineata	1	0	1	0	0	0	0	0	1	0	1	0	2	0	1	1	0	1	0	0
Aloe lineata var. *muirii*	1	0	1	0	0	0	0	0	1	0	1	0	2	0	1	1	0	1	0	0
Aloe lutescens	0	0	1	0	0	0	1	0	1	1	1	0	2	0	1	1	0	0	0	0
Aloe melanacantha	0	0	1	0	0	0	0	0	1	1	1	0	2	1	1	1	0	0	0	0
Aloe microstigma	0/1	0	1	1	0	0	0	0	1	1	1	0	2	1	0/1	1	0	0	0	0
Aloe munchii	2	0	1	0	0	0	1	0	1	0	1	0/1	2	1	1	1	1	0	0	0
Aloe nubigena	1	0/1	1	1	0	0	0	0	1	0	1	1	2	0	0/1	1	0	0	0	0
Aloe pearsonii	1	0	1	0	0	0	1	0	0	0	1	1	2	1	1	1	0	0	2	0
Aloe perfoliata	1	0	1	0/1	0	0	1	0	0	0	1	1	2	0	0	1	0	0	0	0
Aloe petricola	0	0	1	0	0	0	0/1	0/1	1	1	1	0	2	1	1	0/1	1	0	0	0
Aloe pictifolia	1	0	1	1	0	0	0	0	1	0	1	0	2	0	0	1	0	0	0	0
Aloe plicatilis	2	1	0	0	0	1	0	0	1	0	1	0	2	0	1	1	0	0	0	0
Aloe plicatilis	2	1	0	0	0	1	0	0	1	0	1	0	2	0	1	1	0	0	0	0
Aloe propagulifera	0	0	1	1	0	0	0	0	1	0	1	0	2	0	1	0	0	0	2	0
Aloe reynoldsii	0/1	0	1	1	0	0	1	0	1	0	0/1	1	2	0	1	1	0/1	1	2	0
Aloe rupestris	2	0	1	0	0	0	1	0	1	1	1	0	2	0	1	1	0	0	0	0
Aloe saundersiae	0	0	1	0/1	0	0	0	0	1	0	0/1	1	2	1	1	1	0	0	0	1
Aloe spicata	2	0	1	0	0	0	0	0	1	1	0	0	1	0	1	1	0	0	0	0
Aloe striata	0/1	0	0	0	0	0	1	0	1	0/1	1	1	2	0	1	1	0/1	1	2	0
Aloe striata subsp. *karasbergensis*	0	0	0	0	0	0	1	0/1	1	0/1	1	0/1	2	1	1	1	0/1	1	2	0
Aloe striata subsp. *kommagasensis*	0	0	0	0	0	0	1	0	1	0/1	1	1	2	1	1	1	0/1	1	2	0
Aloe striatula	1	0	1	0	0	0	0	0	1	1	1	0	2	0	1	1	0	0	0	0
Aloe succotrina	0/1	0	1	0	0	0	0	0	1	0	1	0	2	0	1	1	0	0	0	0

Aloe tenuior	1	0	1	0	0	0	?	0	1	1	0/1	0	2	0	1	1	0	0	2	0
Aloe thraskii	2	0	1	0	0	0	1	0	1	0/1	0	0	2	0/1	1	1	1	0	0	0
Aloe verecunda	1	0/1	1	1	1	0	0	0	1	0	1	1	2	0	0/1	1	0	0	0	0
Aloe vossii	0/1	0	1	1	0	0	0	0	0	0	1	1	2	0	0	1	0	0	0	0
Aloe vryheidensis	1/2	0	1	0	0	0	0	0	1	1	0	0	1	0	1	1	0	1	0	0
Anthericum liliago	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Asphodeline lutea	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Astroloba corrugata	1	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	1
Astroloba foliolosa	1	0	0	0	0	0	0	0	1	0	0	0	2	0	1	0	0	0	0	1
Astroloba herrei	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1
Astroloba rubriflora	1	0	1	0	0	0	0	1	1	0	0/1	0	2	0	1	1	1	0	1	1
Bulbine frutescens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bulbine semibarbata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chortolirion angolense	0	0	1	1	1	0	0	0	1	0	0	0	2	1	1	0	0	0	1	1
Eremurus spectabilis	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Gasteria acinacifolia	0	0	0/1	1	0/1	0	0	1	1	0	1	0	2	1	1	2	1	1	1	1
Gasteria batesiana var. *dolomitica*	0	0	1	1	1	0	0	1	1	0	1	0	2	1	1	2	1	1	1	1
Gasteria carinata var. *carinata*	0	0	0	0	1	0	0	1	1	0	1	0	2	1	1	2	1	1	1	1
Gasteria carinata var. *retusa*	0	1	0	0	1	0/1	0	1	1	0	1	0	2	1	1	2	1	1	1	1
Gasteria croucheri	0	0	0	1	0	0	0	1	1	0	1	0	2	1	1	2	1	1	1	0
Gasteria disticha	0	1	0	1	1	1	0/1	0	1	0	1	0	2	1	1	2	1	1	1	1
Gasteria doreeniae	0	1	0	1	1	0	0	0	1	0	1	0	2	1	1	2	1	1	0/1	1
Gasteria ellaphieae	0	0	1	0	1	0	1	1	1	0	1	0	2	1	1	2	1	1	1	1
Gasteria excelsa	0	0	0	1	0	0/1	0	1	1	0	1	0	2	1	1	2	1	1	1	1
Gasteria glauca	0	0	1	0	1	0	0	1	1	0	1	0	2	1	1	2	1	?	?	?
Gasteria glomerata	0	1	0	0	1	1	0/1	0/1	1	0	1	0	2	1	1	2	1	1	1	1
Gasteria nitida var. *armstrongii*	0	1	0	0	1	1	0	1	1	0	1	0	2	1	1	2	1	1	1	1
Gasteria obliqua	1	0/1	0	1	0/1	1	1	0/1	1	0	1	0	2	1	1	2	1	1	1	1

Gasteria pillansii var. *ernesti-ruschii*	0	1	0	1	1	0/1	0	1	1	0	1	0	2	1	1	2	1	1	0	0
Gasteria pillansii var. *pillansii*	0	1	0	1	1	0/1	0	1	1	0	1	0	2	1	1	2	1	1	0	0
Gasteria polita	0	0	1	1	0	1	0	1	1	0	1	0	2	1	1	2	1	1	1	0
Gasteria pulchra	0	0	1	1	0	0	0	1	1	0	1	0	2	1	1	2	1	1	1	1
Gasteria rawlinsonii	1	1	0/1	0/1	0	1	0	1	1	0	1	0	2	1	1	2	1	1	1	1
Gasteria tukhelensis	0	0	0/1	1	0	0	1	1	1	0	1	0	2	1	1	2	1	1	1	0/1
Gasteria vlokii	0	0	1	1	0	1	0	1	1	0	1	0	2	1	1	2	1	1	1	1
Haworthia angustifolia	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	?
Haworthia arachnoidea	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia arachnoidea	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia attenuata	0	0	0	0	1	0	0/1	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia bayeri	0	0	0/1	0	0	1	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia blackburniae	0	0	0/1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia blackburniae var. *derustensis*	0	0	0/1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia blackburniae var. *graminifolia*	0	0	0/1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia bruynsii	0	0	0	0	0/1	1	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia chloracantha	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia coarctata var. *adelaidensis*	1	0	0	0	1	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia coarctata	1	0	0	0	1	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia cooperi	0	0	0/1	0	0	0/1	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia cooperi var. *doldii*	0	0	0/1	0	0	0/1	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia cymbiformis	0/1	0	0	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia cymbiformis var. *ramosa*	0/1	0	0	0	0	?	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia cymbiformis var. *setulifera*	0/1	0	0	0	0	?	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia decipiens	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia decipiens var. *pringlei*	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia decipiens var. *virella*	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia emelyae	0	0	0	0	1	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1

Haworthia emelyae var. *comptoniana*	0	0	0	0	1	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia fasciata	0/1	0	0	0	1	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia floribunda	0	0	0/1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia glauca	1	0	0/1	0	0/1	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia herbacea var. *flaccida*	0	0	1	0	1	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia herbacea var. *paynei*	0	0	1	0	1	0	0	0	1	0/1	0	0	2	2	1	0	0	0	0	1
Haworthia kingiana	0	0	0	0	0/1	0	0/1	0	1	0	0	0	2	1	1	0	0	0	0	1
Haworthia kingiana	0	0	0	0	0/1	0	0/1	0	1	0	0	0	2	1	1	0	0	0	0	1
Haworthia koelmaniorum var. *mcmurtryi*	0	0	1	0	1	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia limifolia	0	0	0	0	0/1	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia limifolia var. *ubomboensis*	0	0	0	0	0/1	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia lockwoodii	0	0	0	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia longiana	0	0	0	0	1	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia maculata	0	0	1	1	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia magnifica var. *dekenahii*	0	0	0	0	0	1	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia marginata	0	0	0	0	0	0	1	0	1	0	0	0	2	1	1	0	0	0	0	1
Haworthia marginata	0	0	0	0	0	0	1	0	1	0	0	0	2	1	1	0	0	0	0	1
Haworthia marumiana var. *archeri*	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia marxii	0	0	0	1	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia mirabilis	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia mirabilis var. *calcarea*	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia mirabilis var. *paradoxa*	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia mirabilis var. *triebneriana*	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia monticola var. *asema*	0	0	1	1	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia mucronata var. *bijliana*	0	0	0	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia mucronata var. *habdomadis*	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia mucronata var. *morrisiae*	0	0	0	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia mucronata var. *rycroftiana*	0	0	0	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1

Haworthia mutica	0	0	0	0	0	1	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia mutica var. *nigra*	0	0	0	0	0	1	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia nigra	1	0	0	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia outeniquensis	0	0	1	0/1	0	1	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia pulchella var. *globifera*	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia pumila	0	0	0	0	1	0	1	0	1	0	0	0	2	1	1	0	0	0	0	1
Haworthia pygmaea	0	0	0	0	0	1	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia reinwardtii	1	0	0	0	1	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia reinwardtii var. *brevicula*	1	0	0	0	1	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia reticulata	0/1	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia retusa	0	0	0/1	0	0	1	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia semiviva	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia sordida	0	0	0	0	0	0/1	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia truncata	0	1	0	0	1	1	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia variegata	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia venosa	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia venosa var. *granulata*	0/1	0	0	0	0	0	0	1	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia vlokii	0	0	1	1	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia wittebergensis	0	0	1	0	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Haworthia zantneriana	0	0	0	0/1	0	0	0	0	1	0	0	0	2	2	1	0	0	0	0	1
Jodrellia fistulosa	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Kniphofia galpinii	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Kniphofia uvaria	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Tecophilaea cyanocrocus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Xanthorrhoea resinosa	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Xanthorrhoea sp.	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Zephyra elegans	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?

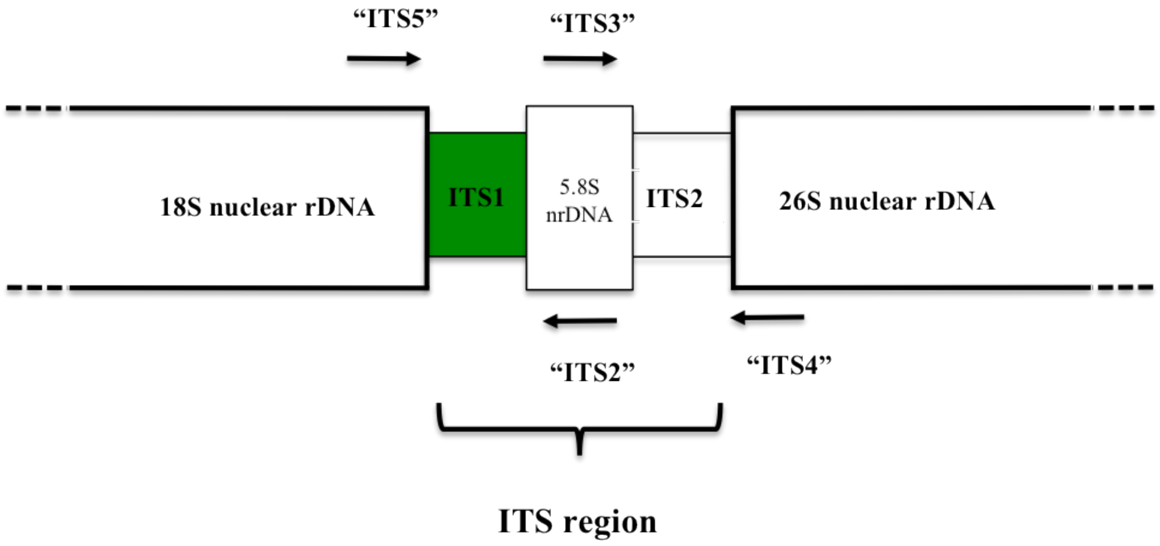


Figure 2.1 Organisation of the ITS region. Green area represents ITS region sequenced in the study. Arrows indicate orientation and approximate position of primer sites. Adapted from Baldwin *et al.* (1995).

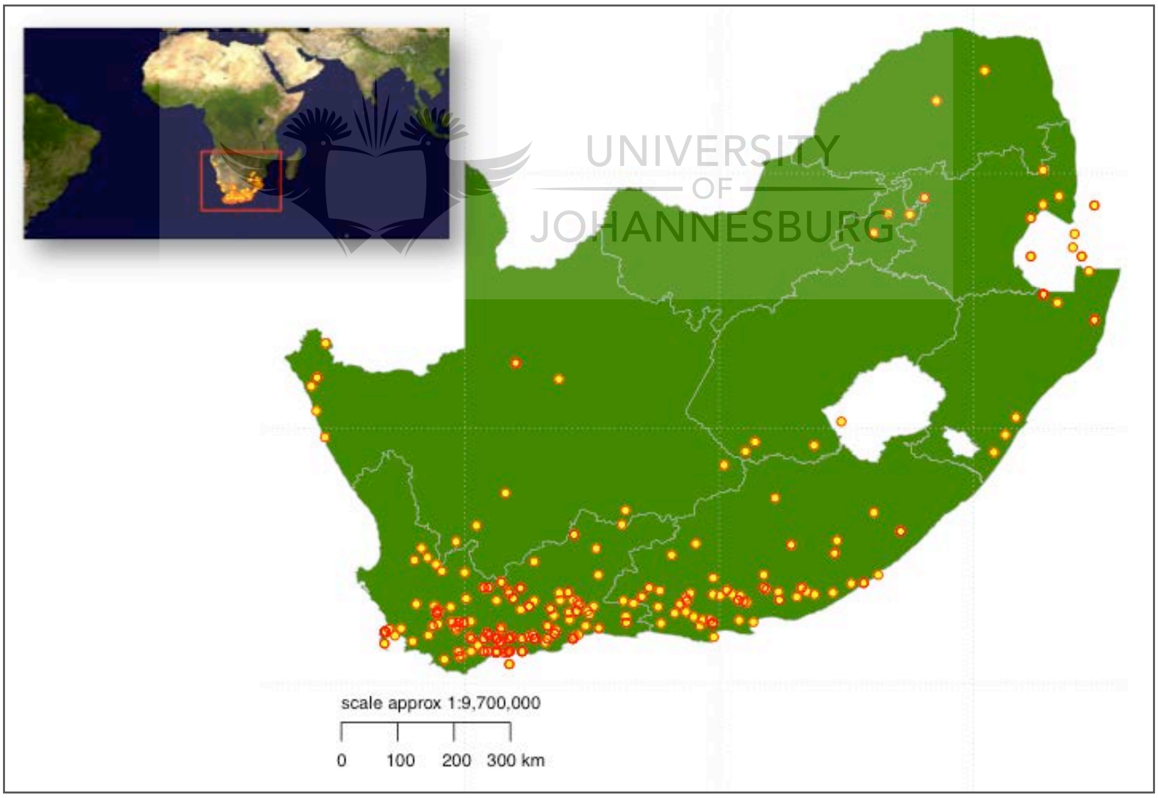
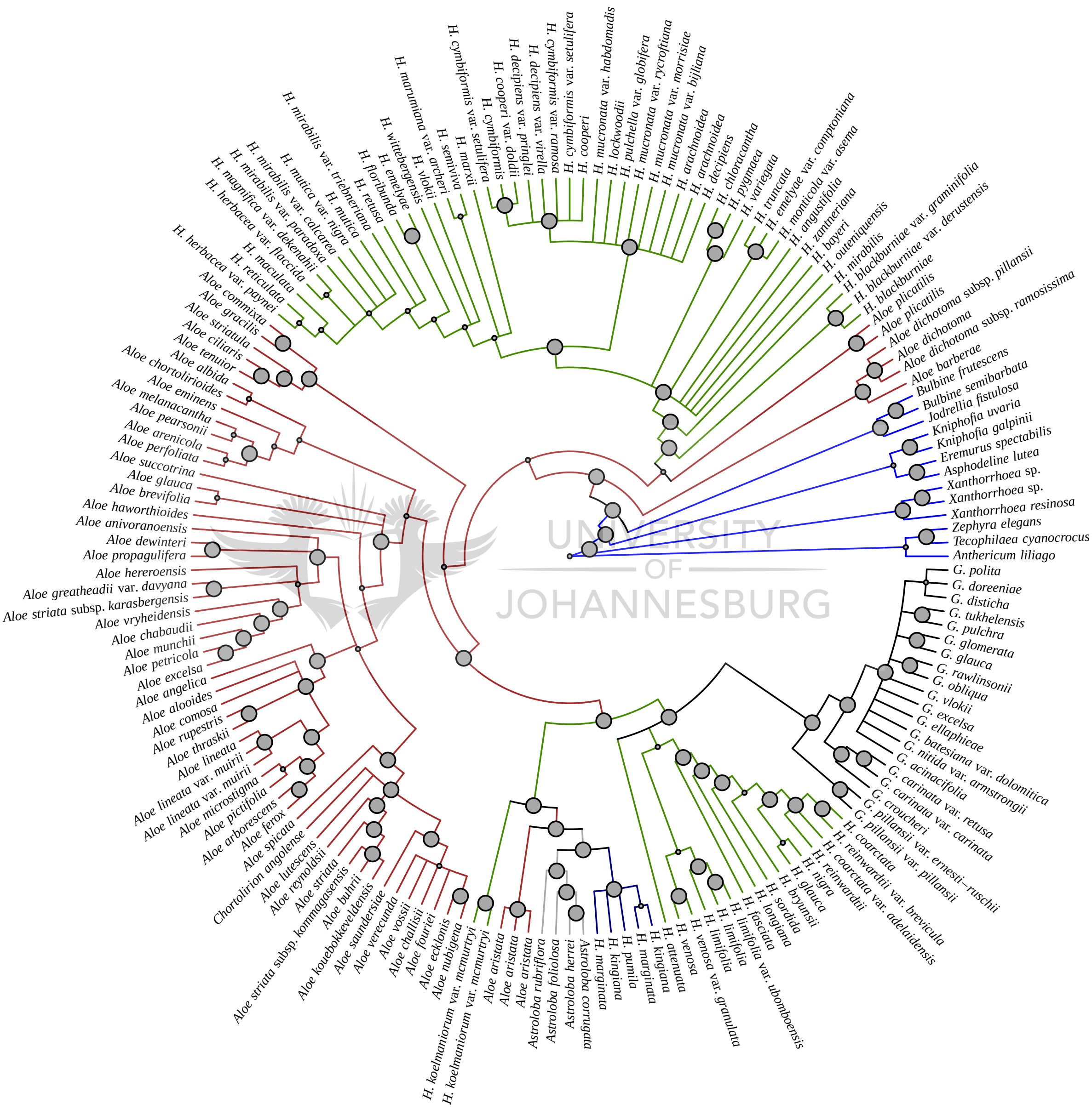


Figure 2.2 Distribution of Alooideae collected in the wild and grown at Sheilam Nursery, Gariep Nursery and Kirstenbosch Botanical Garden, used in this study (yellow circles).

CHAPTER THREE

Results



3. RESULTS

3.1 Molecular Evolution

The statistics from MP analyses for the single plastid analysis, combined plastid (*rbcLa* + *matK* + *trnH-psbA*), ITS1 and the combined plastid and nuclear datasets are shown in Table 3.1. Of the plastid regions used, *matK* had a significantly higher number of variable sites (33%; Table 3.1) than *trnH-psbA* (16%) or *rbcLa* (14%). The number of potentially informative characters for *matK* (21%) is much higher than for the two other plastid regions, *rbcLa* and *trnH-psbA* (10% and 9% respectively). The ITS1 region had significantly more variable sites (40%) compared to the plastid regions. It also evolves at a much faster rate than the plastid genes (2.1 changes per variable site) with a lower consistency index (CI).

Results from the following analyses are presented: simplified tree topologies of the combined plastid regions (*rbcLa* + *matK* + *trnH-psbA*), ITS1 and combined plastid and nuclear regions (Fig. 3.1), combined plastid regions (*rbcLa* + *matK* + *trnH-psbA*; Fig. 3.2), ITS1 (Fig. 3.3) and combined plastid and ITS1 (*rbcLa* + *matK* + *trnH-psbA* + ITS1; Fig. 3.4). The MP analyses were largely congruent with the Bayesian analyses, and therefore results are displayed on the same tree (Fig. 3.4).

3.2 Combined Plastid Data

Individual plastid sequence analyses (results not shown) were topologically consistent (negligible to zero incongruence), and for the purpose of the results and discussion were combined and treated as a single analysis. The MP analysis yielded 661 most parsimonious trees of 752 steps with a CI of 0.72 and a RI of 0.91 (Table 3.1). One of the most parsimonious trees is illustrated in Fig. 3.2. Alooideae is strongly supported as monophyletic (BP = 94; Fig. 3.2). Although resolution in the plastid tree were relatively poor, possibly due to a lack of sufficient informative sequence variation, we observed eight clades (A, B, C, D, E, F, G and H) while the internal groups for each clade were retrieved as a polytomy.

3.3 ITS1 data

Analyses resulted in 314 equally most parsimonious trees with TL = 346, CI = 0.64, and RI = 0.92. One of the most parsimonious trees is illustrated in Fig. 3.3. Seven major clades were recovered; *Haworthia* subg. *Haworthia, H.* subg. *Robustipenduncculares, Gasteria, Astroloba, Aloe* sect. *Macrifoliae,* tree *Aloes,* and remaining *Aloe* species. The tree *Aloe* species (*A. barberae, A. eminens* and *A. dichotoma*) were retrieved as the early diverging lineage to the subfamily Alooideae.

3.4 Combined plastid and nuclear dataset (*rbcLa*+*matK*+*trnH-psbA*+ ITS1)

Results of the combined plastid analysis are topologically congruent with those of the ITS analysis, and all data were therefore combined. Congruence between the ITS and plastid datasets was addressed by comparisons of bootstrap percentages from separate analyses. Bootstrap trees were considered incongruent only when hard (i.e. with high bootstrap support) instead of "soft" (with low bootstrap support) incongruence was displayed (See Wiens, 1998). The aligned combined dataset (*rbcLa* + *matK* + *trnH-psbA* + ITS1) included 2518 characters of which 604 (24%) are variable. The MP analysis yielded 5020 equally most parsimonious trees of 1159 steps with a CI of 0.63 and a RI of 0.89 (Table 3.1). A monophyletic Alooideae was recovered with high support (Fig. 3.4; BP = 93; PP = 1.00). It resolves eight major clades represented in different colours in Fig. 3.4: *Aloe plicatilis* and the remaining tree *Aloes*, *Haworthia* subg. *Haworthia*, the climbing *Aloes*, the true *Aloe* species, '*Haworthioid*' clade (*H.* subg. *Robustipenduncculares* + *Astroloba* + *Aloe aristata*), *Haworthia* subg. *Hexangulares*, and *Gasteria*. While the combined data recovered eight clades, the plastid data retrieved five with clades A, B, D, and E, grouping together. The ITS1 data recovered seven clades compared to eight in the combined data.

The tree Aloe species — The analysis indicates an isolated position for *Aloe plicatilis* in a polytomy with *Haworthia* subg. *Haworthia* but without BP support and weak support in the BI analysis (PP = 0.58); and for *A. eminens* in the clade including *A. perfoliata* but without BP support and weak support in the BI analysis (PP = 0.58). The other tree *Aloe* species (*A. barberae, A. dichotoma*, and *A. pillansii*) were retrieved as a monophyletic clade with moderate support in MP analysis and strong support in the BI analysis (BP = 76; PP = 1.00). The *A. plicatilis, A. eminens* and *A. barberae* lineages constitute the early diverging elements in subfamily Alooideae.

Haworthia subg. Haworthia — This clade is well supported in the MP and BI analyses (BP = 98; PP = 1.00). This clade resolved (BP = 96; PP = 1.00) the grass-like *Haworthia blackburniae* as the earliest-diverging lineage to the other *Haworthia* species, which were unresolved.

Climbing Aloes — This group, representing *Aloe* sect. *Macrifoliae* (*Aloe ciliaris, A. commixta, A. gracilis, A. striatula*, and *A. tenuior*) was recovered in both analyses as a strongly supported clade (BP = 95; PP = 1.00).

The true Aloes — This group, which comprises the majority of *Aloe* species plus *Chortolirion* (coloured brown; Fig. 3.4) was retrieved as a group with strong support (PP = 1.00) in the BI. Several internal clades, none of which correspond exactly to current sections recognised in the taxonomy of the genus, were well supported in the BI analysis.

Among these, the majority of the grass *Aloes* (sect. *Leptoaloe*) grouped together (BP = 89; PP = 1.00) in a clade comprising *Chortolirion angolense, A. kouebokkeveldensis, A. buhrii, A. lutescens, A. reynoldsii, A. spicata, A. striata* subsp. *komaggasensis*, and *A. striata* subsp. *striata*. In the MP tree, it was recovered as part of a larger polytomy with no bootstrap support.

A second clade comprised several of the 'single-stemmed Aloes' (*Aloe* sect. *Pachydendron*) plus other species, with weak MP support (BP = 61) but strong support in the BI analysis (PP = 1.00).

A third clade comprising a heterogenous assemblage of *Aloe* species (*A. excelsa, A. petricola, A. munchii, A. chabaudii* and *A. vryheidensis*) was well supported in the BI (BP = 53; PP = 0.99).

The fourth clade also recovered a heterogenous mix of *Aloe* species containing the type of the genus, *A. perfoliata* with weak to moderate support (BP = 53; PP = 0.92).

'Haworthioid' clade (Haworthia subg. Robustipendunculares, Astroloba, Aloe aristata, and H. koelmaniorum) — This clade was strongly supported (BP = 92, PP = 1.00) as sister to the *Gasteria–Hexangulares* clade but was only moderately supported as monophyletic in the BI (BP = 58; PP = 0.68). *Haworthia koelmaniorum* var. *mcmurtryi* (BP = 99; PP = 1.00), also *Hexangulares* was retrieved with weak BI support (PP = 0.68) as the earliest-diverging lineage in the clade. There is moderate to strong support (BP = 77, PP = 0.95) for a sister relationship between *Aloe aristata* and *H.* subg. *Robustipendunculares* + *Astroloba*. The remaining species of *H.* subg. *Robustipendunculares* (highlighted in chartreuse) were recovered with strong support (BP = 100; PP = 1.00) but are only weakly supported in BI (PP = 0.74) as sister to a moderately to well supported (BP = 79; PP = 1.00) *Astroloba*.

Haworthia subg. Hexangulares — *H.* subg. *Hexangulares* (Fig. 3.4) was recovered in both MP and BI with moderate to strong support (BP = 80; PP = 1.00) in a clade with *Gasteria*. Relationships between *Haworthia attenuata*, the rest of subg. *Hexangulares* and *Gasteria* remain unresolved.

***Gasteria* —** *Gasteria* species form a well-supported monophyletic clade in both analyses (BP = 97; PP = 1.00) (Fig. 3.4). The clade is recovered in all trees as one element of a moderately to well supported trichotomy (BP = 80; PP = 1.00) that includes the species of *Haworthia* subg. *Hexangulares.*

3.5 Evolution of morphological traits

Among 20 morphological traits that were analysed, habit, leaf arrangement, leaf maculation and tuberculation, inflorescence branching and orientation, inflorescence shape and colour, perianth orientation at anthesis, perianth symmetry and colour, perianth shape and curvature, tepal connation, and stamen length are all shown to be homoplasious (Tables 2.3 and 2.4; Fig. 3.5).

Table 3.2 presents summary of the characters and character states constructed for nine of the 20 morphological characters for Alooideae. These traits are selected because of their importance in generic and infrageneric delimitation within Alooideae (see also Figs. 3.6Aabc, 3.7abc and 3.8abc).

In *Gasteria*, only *G. obliqua* and *G. rawlinsonii* exhibit caulescence (character 1; Fig. 3.6a). Among members of *Haworthia* subg. *Hexangulares* almost equal numbers exhibit caulescence and acaulescence. Members of subg. *Robustipendunculares* are all stemless but all *Astroloba* species (including *Poellnitzia*) are caulescent. An arborescent habit is characteristic of only a few species of *Aloe*, namely *A. plicatilis* (sect. *Kumara*) and members of sect. *Pachydendron* and *Dracoaloe*.

Most members of Alooideae have a polystichous leaf arrangement (character 2; Fig. 3.6b). Exceptions include a few *Gasteria* species, which show a distichous leaf arrangement in the juvenile stage. Most become polystichous later in the adult stage with the exception of

Gasteria obliqua, which retains the distichous leaf arrangement into the adult stage. Other species with a distichous leaf arrangement are *Aloe nubigena*, *A. plicatilis*, *A. verecunda* and *Haworthia truncata*.

A toothed or bristled leaf margin (character 3) is characteristic of all species except members of *Haworthia* subg. *Hexangulares*, in which the leaf margins are entire.

Maculate leaves (character 4) are consistently present in all *Gasteria* species and also in several aloes, including *Aloe aristata* and the grass *Aloes* (sect. *Leptoaloe*).

Leaf tubercles (character 5; Fig. 3.6c) are also characteristic of all *Gasteria* species plus *Aloe aristata*, and also all species of *Haworthia* subg. *Hexangulares* and *Robustipedunculares.*

Most members of Alooideae have acute leaf apices (character 6) except some *Gasteria* and *Haworthia* species, which have truncate to blunt apices.

Five of the 20 *Gasteria* species (*G. disticha*, *G. tukhelensis*, *G. glomerata*, *G. obliqua* and *G. ellaphieae*) have branched inflorescences (character 7) and this trait is also found in all species of *Astroloba* and *Haworthia* subg. *Robustipendunculares*, but not in subg. *Hexangulares* and *Haworthia.* Various species of *Aloe* also have branched inflorescences.

A secund inflorescence (character 8) is characteristic of all *Gasteria* species, and also of *Astroloba* (*Poellnitzia*) *rubriflora*, *Haworthia venosa* var. *granulata*, *Aloe excelsa*, *A. petricola* and *A. striata* var. *karasbergensis.*

Relatively lax inflorescences (character 9) are characteristic of most Alooideae. Only a few *Aloe* species exhibit bicoloured racemes (character 10; e.g. *A. striata*, *A. lutescens*, *A. spicata*, *A. pictifolia*, *A. thraskii*, *A. rupestris*, *A. comosa*). Pendulous flowers (character 11; Fig. 3.7a) are found in most *Aloe* species and in *Gasteria*.

Capitate inflorescences (character 12) are found among relatively few, unrelated *Aloe* species (sect. *Leptoaloe* plus *A. chabaudii*, *A. munchii* and *A. striata* subsp. *karasbergensis*).

Strongly bilabiate flowers (character 14; Fig. 3.7b) are diagnostic for *Haworthia* subg. *Haworthia* and *Hexangulares* plus *Chortolirion angolense* but *H.* subg. *Robustipendunculares* and several *Aloe* species, notably *A. albida*, have ± zygomorphic flowers. A short pedicel (character 15) is characteristic of all Alooideae. Flower colouring is strongly correlated with current generic circumscriptions. All the *Gasteria* species have unusual, bicoloured flowers; and all *Haworthia* species; *Chortolirion* and all but one *Astroloba* species have white/cream/greenish flowers. The great majority of *Aloe* species have orange/red flowers but isolated species have whitish flowers. Perianth curvature (character 17; Fig. 3.7c) is also associated with current generic circumscriptions. All species of *Chortolirion, Gasteria* and *Haworthia* subg. *Haworthia* and *Hexangulares* have curved perianth tubes whereas *Astroloba*, *H.* subg. *Robustipendunculares* and the majority of *Aloe* species have straight perianth tubes. Basally inflated flowers (character 18; Fig. 3.7a) are characteristic of *Gasteria* but also of several *Aloe* species. All *Gasteria* species and some *Aloe* species have both tepal whorls connate for half or more of their length (character 19; Fig. 3.7c) but a number of *Aloe* species have flowers in which only the outer tepals are connate for half or more of length. Included stamens (character 20; Fig. 3.7b) are typical of *Chortolirion, Gasteria* and *Haworthia* but are also found in various *Aloe* species.

Table 3.1 Statistics from MP analyses obtained from separate and combined datasets.

	rbcLa	*matK*	*trnH-psbA*	Combined plastid (*rbcLa* + *matK* + *trnH-psbA*)	ITS1	Combined plastid + ITS1
No. of taxa included	172	172	157	172	156	172
No. of included characters	552	789	768	2109	409	2518
No. of constant characters	472	531	645	1648	244	1914
No. of variable sites	80 (14%)	258 (33%)	123 (16%)	461 (22%)	165 (40%)	604 (24%)
No. of parsimony informative sites	55 (10%)	165 (21%)	70 (9%)	290 (14%)	92 (22%)	376 (15%)
No. of trees (Fitch)	523	408	101	661	314	5020
No. of steps (Tree length)	120	408	194	752	346	1159
CI	0.74	0.75	0.74	0.72	0.64	0.63
RI	0.91	0.92	0.92	0.91	0.92	0.89
Average number of changes per variable site (number of steps/ number of variable sites)	1.5	1.6	1.6	1.6	2.1	1.9
Model selected by AIC	HKY+ G	TVM+G	TVM+I+ G		TRN+G	GTR+I+G

AIC = Akaike information criterion; CI = consistency index; RI = retention index

Table 3.2 Characters states for nine selected autapomorphic traits which have been employed in previous Alooideae systematics.

Character	Character state	Species
Growth habit	Stemless	Most *Gasteria;* half of *Hexangulares*; *Robustipedunculares*
	Caulescent	*Gasteria obliqua*; half of *Hexangulares;* all *Astroloba* (including *Poellnitzia*);
	Arborescent	*A. plicatilis* (sect. *Kumara*); sect. *Pachydendron* and *Dracoaloe*.
Leaf insertion	Spiral/polystichous	Most Alooideae;
	Distichous	*Gasteria obliqua; Aloe nubigena, A. plicatilis, A. verecunda* and *Haworthia truncata.*
Leaf maculation	Immaculate	Most Alooideae
	Maculate	All *Gasteria;* several *Aloe* species e.g. *A. aristata* and *Aloe* sect. *Leptoaloe*.
Flower orientation at anthesis	Spreading	*Chortolirion, Astroloba, Haworthia* and a few *Aloe*
	Pendulous	Most *Aloe* and *Gasteria* species.
Perianth symmetry	Actinomorphic	*Robustipedunculares* and several *Aloe* e.g. *A. albida*
	Weakly bilabiate	*Gasteria* species
	Strongly bilabiate	*Haworthia* subg. *Haworthia* and *Hexangulares* plus *Chortolirion angolense*
Perianth curvature	Straight	*Astroloba*, *Robustipedunculares* and majority of *Aloe*
	Curved upwards	*Chortolirion, Gasteria; Haworthia* subg. *Haworthia* and *Hexangulares*
Perianth	Cylindrical and tapering into pedicel	Most *Aloe; Astroloba; Chortolirion; Haworthia* subg. *Haworthia* and *Hexangulares*
	Inflated basally or truncated	*Gasteria* and several *Aloe* species.
Stamen inclusion	Exserted	Most *Aloe* species
	Inserted	*Chortolirion, Gasteria* and the three *Haworthia* subgenera
Tepal connation	Both whorls connate basally	The three *Haworthia* subgenera; *Astroloba* and some *Aloe* species
	Both whorls connate ½ or more of length	*Gasteria* and some *Aloe* species
	Only outer whorls connate ½ or more of length	A few *Aloe* species

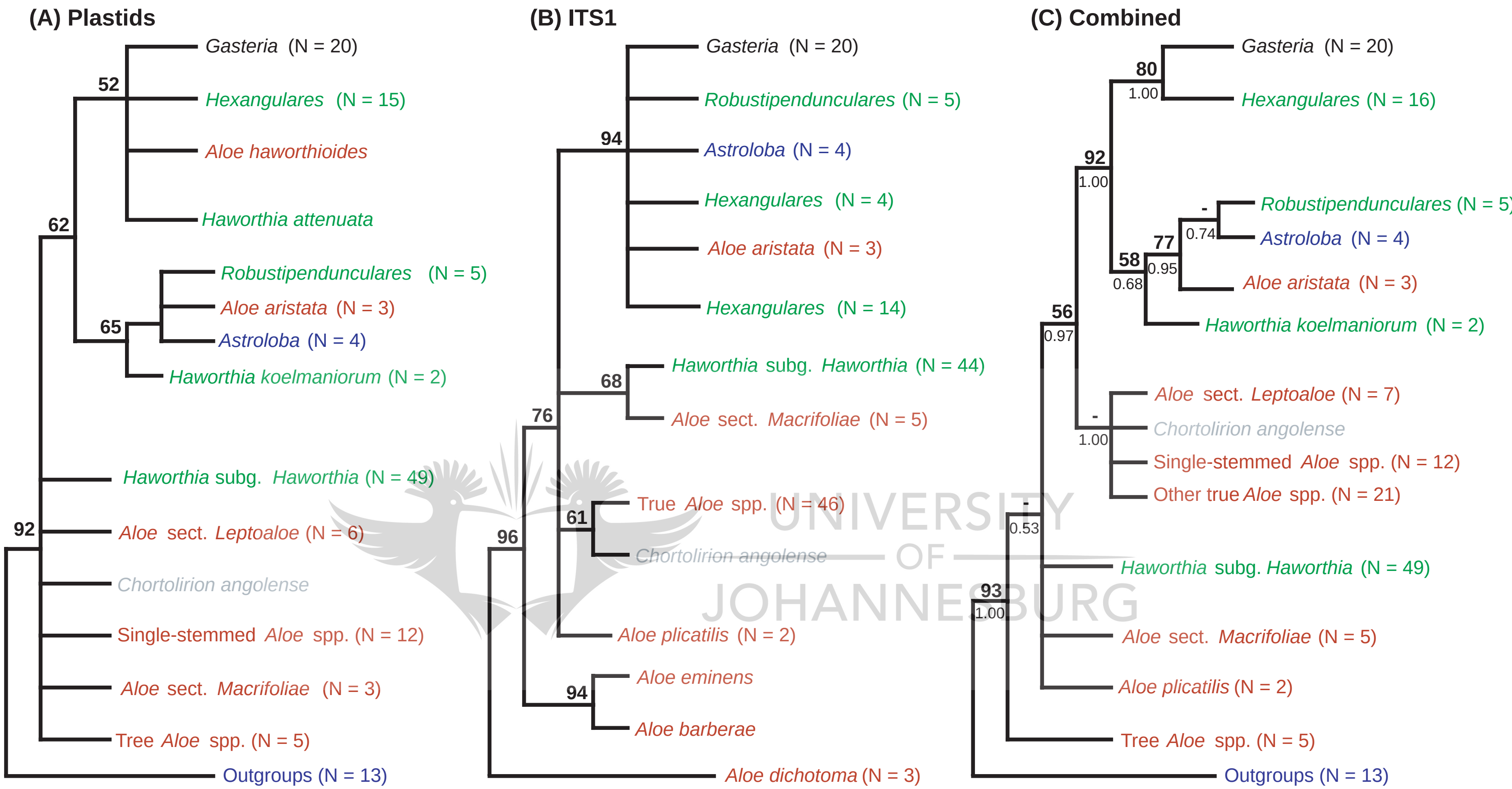
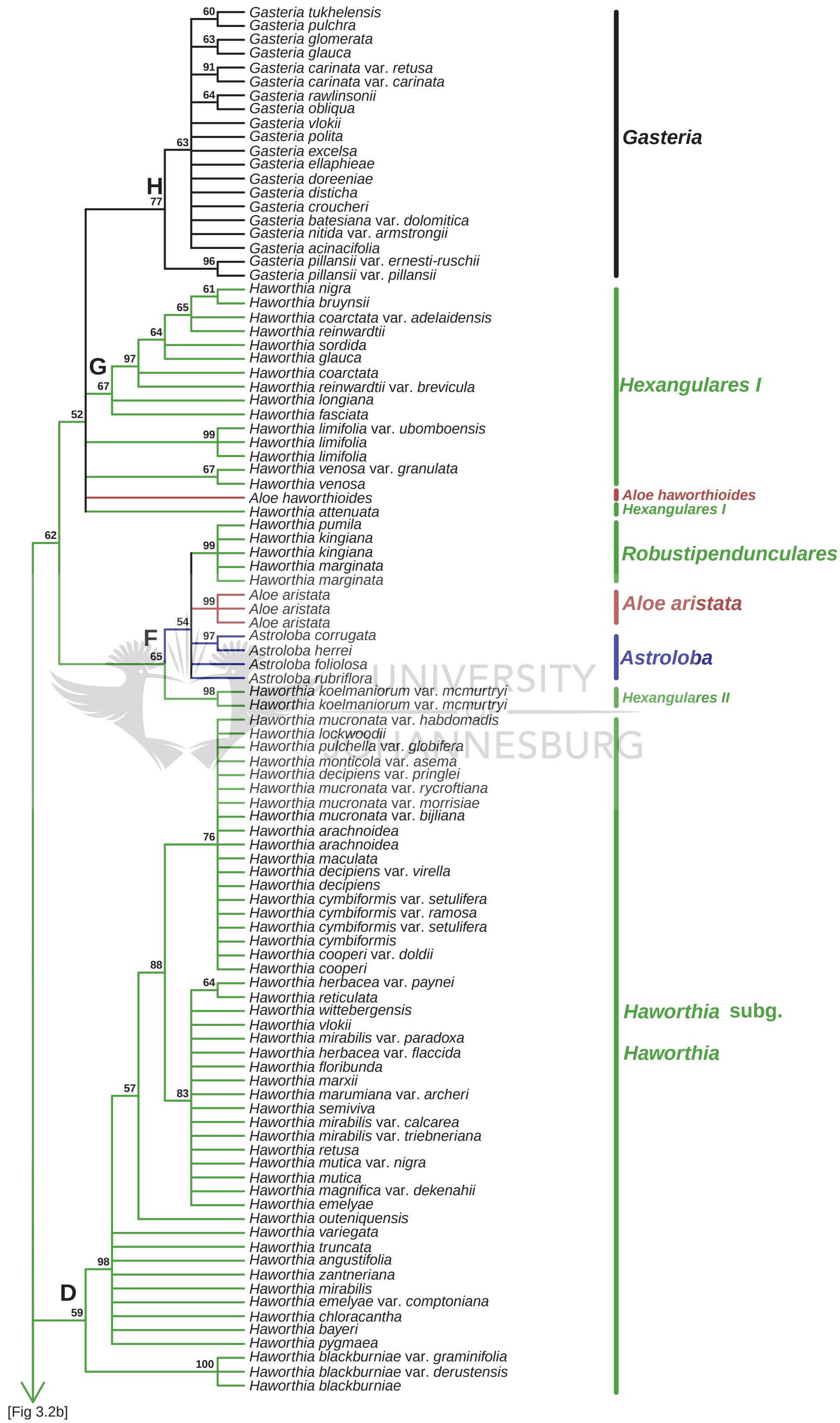


Figure 3.1 Simplified tree topologies showing relationships among Alooideae genera for the plastids, nuclear and combined dataset.


Gasteria tukhelensis
Gasteria pulchra
Gasteria glomerata
Gasteria glauca
Gasteria carinata var. retusa
Gasteria carinata var. carinata
Gasteria rawlinsonii
Gasteria obliqua
Gasteria vlokii
Gasteria polita
Gasteria excelsa
Gasteria ellaphieae
Gasteria doreeniae
Gasteria disticha
Gasteria croucheri
Gasteria batesiana var. dolomitica
Gasteria nitida var. armstrongii
Gasteria acinacifolia
Gasteria pillansii var. ernesti-ruschii
Gasteria pillansii var. pillansii
Haworthia nigra
Haworthia bruynsii
Haworthia coarctata var. adelaidensis
Haworthia reinwardtii
Haworthia sordida
Haworthia glauca
Haworthia coarctata
Haworthia reinwardtii var. brevicula
Haworthia longiana
Haworthia fasciata
Haworthia limifolia var. ubomboensis
Haworthia limifolia
Haworthia limifolia
Haworthia venosa var. granulata
Haworthia venosa
Aloe haworthioides
Haworthia attenuata
Haworthia pumila
Haworthia kingiana
Haworthia kingiana
Haworthia marginata
Haworthia marginata
Aloe aristata
Aloe aristata
Aloe aristata
Astroloba corrugata
Astroloba herrei
Astroloba foliolosa
Astroloba rubriflora
Haworthia koelmaniorum var. mcmurtryi
Haworthia koelmaniorum var. mcmurtryi
Haworthia mucronata var. habdomadis
Haworthia lockwoodii
Haworthia pulchella var. globifera
Haworthia monticola var. asema
Haworthia decipiens var. pringlei
Haworthia mucronata var. rycroftiana
Haworthia mucronata var. morrisiae
Haworthia mucronata var. bijliana
Haworthia arachnoidea
Haworthia arachnoidea
Haworthia maculata
Haworthia decipiens var. virella
Haworthia decipiens
Haworthia cymbiformis var. setulifera
Haworthia cymbiformis var. ramosa
Haworthia cymbiformis var. setulifera
Haworthia cymbiformis
Haworthia cooperi var. doldii
Haworthia cooperi
Haworthia herbacea var. paynei
Haworthia reticulata
Haworthia wittebergensis
Haworthia vlokii
Haworthia mirabilis var. paradoxa
Haworthia herbacea var. flaccida
Haworthia floribunda
Haworthia marxii
Haworthia marumiana var. archeri
Haworthia semiviva
Haworthia mirabilis var. calcarea
Haworthia mirabilis var. triebneriana
Haworthia retusa
Haworthia mutica var. nigra
Haworthia mutica
Haworthia magnifica var. dekenahii
Haworthia emelyae
Haworthia outeniquensis
Haworthia variegata
Haworthia truncata
Haworthia angustifolia
Haworthia zantneriana
Haworthia mirabilis
Haworthia emelyae var. comptoniana
Haworthia chloracantha
Haworthia bayeri
Haworthia pygmaea
Haworthia blackburniae var. graminifolia
Haworthia blackburniae var. derustensis
Haworthia blackburniae
Gasteria
Hexangulares I
Aloe haworthioides
Hexangulares I
Robustipendunculares
Aloe aristata
Astroloba
Hexangulares II
Haworthia subg.
Haworthia
H
G
F
D
[Fig 3.2b]

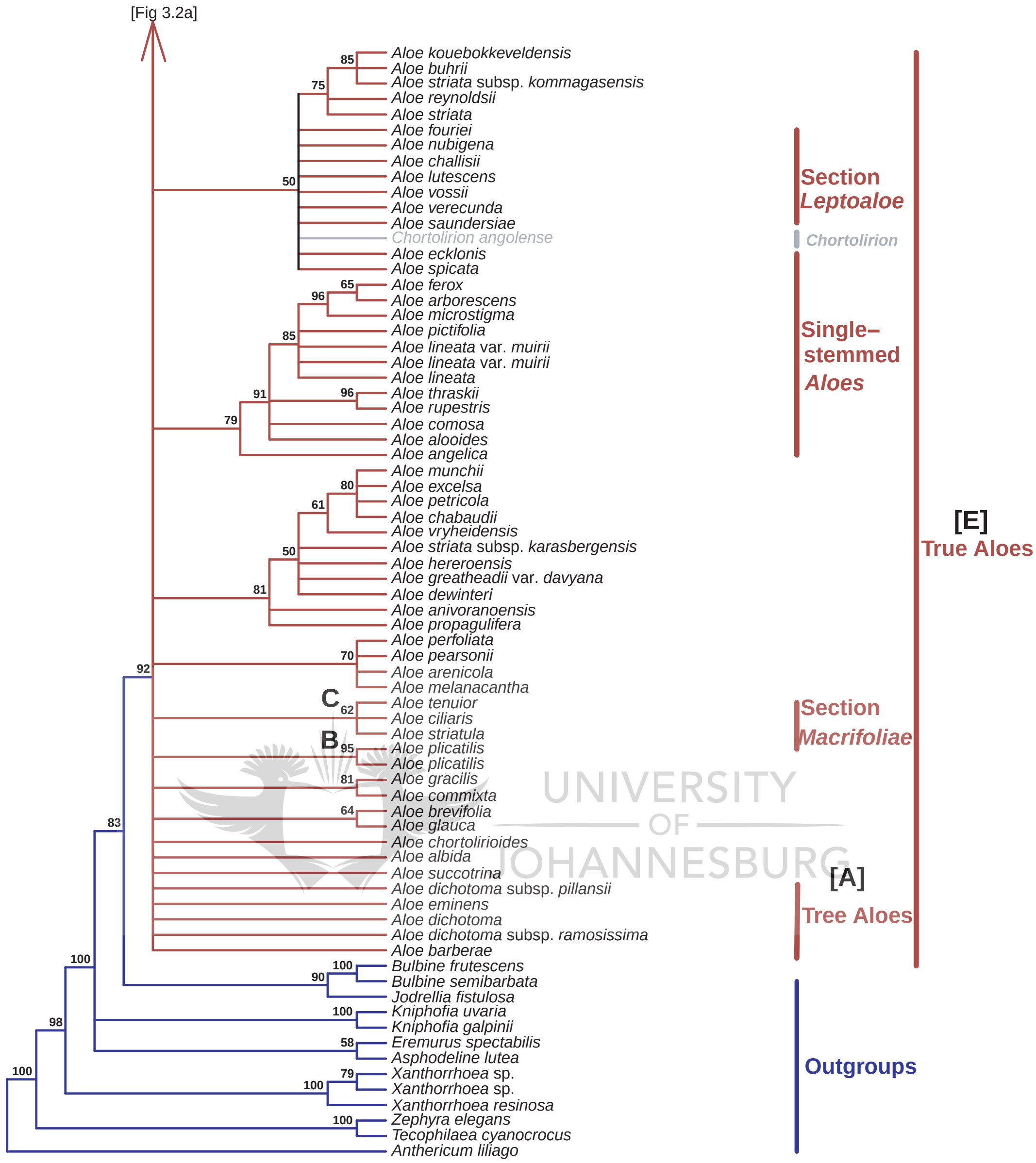
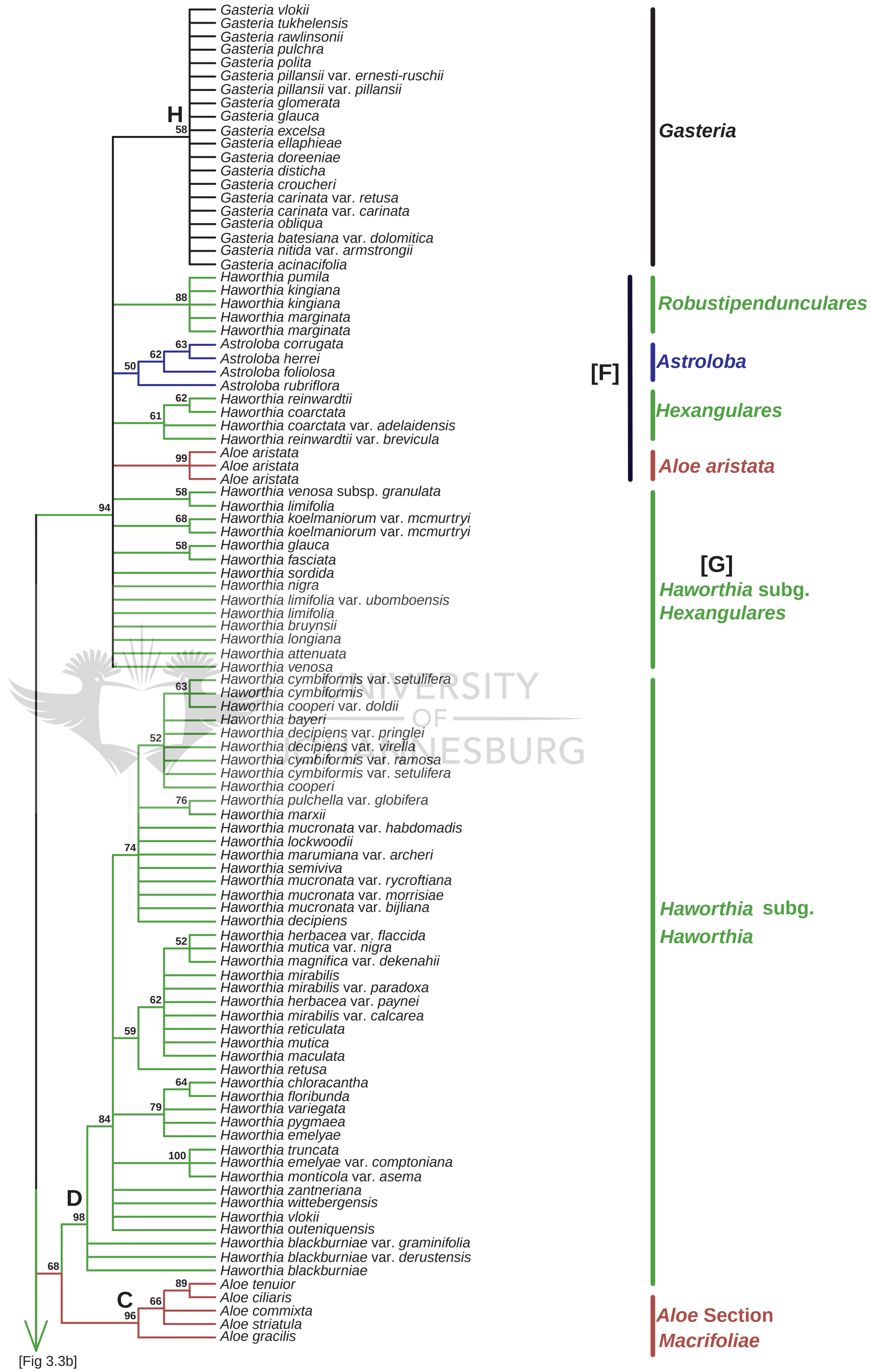


Figure 3.2 One of the most parsimonious trees obtained from the maximum parsimony (MP) analyses of the combined plastid data set (*rbcLa* + *matK* + *trnH-psbA*) for Alooideae plus outgroups (TL = 752; CI = 0.72; RI = 0.91). Numbers above the branches are MP bootstrap support.


Gasteria vlokii
Gasteria tukhelensis
Gasteria rawlinsonii
Gasteria pulchra
Gasteria polita
Gasteria pillansii var. ernesti-ruschii
Gasteria pillansii var. pillansii
Gasteria glomerata
Gasteria glauca
Gasteria excelsa
Gasteria ellaphieae
Gasteria doreeniae
Gasteria disticha
Gasteria croucheri
Gasteria carinata var. retusa
Gasteria carinata var. carinata
Gasteria obliqua
Gasteria batesiana var. dolomitica
Gasteria nitida var. armstrongii
Gasteria acinacifolia
Haworthia pumila
Haworthia kingiana
Haworthia kingiana
Haworthia marginata
Haworthia marginata
Astroloba corrugata
Astroloba herrei
Astroloba foliolosa
Astroloba rubriflora
Haworthia reinwardtii
Haworthia coarctata
Haworthia coarctata var. adelaidensis
Haworthia reinwardtii var. brevicula
Aloe aristata
Aloe aristata
Aloe aristata
Haworthia venosa subsp. granulata
Haworthia limifolia
Haworthia koelmaniorum var. mcmurtryi
Haworthia koelmaniorum var. mcmurtryi
Haworthia glauca
Haworthia fasciata
Haworthia sordida
Haworthia nigra
Haworthia limifolia var. ubomboensis
Haworthia limifolia
Haworthia bruynsii
Haworthia longiana
Haworthia attenuata
Haworthia venosa
Haworthia cymbiformis var. setulifera
Haworthia cymbiformis
Haworthia cooperi var. doldii
Haworthia bayeri
Haworthia decipiens var. pringlei
Haworthia decipiens var. virella
Haworthia cymbiformis var. ramosa
Haworthia cymbiformis var. setulifera
Haworthia cooperi
Haworthia pulchella var. globifera
Haworthia marxii
Haworthia mucronata var. habdomadis
Haworthia lockwoodii
Haworthia marumiana var. archeri
Haworthia semiviva
Haworthia mucronata var. rycroftiana
Haworthia mucronata var. morrisiae
Haworthia mucronata var. bijliana
Haworthia decipiens
Haworthia herbacea var. flaccida
Haworthia mutica var. nigra
Haworthia magnifica var. dekenahii
Haworthia mirabilis
Haworthia mirabilis var. paradoxa
Haworthia herbacea var. paynei
Haworthia mirabilis var. calcarea
Haworthia reticulata
Haworthia mutica
Haworthia maculata
Haworthia retusa
Haworthia chloracantha
Haworthia floribunda
Haworthia variegata
Haworthia pygmaea
Haworthia emelyae
Haworthia truncata
Haworthia emelyae var. comptoniana
Haworthia monticola var. asema
Haworthia zantneriana
Haworthia wittebergensis
Haworthia vlokii
Haworthia outeniquensis
Haworthia blackburniae var. graminifolia
Haworthia blackburniae var. derustensis
Haworthia blackburniae
Aloe tenuior
Aloe ciliaris
Aloe commixta
Aloe striatula
Aloe gracilis
H
58
88
63
62
50
62
61
99
58
94
68
58
63
52
76
74
52
62
59
64
79
84
100
D
98
68
C
89
66
96
[Fig 3.3b]
Gasteria
[F]
Robustipendunculares
Astroloba
Hexangulares
Aloe aristata
[G]
Haworthia subg.
Hexangulares
Haworthia subg.
Haworthia
Aloe Section
Macrifoliae

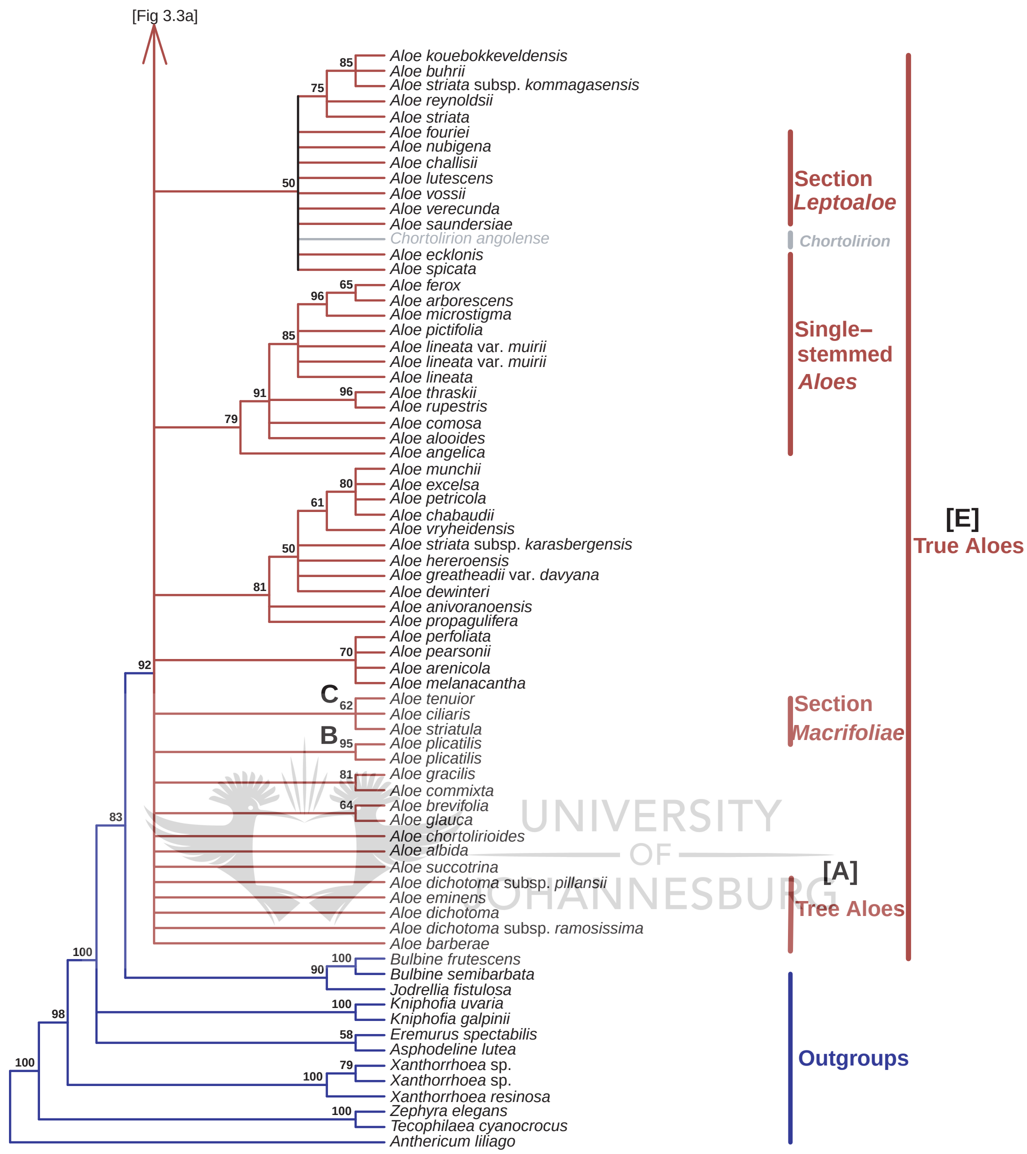
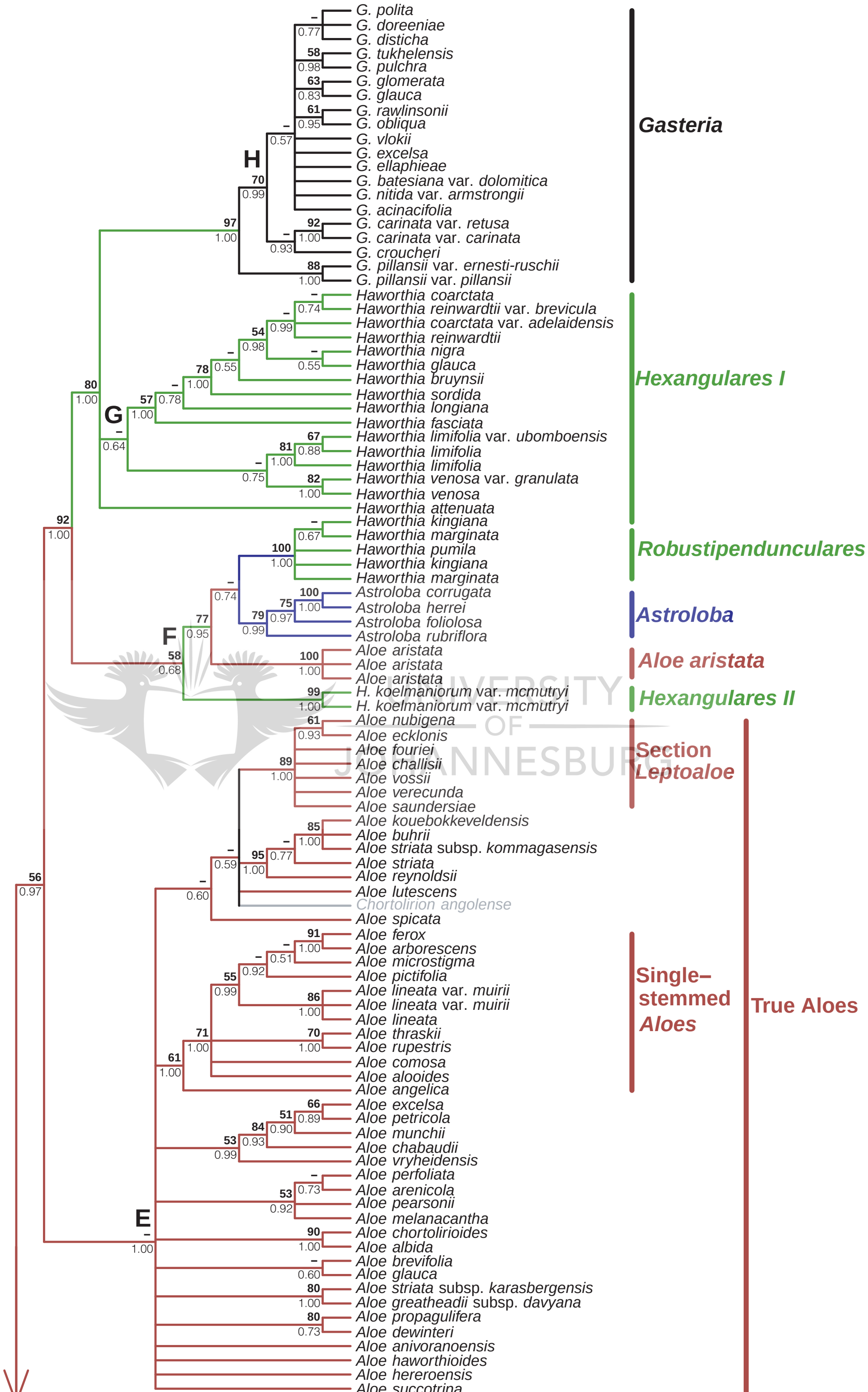


Figure 3.3 One of the most parsimonious trees obtained from the maximum parsimony (MP) analyses of the ITS1 data set for Alooideae (TL = 346; CI = 0.64; RI = 0.92).


G. polita
G. doreeniae
G. disticha
G. tukhelensis
G. pulchra
G. glomerata
G. glauca
G. rawlinsonii
G. obliqua
G. vlokii
G. excelsa
G. ellaphieae
G. batesiana var. dolomitica
G. nitida var. armstrongii
G. acinacifolia
G. carinata var. retusa
G. carinata var. carinata
G. croucheri
G. pillansii var. ernesti-ruschii
G. pillansii var. pillansii
Haworthia coarctata
Haworthia reinwardtii var. brevicula
Haworthia coarctata var. adelaidensis
Haworthia reinwardtii
Haworthia nigra
Haworthia glauca
Haworthia bruynsii
Haworthia sordida
Haworthia longiana
Haworthia fasciata
Haworthia limifolia var. ubomboensis
Haworthia limifolia
Haworthia limifolia
Haworthia venosa var. granulata
Haworthia venosa
Haworthia attenuata
Haworthia kingiana
Haworthia marginata
Haworthia pumila
Haworthia kingiana
Haworthia marginata
Astroloba corrugata
Astroloba herrei
Astroloba foliolosa
Astroloba rubriflora
Aloe aristata
Aloe aristata
Aloe aristata
H. koelmaniorum var. mcmutryi
H. koelmaniorum var. mcmutryi
Aloe nubigena
Aloe ecklonis
Aloe fouriei
Aloe challisii
Aloe vossii
Aloe verecunda
Aloe saundersiae
Aloe kouebokkeveldensis
Aloe buhrii
Aloe striata subsp. kommagasensis
Aloe striata
Aloe reynoldsii
Aloe lutescens
Chortolirion angolense
Aloe spicata
Aloe ferox
Aloe arborescens
Aloe microstigma
Aloe pictifolia
Aloe lineata var. muirii
Aloe lineata var. muirii
Aloe lineata
Aloe thraskii
Aloe rupestris
Aloe comosa
Aloe alooides
Aloe angelica
Aloe excelsa
Aloe petricola
Aloe munchii
Aloe chabaudii
Aloe vryheidensis
Aloe perfoliata
Aloe arenicola
Aloe pearsonii
Aloe melanacantha
Aloe chortolirioides
Aloe albida
Aloe brevifolia
Aloe glauca
Aloe striata subsp. karasbergensis
Aloe greatheadii subsp. davyana
Aloe propagulifera
Aloe dewinteri
Aloe anivoranoensis
Aloe haworthioides
Aloe hereroensis
Aloe succotrina
H
G
F
E
Gasteria
Hexangulares I
Robustipendunculares
Astroloba
Aloe aristata
Hexangulares II
Section Leptoaloe
Single–stemmed Aloes
True Aloes

[Fig 3.4b]

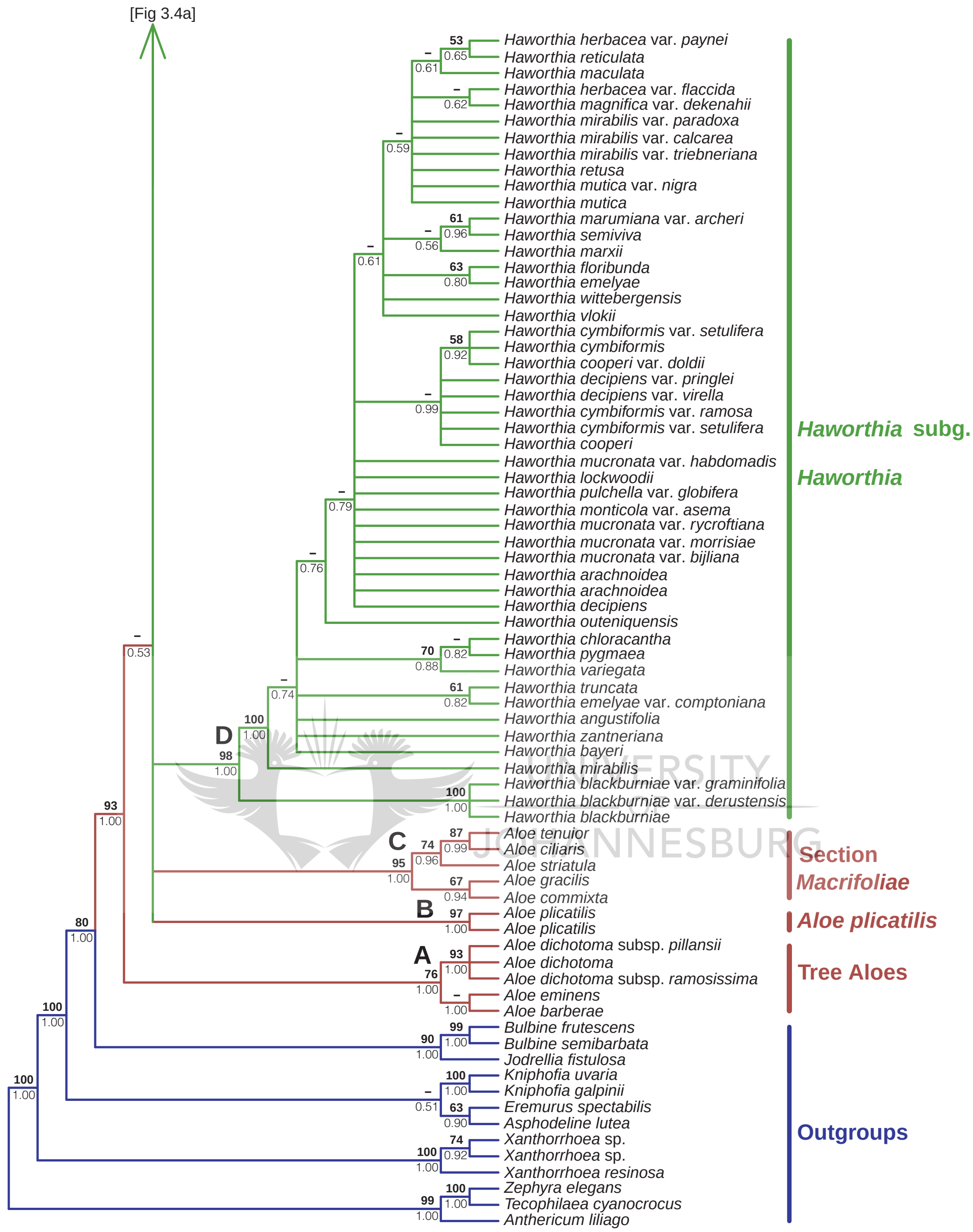


Figure 3.4 Majority-rule consensus tree obtained from the Bayesian analysis of the combined plastid and nuclear internal transcribed spacer (ITS1) data set for Alooideae plus outgroup (TL = 1159; CI = 0.63; RI = 0.89). Numbers above the branches are MP bootstrap support and ones below are BI posterior probabilities.

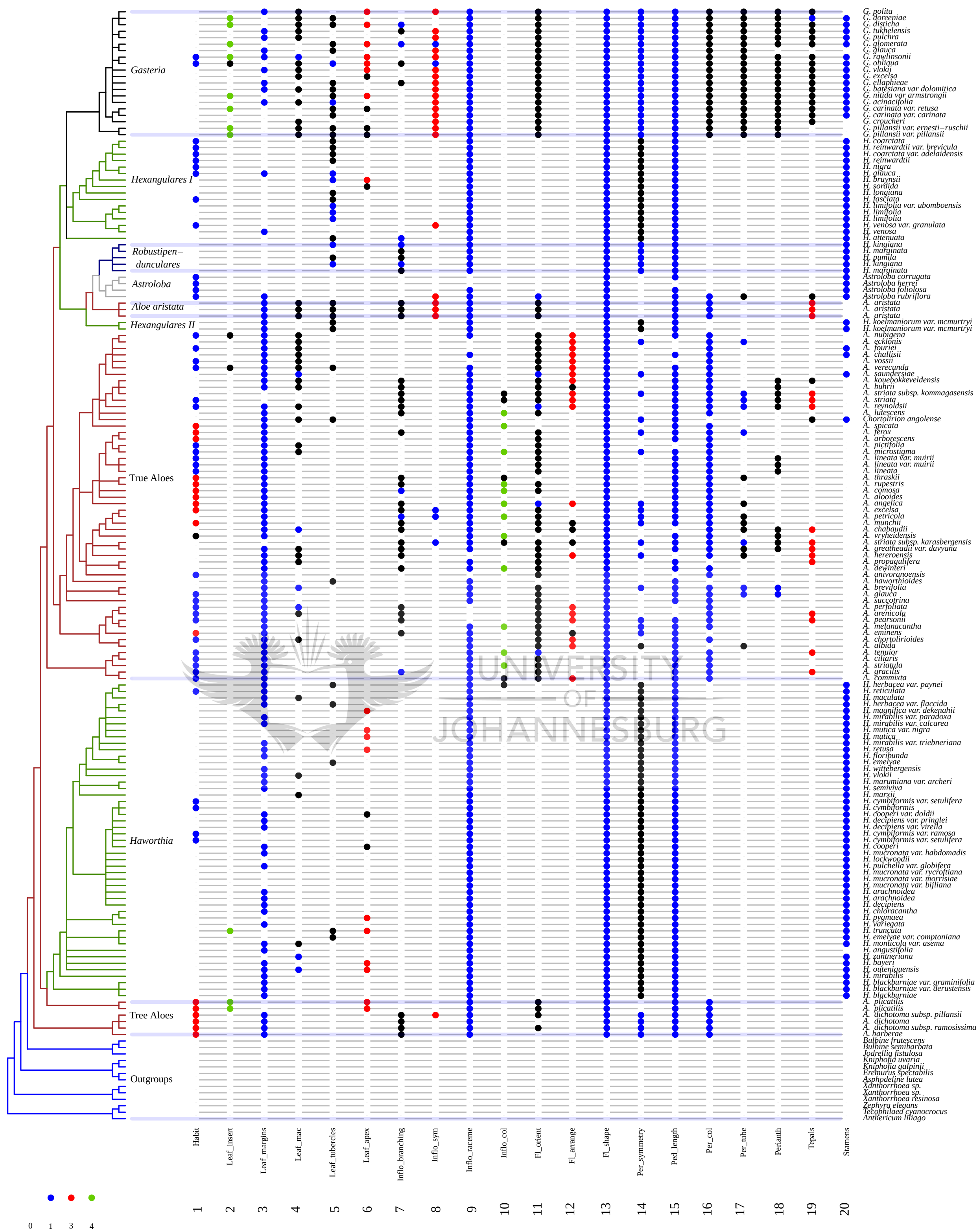


Figure 3.5 Distribution of morphological traits along phylogeny of Alooideae. All trait values are centred and scaled, resulting in circles of different colours. The colours of the symbols are proportional to the absolute value of each trait.

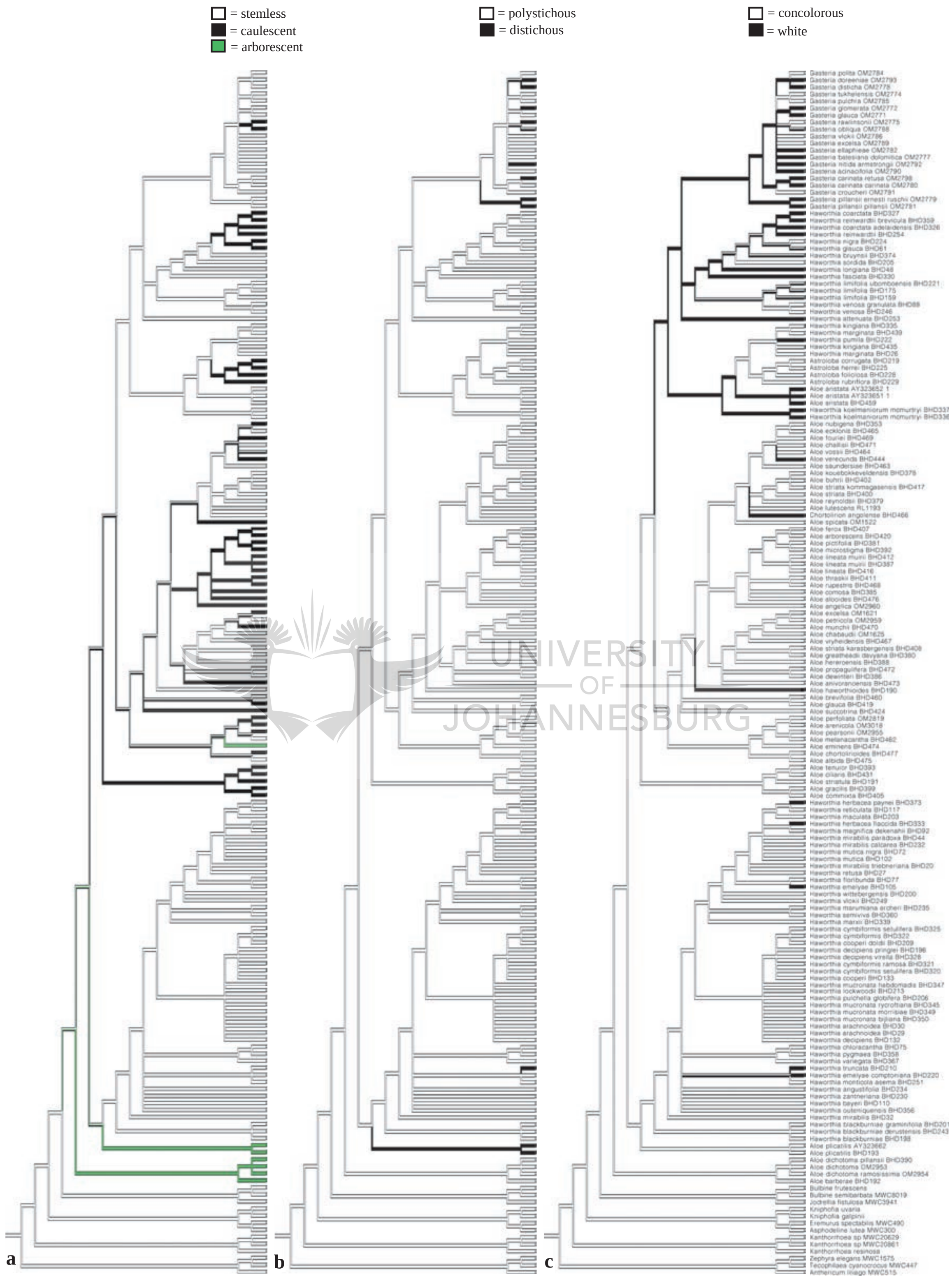


Figure 3.6 Parsimony-based reconstructions of a) habit character (character 1), b) leaf insertion (character 2), and c) leaf tubercles (character 5) on the majority-rule consensus tree from the combined molecular data.

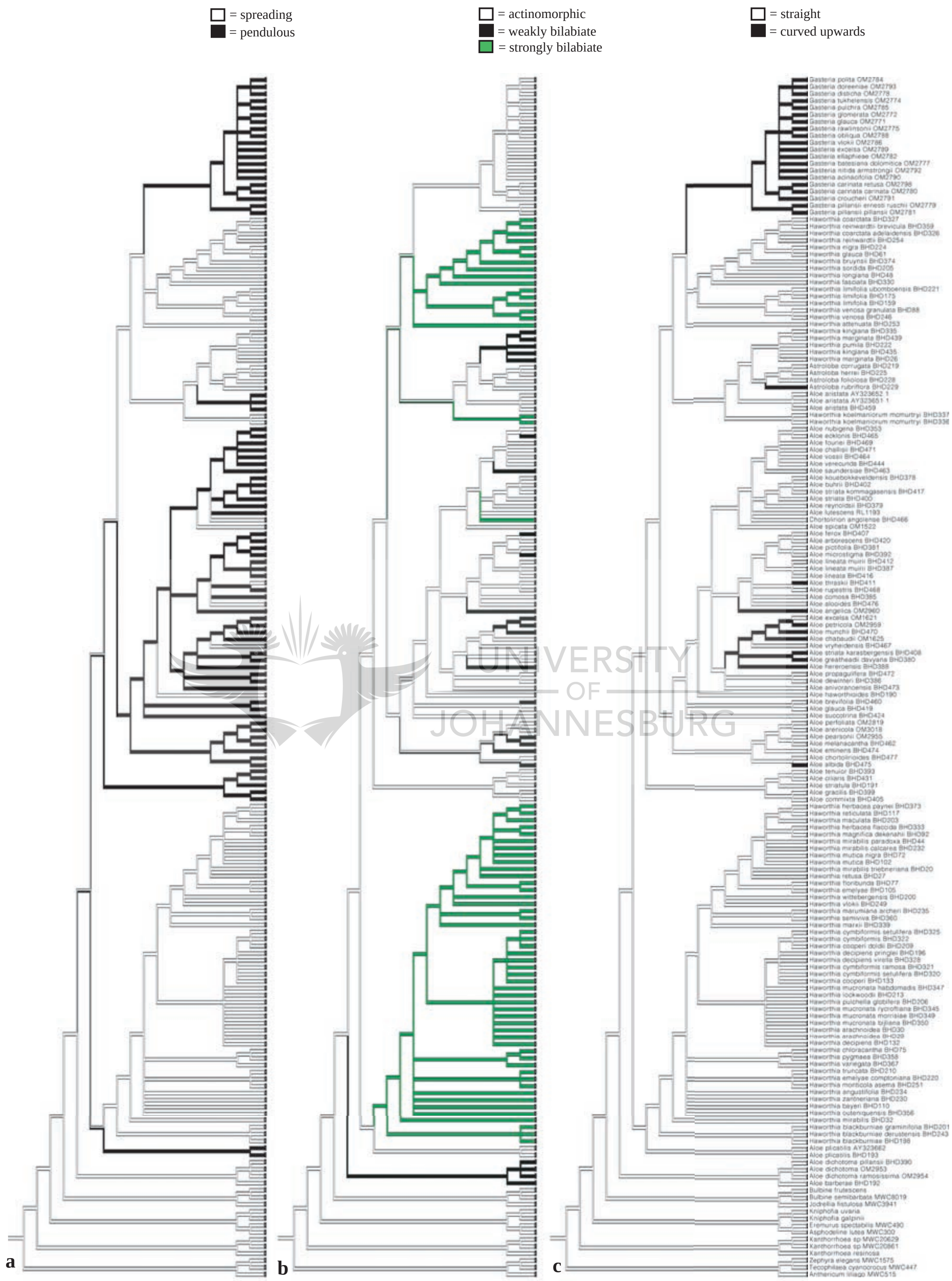


Figure 3.7 Parsimony-based reconstructions of a) flower orientation (character 11), b) periath symmetry (character 14), and c) perianth tube (character 17) on the majority-rule consensus tree from the combined molecular data.

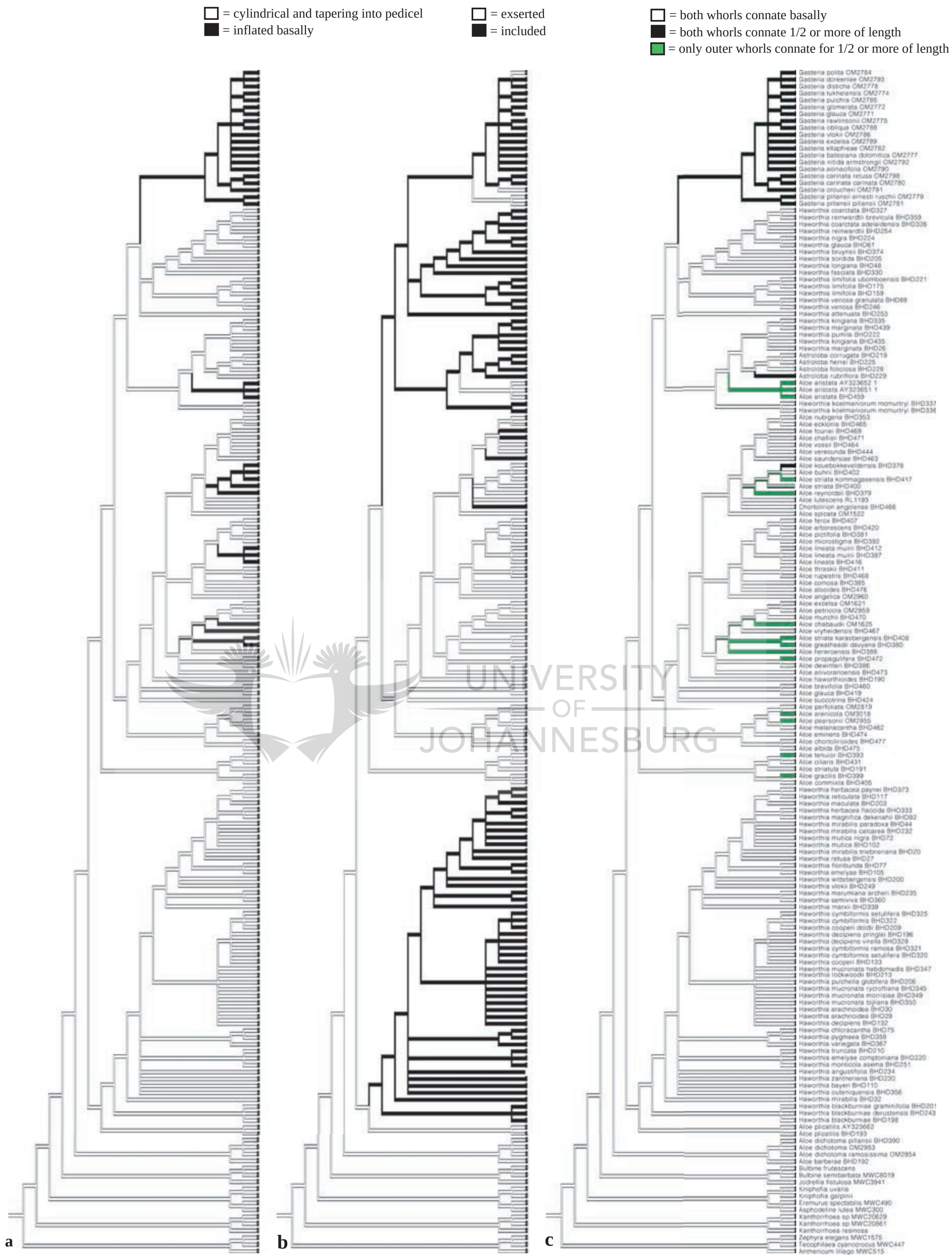


Figure 3.8 Parsimony-based reconstructions of a) periath shape (character 18), b) stamen inclusion (character 20), and c) tepal fusion (character 19) on the majority-rule consensus tree from the combined molecular data.

CHAPTER FOUR

Discussion and Conclusion

4. DISCUSSION AND CONCLUSIONS

4.1 Phylogenetic Relationships within Alooideae

Based on congruence between the plastid and nuclear DNA data sets, the discussion is restricted to results obtained from the combined data set (Fig. 3.4). Results strongly confirm the monophyly of Alooideae as previously shown by Linnaeus (1753) and more recently by Treutlein *et al.* (2003a, b) and Ramdhani *et al*. (2011). Furthermore, the genus *Aloe* is polyphyletic as currently circumscribed, with all other genera of Alooideae nested in different parts of *Aloe*. The analysis identifies seven primary monophyletic lineages in Alooideae, corresponding to the following currently recognised generic and infrageneric groups:

1. *Aloe* sect. *Dracoaloe* + *Aloe* sect. *Aloidendron*
2. *Aloe* sect. *Macrifoliae*
3. *Haworthia* subg. *Haworthia*
4. *Haworthia* subg. *Robustipendunculares* + *Astroloba* + *Aloe* sect. *Aristatae*
5. *Haworthia* subg. *Hexangulares*
6. *Gasteria*
7. Remaining *Aloe* spp.

This topology is congruent with that derived earlier by Treutlein *et al*. (2003b) with much smaller taxon sampling and analysis of just two plastid gene regions. Among the smaller segregate genera, only *Gasteria* and *Astroloba* are retrieved as monophyletic (*Chortolirion* is monotypic). The analysis indicated that *Haworthia* is not monophyletic but rather represents three lineages corresponding more or less to the subgenera proposed by Bayer (1982, 1999).

4.1.1 Relationships within *Aloe*

Relationships within Aloe sect. Dracoaloe + sect. Aloidendron **—** The analysis did not retrieved the 'tree aloes' sensu Van Wyk & Smith (2003) as a monophyletic group, but rather as two separate lineages. Lineage 1 includes sections *Aloidendron* + *Dracoaloe* which appears to be the earliest diverging lineage within the subfamily. *Aloe* section *Dracoaloe* (*A. dichotoma, A. dichotoma* subsp. *ramosissima,* and subsp. *pillansii*) known as the "Quiver tree" (Van Wyk & Smith, 2003), occurs in the Northern Cape region and Namibia. Capitate raceme is typical of this group (Glen & Hardy, 2000). The section *Aloidendron* comprise only of *Aloe barberae* Dyer, which is the largest African *Aloe* species (growing up to 10-18 m tall). It is distributed along the eastern coast of southern Africa (Mpumalanga, Swaziland, Kwazulu-Natal, Eastern Cape and Mozambique). *Aloe plicatilis* (lineage 2) (also tree *Aloe* sensu Van Wyk & Smith, 2003, endemic to the Cape Floristic region) did not group with the other 'tree aloes', but was retrieved as the early diverging lineage to *Haworthia* subgenus *Haworthia.* Although all three sections share a tree-like growth form, arborescence appears to have developed convergently in several *Aloe* lineages. I also recovered a strong support for *A. eminens* grouping with *A. barberae* (*A. bainesii* sensu Reynolds, 1966). This grouping might be a confirmation of their similar arborescent habit, although they are geographically isolated (*A. barberae* from South Africa and *A. eminens* from Somalia) plus the slight differences in some floral details, my analysis places them together as part of the earliest diverging lineage within Alooideae.

Relationships within Aloe section Macrifoliae **—** *Aloe* sect. *Macrifoliae,* the 'rambling Aloes' (sensu Van Wyk & Smith, 2003), comprising five closely related species (*A. ciliaris, A. commixta, A. gracilis, A. striatula,* and *A. tenuior*), is recovered as a strongly supported clade sister to the true aloes + all segregate genera. The cane-like stems of these species, with their slender, sheathing, unspotted and mesophytic leaves with minute marginal teeth are unique to members of this section (Glen & Hardy, 2000).

Relationships within the true Aloe species — The relationships among the remaining species of *Aloe* included in the analysis are poorly resolved. Although some of the currently recognised sections may be monophyletic, other sections are clearly not, and a much more extensive sampling is required to evaluate taxonomic and evolutionary relationships among them. The position of *Chortolirion* is noteworthy, where it is deeply embedded within this group, as part of a clade including most of the grass aloes. However the precise relationships are still unclear. Close morphological similarity in vegetative parts, namely the grass-like leaves with bulb-like swelling, and the small, bilabiate flowers, to species such as *A. bowiea* and *A. inconspicua* suggest a close relationship to sect. *Leptoaloe*. This resemblance between *Chortolirion* and the grass aloes was also pointed out in previous studies (e.g. Mabberley, 2008; Treutlein *et al.*, 2003b).

4.1.2 Relationships within *Haworthia*

Relationships within Haworthia subg. Haworthia — Species of *H.* subgenus *Haworthia* comprise a strongly supported monophyletic clade, defined morphologically by the basally triangular perianth, obclavate flowers and upcurved style (Bayer, 1982, 1999). Additional support for this comes from Smith *et al*. (2001), which reported the occurrence of lower (< than 50 %) sucrose concentrations in *H.* subg. *Haworthia* in contrast to higher (> 60 %) sucrose concentrations in subg. *Hexangulares* and *Robustipendunculares.*

Relationships within Haworthia subg. Robustipendunculares + Astroloba + Aloe sect. Aristatae — *Haworthia subg. Robustipendunculares* is a small group of three species (*Haworthia marginata, H. kingiana,* and *H. pumila*) that is well-supported as monophyletic and sister to *Astroloba*. Members of subg. *Robustipendunculares* are often large in size, about 100-300 mm across (with the largest been *H. pumila*) and restricted to the southern parts of the Western Cape, from Worcester to Mossel Bay. It is defined morphologically by its more or less straight perianth abruptly joined to the pedicel (Bayer, 1982, 1999). The flower type found in *H*. subg. *Robustipendunculares* is not dissimilar to that in *Astroloba*, differing essentially in its slight zygomorphy. Members of *Robustipendunculares* are often robust with attenuate leaves, often scabrid and patterned with white tubercles (Bayer, 1999). Sister to subg. *Robustipendunculares* + *Astroloba* is *Aloe aristata*, which is unique in the genus *Aloe* by having "*Haworthia*-like" leaves with dry awn-tipped apices and white tubercles (Glen & Hardy, 2000). The sister relationship between *Astroloba* and *Haworthia* subg. *Robustipendunculares* is further supported by similarities in nectar sucrose proportions (Van Wyk *et al*., 1993).

Relationships within Haworthia subg. Hexangulares — Bayer (1999) recognised 16 species within *H*. subg. *Hexangularis***.** The current analysis however, indicates that *H*. subg. *Hexangulares* is polyphyletic, with *H. koelmanniorum* more closely related to *H*. subg. *Robustipendunculares*. *Haworthia koelmanniorum* occupies an isolated position sister to subg. *Robustipendunculares* + *Astroloba* + *Aloe aristata.* The geographical distribution of this species is also disjunct from that of other species of *Haworthia*. Significantly, species of *Hexangulares* display the largest vegetative diversity in *Haworthia* with some closely resembling members of *Astroloba* and *Robustipendunculares* respectively, in their vegetative morphology.

4.1.3 Relationships within smaller segregate genera

***Relationships within Astroloba* —** *Astroloba*, consisting of 6 species, are retrieved as a monophyletic clade sister to *Haworthia* subg. *Robustipendunculares*. The genus is morphologically defined by its caulescent habit with stiff, imbricate leaves, and small, actinomorphic flowers with included stamens. Results from this study confirm the inclusion of *Poellnitzia rubriflora within Astroloba.*

***Relationships within Gasteria* —** *Gasteria* forms a strongly supported monophyletic clade with several morphological synapomorphies, notably the unarmed, verrucose leaves and inclined secund inflorescences of pendulous, gasteriform flowers with a well-developed floral tube (Van Jaarsveld, 2007). Bimodal karyotype is uniform in all *Gasteria* species and consists of eight large and six chromosomes (Vosa & Bennett, 1990).

4.2 Evolution of selected morphological characters in Alooideae

I analysed 20 morphological characters commonly used to differentiate between Alooideae species, following extensive literature review (as presented in section 2.5). I then reconstructed the likely evolutionary history of nine morphological characters that are important in generic and infrageneric delimitation within the subfamily Alooideae onto the majority rule consensus tree from the BI. The importance of these selected characters in Alooideae systematics are not only employed in previous Alooideae systematics (e.g. Bayer, 1999; Smith & van Wyk, 1991; Van Jaarsveld, 1994), they are shown to be unique to certain clades in this study.

***Habit.* —** Acaulescence is plesiomorphic within Alooideae, with certain lineages characterised by secondary caulescence and arborescence (Fig. 3.6a). Caulescence has developed several times in the subfamily, including *Aloe* itself (notably section *Macrifoliae*) and in some of the segregate genera, notably *Astroloba* and also some species of *Gasteria* and *Haworthia* subg. *Hexangulares*. Arborescence is relatively uncommon in the subfamily but is characteristic of species in *Aloe* sect. *Aloidendron* and *Dracoaloe*, the clade including *A. eminens*. It is implicit in previous classifications (e.g. Reynolds, 1966; Holland, 1978) that arborescence in *Aloe* is the derived state. Although the cytogenetic analysis by Brandham (1983) proposed that scandent *Aloe* species with usually relatively mesophytic leaves, e.g. *A. tenuior* Haw. and *A. ciliaris* Haw. (sect. *Macrifoliae*), represent the primitive state in *Aloe* species, our analysis suggests that caulescence is derived in the genus. Smith & van Wyk (1991) argued that both small, highly succulent taxa and arborescent forms were derived from a mesophytic, comparatively acaulescent taxon. The current study supports this hypothesis, showing that arborescence in *Aloe* is found not only in early diverging lineages but also in others deeply embedded within *Aloe*, and that the grass *Aloes* (sect. *Leptoaloe*) are clearly derived.

***Leaf insertion* —** Polystichous leaf insertion is common within the subfamily, and distichous leaf insertion is rare, having evolved independently several times in *Aloe*, once in *Haworthia* and possibly once in *Gasteria* (Fig. 3.6b). Distichy is evidently the juvenile condition, present in all *Aloe* and *Gasteria* seedlings, and its persistence in adult plants is best interpreted as neoteny. The ecological advantages are unclear.

***Leaf tuberculation* —** The presence of white tubercles on the leaves is largely restricted to *Gasteria* and *Haworthia* subg. *Hexangulares* and *Robustipendunculares* but tubercules are also developed in some *Aloe* species (e.g. *Aloe aristata*, *A. haworthioides* and *A. verrecunda*) and in the genus *Chortolirion* (Fig. 3.6c). Tuberculation is certainly a derived condition, as hypothesised by Smith & van Wyk (1991). In *Gasteria*, Van Jaarsveld (1994) has proposed that its evolution was driven by the absence of the bitter constituent typical of *Aloe* species, suggesting that the rigid tubercles may make the leaves less palatable. This remains to be tested.

***Perianth colour* —** Vivid, yellow, orange or reddish flowers are clearly plesiomorphic for the subfamily, and are strongly associated with ornithophily. The whitish or greenish perianth that is characteristic of *Haworthia* and *Astroloba* (excluding *Poellnitzia*) and several species of *Aloe*, especially several members of sect. *Leptaloae*, appear to be adaptations to entomophily (Botes *et al*., 2008; Hargreaves *et al*., 2008) and are derived.

***Flower orientation at anthesis* —** Representatives of *Astroloba*, *Chortolirion* and *Haworthia*, bear flowers ascending on vertical peduncles. This is hypothesised by Smith & Van Wyk (1991) to be the derived condition, which developed in response to a specialised, insect pollination syndrome. Pendulous flowers at anthesis are found in all *Gasteria* and some *Aloe* species. Flower orientation in *Gasteria* appears to be an adaptation to bird pollination, the horizontal peduncles acting as perches for bird visitors such as sunbirds (Nectariidae; Smith & Van Wyk, 1991), enabling easy access to nectar reward.

***Tepal connation* —** The perianth in Alooideae comprises six tepals arranged in two whorls of three each, variously connate into a short or prominent tube. The plesiomorphic condition is for the tepals to be basally connate only, and this is charateristic even in bird pollinated *Aloe* species (Fig. 3.8c). In several of the ornithopilous *Aloe* species, however, the outer tepal whorl is connate in the basal half, forming a distinct perianth tube. Tepals with both whorls connate for half or more of their length are diagnostic for *Gasteria*, *Astroloba* (*Poellnitzia*) *rubriflora*, and *Aloe kouebokkeveldensis* and appear to have evolved independently in these three lineages from ancestral types with tepals connate at the base only. Floral syndromes indicate that all of these taxa are bird pollinated and the results from this study suggest that in *Gasteria* and *Astroloba* these are secondary modification of an entomophilous *Haworthia*-type flower rather than developments from a more typical *Aloe*-type flower.

Haworthia-type flowers are small, spreading flowers with whitish, sometimes strongly bilabiate perianths, and included anthers are diagnostic of the genus *Haworthia* (Bayer, 1999). Cladistic analysis suggests that this flower type is homoplasious, and that it has evolved independently at least three and possibly four times (once each in *Chortolirion* and *H.* subg. *Haworthia* and once or twice in subg. *Hexangulares/Robustipendunculares* with possible reversals in *A. aristata* and *Gasteria*) (Figs. 3.7a & b). Relatively short-tubed whitish or sometimes cream flowers in some *Aloe* species (e.g. *Aloe inconspicua*) have been revealed as an adaptation to insect pollination (Botes *et al*., 2009). Although still recognisably '*Aloe*-like', the flowers of bee-pollinated *Aloe* species (sensu Botes *et al.*, 2009) such as *A. linearifolia* and *A. minima*, display several characteristics of the *Haworthia*-flower apart from reduced size, namely their nearly horizontal orientation, whitish and weakly bilabiate perianth, and sometimes included stamens. In term of perianth symmetry, Smith & van Wyk (1991) suggested that zygomorphy (as revealed from the flowers of *Astroloba, Chortolirion* and *Haworthia*) represents an advanced state derived from the plesiomorphic actinomorphic pattern, and the results in this study confirmed this hypothesis.

Gasteria-type flowers are curved and flask-shaped with an ovoid, inflated tube at least half as long as the perianth, and included or shortly exserted stamens, is characteristic of the genus *Gasteria*. The flowers in the genus are also often bicoloured, with greenish tips to the tepals, and are borne secund on inclined racemes. Flask-shaped flowers with inflated bases occur widely in *Aloe* and have evidently evolved several times (Fig. 3.8a), although in this genus the stamens are often but not always exserted (Fig. 3.8b). Similar flowers are characteristic also of *Poellnitzia* but here are uniquely held erect on an inclined raceme. A well-developed perianth tube formed by the fusion of both tepal whorls has evolved independently in *Gasteria*, *Astroloba* and *Aloe kouebokkeveldensis* (Fig. 3.8c).

4.3 Conclusions

Results of this study were largely conducted with new data collected for four gene regions (*rbcLa*, *matK*, *trnH-psbA* and ITS1), with greater sampling than in any previously published analyses (e.g. Treutlein *et al*., 2003a, b; Ramdhani *et al*., 2011) and essentially congruent with those of earlier studies. Evidence from morphological (Smith & Steyn, 2004), cytological (Taylor, 1925), cladistic (Smith & Van Wyk, 1991; Klopper *et al*., 2010) and chemical analyses (Viljoen *et al*., 1998; Viljoen, 1999) have been routinely applied to the current classification of Alooideae, which recognises five genera. These studies, although unable to resolve the relationships within the subfamily, provided a working hypothesis for this study.

Gasteria is well defined morphologically by its verrucose, edentate leaves and inclined inflorescences with ± secund, 'gasteriform' flowers, and it is not coincidental that it was one of the earliest groups of species to be recognised. The phylogenetic analysis places *Gasteria* sister to *Haworthia* subg. *Hexangulares*, which includes species with remarkably similar leaves, and the unique *Gasteria*-type flowers, are most parsimoniously interpreted as a reversion to bird-pollination from the entomophilous *Haworthia*-type flower with included stamens. One of the most unexpected results of the analyses is the apparent convergent evolution of the *Haworthia*-type flower. It appears to have evolved at least three times: at least twice and possibly three times in *Haworthia* itself and independently in *Chortolirion*. The close phylogenetic association between *Poellnitzia*, *Astroloba* and *Haworthia* subg. *Robustipendunculares* is reflected in the vegetative similarity among these three genera, and the floral differences between them are in effect very minor, relying on differences in perianth symmetry, size and colour. Indeed, Manning & Smith (2000) proposed the inclusion of *Poellnitzia* in *Astroloba* and this was confirmed in the current study. It is significant that *Aloe aristata*, treated as the monoptypic sect. *Aristatae* on the basis of its distinctive foliage (edentate, aristate and strikingly marked with transverse verrucae), is shown to be closely allied with *H.* sect. *Robustipendunculares* + *Astroloba*. Vegetatively, *A. aristata* would fit perfectly in *H.* sect. *Robustipendunculares*.

It is thus clear that few of the characters used to define genera within Alooideae at present are autapomorphies, and *Aloe* already includes most of the variation evident in the subfamily. Furthermore, *Aloe* is shown to be polyphyletic with the other four Alooideae genera nested in different parts of *Aloe*. There is an increasing trend to align classifications with phylogenies at all taxonomic levels, and for taxa to be circumscribed as monophyletic lineages (Backlund & Bremer, 1998). In Alooideae there are essentially just two options available. The first — splitter's approach — requires the recognition of at least four and possibly more additional segregates among *Aloe* in order to render a rump *Aloe* monophyletic, plus the fragmentation of *Haworthia* into three smaller genera. The second — lumper's approach — requires combining all of the genera into a single genus *Aloe*, with appropriate infrageneric groups.

Finally, the results present a milestone in Alooideae systematics with a phylogeny-driven generic classification proposed in which *Aloe* is expanded to include all members of Alooideae (Appendices II & III).

CHAPTER FIVE

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APPENDIX I

This appendix includes all abstracts of papers presented at conferences.

PAPER PRESENTATIONS

Molecular phylogeny of *Haworthia* (Asphodelaceae): Evidence from plastid and nuclear DNA sequences
Barnabas H. Daru[1], Olivier Maurin[1], Norman Maclean[2], Bruce M. Bayer[3], and Michelle van der Bank[1] [1]African Centre for DNA Barcoding, Faculty of Science, University of Johannesburg, P. O. Box 524 Auckland Park, 2006 Johannesburg, South Africa. [2]School of Biological Sciences, University of Southampton, Highfield, Southampton, Hants, SO16 7PX, United Kingdom. [3]PO Box 960, 7579 Kuilsriver, Western Cape, South Africa.
Haworthia Duval (Asparagales: Asphodelaceae: Alooideae) is endemic to southern Africa with modifications to withstand relatively waterless or desert environments. There is considerable variation in form between members of the genus and also, to a lesser extent, between populations of the same species. It is likely that cryptic species are yet to be described. Despite their importance in commercial horticulture, little is known about their evolutionary history and their genetic diversity, and the taxonomy remains unresolved. In the current study, generic limits of *Haworthia* were addressed using molecular sequenced data from three plastid (*rbcLa*, *matK* and *trnH-psbA*) and the nuclear ribosomal internal transcribed spacer (ITS1). Representatives of other genera within Alooideae were also included to infer the placement of the three subgenera of *Haworthia* within Alooideae. Preliminary findings were discussed.
Oral Presentation: 2011 Congress of the Southern African Society for Systematics Biologists (SASSB), Rhodes University, Grahamstown, South Africa. **Awarded the** *Best MSc Oral presentation SASSB Congress 2011*.

Phylogeny of the subfamily Alooideae (Asphodelaceae): Paraphyly of *Aloe* and *Haworthia* and consequences for classification

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Abstract

This study addresses generic delimitation of subfamily Alooideae (Asphodelaceae) using DNA sequences from plastid (*rbcLa*, *matK* and *trnH-psbA*) and nuclear (ITS1) regions plus morphological trait data. The plastid and nuclear DNA were analysed using maximum parsimony and bayesian statistics. The morphological data was mapped unto the molecular phylogeny. Parsimony and bayesian analyses of cpDNA and ITS1 combined together yielded two clades in Alooideae. The findings were discussed.

Keywords—Asphodelaceae, ITS1, *matK*, molecular phylogeny, morphology, nomenclature, *rbcLa*, *trnH-psbA*, taxonomy

Oral Presentation: South African Academy for Science and Arts Conference, University of Johannesburg, South Africa (2011)

Phylogeny of the subfamily Alooideae (Asphodelaceae): Paraphyly of *Aloe* and *Haworthia* and consequences for classification

Barnabas H. Daru[1,3,4], John C. Manning[2], James S. Boatwright[2,3], Olivier Maurin[1], and Michelle van der Bank[1]

[1]African Centre for DNA Barcoding, University of Johannesburg, P. O. Box 524 Auckland Park, 2006 Johannesburg, South Africa.

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[3]Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, Johannesburg, South Africa.

[4]Author for correspondence (darunabas@gmail.com)

Abstract

Alooideae (Asparagales–Asphodelaceae) currently comprises five genera, four of which are endemic to southern Africa. Despite their importance in commercial horticulture, evolutionary relationships among the genera are still incompletely understood, and the generic taxonomy remains unresolved. This study addresses generic delimitation in subfamily Alooideae using an expanded molecular sequenced datasets from three plastid regions (*rbcLa*, *matK* and *trnH-psbA*) and the first subunit of the nuclear ribosomal internal transcribed spacer (ITS1). Sequence data were analysed using maximum parsimony and bayesian statistics, and selected morphological traits were mapped onto the molecular phylogeny. Among the polytypic genera, only *Astroloba* and *Gasteria* were retrieved as monophyletic. *Haworthia* is shown to be polyphyletic and to comprise three main clades largely correllated with current subgeneric circumscriptions. The taxonomic implications of these findings are examined and a revised, phylogeny-driven generic classification is proposed in which the genus *Aloe* is expanded to include all members of Alooideae.

Keywords**—**Asphodelaceae, ITS1, *matK*, molecular phylogeny, morphology, nomenclature, *rbcLa*, *trnH-psbA*, taxonomy

Oral Presentation: South African Association of Botanists (SAAB) 38th Annual Conference, University of Pretoria, South Africa (2012).

APPENDIX II

Paper submitted to *Taxon* – 15 December 2011 (under review).

Phylogeny of the subfamily Alooideae (Asphodelaceae): Paraphyly of *Aloe* and *Haworthia* and consequences for classification

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Abstract
Alooideae (Asparagales–Asphodelaceae) currently comprises five genera, four of which are endemic to southern Africa. Despite their importance in commercial horticulture, evolutionary relationships among the genera are still incompletely understood, and the generic taxonomy remains unresolved. This study addresses generic delimitation in subfamily Alooideae using an expanded molecular sequenced datasets from three plastid regions (*rbcLa*, *matK* and *trnH-psbA*) and the first subunit of the nuclear ribosomal internal transcribed spacer (ITS1). Sequence data were analysed using maximum parsimony and bayesian statistics, and selected morphological traits were mapped onto the molecular phylogeny. Among the polytypic genera, only *Astroloba* and *Gasteria* were retrieved as monophyletic. *Haworthia* is shown to be polyphyletic and to comprise three main clades largely correllated with current subgeneric circumscriptions. The taxonomic implications of these findings are examined and a revised, phylogeny-driven generic classification is proposed in which the genus *Aloe* is expanded to include all members of Alooideae.

Keywords Asphodelaceae, ITS1, *matK,* molecular phylogeny, morphology, nomenclature, *rbcLa, trnH-psbA*, taxonomy.

APPENDIX III

The taxonomic changes in Alooideae resulting from the current study.

TAXONOMY

Aloe L., Sp. Pl.: 319. 1753. Type species: *A. perfoliata* L.

I. Section *Aloidendron* A.Berger in Bot. Jahrb. 36: 48. 1905. Type: *Aloe barberae* T.-Dyer

II. Section *Dracoaloe* A.Berger in Bot. Jahrb. 36: 48. 1905. Type: *Aloe dichotoma* Masson

III. Section *Kumara* (Medik.) Baker in J. Linn. Soc. Bot. 18: 155. 1880. Type: *Aloe plicatilis* (L.) Mill.

IV. Section *Reticulatae* Salm-Dyck in Monographia generum Aloes et Mesembryanthemi 1: sect. 10. 1836–1863. Lectotype species, here designated: *Aloe reticulata* Haw.

=*Haworthia* Duval, Pl. Succ. Horto Alencon.: 7. 1809. nom. cons., **syn. nov.** Type: *Haworthia arachnoidea* (L.) Duval.

=*Apicra* Willd., Mag. Neuesten Entdeck. Gesammten Naturk. Ges. Naturf. Freunde Berlin 5: 167. 1811. *nom. illegit*. *pro Haworthia* Duval – Type: not designated.

= *Aloe* section *Retusae* Salm-Dyck in Monographia generum Aloes et Mesembryanthemi 1: sect. 9. 1836–1863., **syn. nov.** – Lectotype species, here designated: *Aloe retusa* L.

= *Aloe* section *Limpidae* Salm-Dyck in Monographia generum Aloes et Mesembryanthemi 1: sect. 11. 1836–1863., **syn. nov.** – Lectotype species, here designated: *Aloe cymbifolia* Schad. [=*Aloe cymbiformis* Haw.]. [note: as there is no nominate species in this section, the first listed species is chosen as lectotype].

= *Aloe* section *Setatae* Salm-Dyck in Monographia generum Aloes et Mesembryanthemi 1: sect. 12. 1836–1863., **syn. nov.** – Lectotype species, here designated: *Aloe setosa* Schult. & Schult.f. [=*Aloe arachnoidea* (L.) Thunb.].

= *Aloe* section *Loratae* Salm-Dyck in Monographia generum Aloes et Mesembryanthemi 1: sect. 13. 1836–1863., **syn. nov.** – Lectotype species, here designated: *Aloe chloracantha* (Haw.) Schult. & Schult.f.

1. ***Aloe arachnoidea*** [as *arachnoides*] (L.) Thunb., Fl. Cap.: 311. 1823. ≡ *Aloe pumila* var. *arachnoidea* L., Sp.Pl.: 322. 1753. ≡ *Haworthia arachnoidea* (L.) Duval., Pl.Succ. Horto Alencon.: 7. 1809. – Type: Illustration in Commelin Praeludea Bot.: t. 27. 1703. (lecto, designated by Scott, 1977); South Africa, [Western Cape], Buitenstekloof, Langvlei, *Bayer 153* (NBG, epi., designated by Breuer & Metzing, 1997).

2. ***Aloe bayeri*** (J.D.Venter & S.A.Hammer) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia bayeri* J.D.Venter & S.A.Hammer in Cact. Succ. J. (US) 69: 75. 1997. –Type: South Africa, [Eastern Cape], hills S of Uniondale, *Steyner s.n.* (NBG, holo.).

3. ***Aloe blackburniae*** (W.F.Barker) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia blackburniae* W.F.Barker, J. S. Afr. Bot 3: 93. 1937. – Type: South Africa, [Western Cape], Calitzdorp, *Reynolds 1842* (NBG, holo.; BOL, PRE, iso.).

4. ***Aloe bolusii*** (Baker) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia bolusii* Baker, J. Linn. Soc. 18: 215. 1880. – Type: South Africa, [Eastern Cape], Graaff-Reinet, *Bolus 158* (K, holo.; BOL, iso.).

5. ***Aloe chloracantha*** (Haw.) Schult. & Schult. f., Syst. Veg. 7(1): 641. 1829. ≡ *Haworthia chloracantha* Haw., Saxifrag. Enum. 2: 57. 1821. – Type: South Africa, [Western Cape], N of Herbertsdale, *Bayer s.n.* (NBG, neo., designated by Breuer & Metzing, 1997).

6. ***Aloe cymbiformis*** Haw., Trans. Linn. Soc. 7: 8. 1804. ≡ *Haworthia cymbiformis* (Haw.) Duval, Pl. Succ. Horto Alencon.: 7. 1809. – Type: South Africa, [Eastern Cape], Walmer, *Smith 2844* (NBG, neo., designated by Breuer & Metzing, 1997).

7. ***Aloe denticulata*** (Haw.) Schult. & Schult. f., Syst. Veg. 7(1): 639. 1829. ≡ *Haworthia denticulata* Haw., Rev. Pl. Succ.: 58. 1821. – Type: Illustration in K (lecto., designated by Bayer, 1999).

=*Haworthia aristata* Haw., Suppl. Pl. Succ.: 51. 1819., non *Aloe aristata* Haw. 1825. – Type: Illustration in K (lecto., designated by Bayer, 1999); South Africa, [Eastern Cape], Dead Man's Gulch, *Smith 3550* (NBG, epi., designated by Bayer, 1999).

8. ***Aloe emelyae*** (Poelln.) Boatwr. & J.C.Manning, comb. nov. ≡ *Haworthia emelyae* Poell., Repert. Spec. Nov. Regni Veg. 42: 271. 1937. – Type: Unpublished photograph "*Haworthia Emelyae* v. P." (B, lecto., designated by Breuer & Metzing, 1997).

9. ***Aloe floribunda*** (Poelln.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia floribunda* Poelln., Repert. Spec. Nov. Regni Veg. 40:149. 1936. – Type: Unpublished photograph "*Haworthia floribunda* v. P." (B, neo., designated by Bayer, 1982); South Africa, [Western Cape], Black Down, 3.2 km N of Heidelberg, *Bayer 158* (NBG, epi., designated by Breuer & Metzing, 1997).

10. ***Aloe heidelbergensis*** (G.G.Sm.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Hawortia heidelbergensis* G.G.Sm. in J. S. Afr. Bot. 14: 42. 1948. – Type: South Africa, [Western Cape], W of Heidelberg, *Smith 6566* (NBG, holo.; NBG, iso.).

11. ***Aloe herbacea*** Mill., Gard. Dict. ed. 8: Aloe No. 18. 1768. ≡ *Haworthia herbacea* (Mill.) Stearn in Cact. J. (Croydon) 7: 40. 1938. – Type: Illustration in Boerhaave, Ind. Alter Hort. Lugd.-Bat. 2: ad p. 131. 1720 (lecto, designated by Bayer, 1972b); South Africa, [Western Cape], N of Ribbokkop, SW of Worcester, *Bayer 161* (NBG, epi., designated by Breuer & Metzing, 1997).

12. ***Aloe lockwoodii*** (Archibald) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia lockwoodii* Archibald in Fl. Pl. S. Afr.: ad t. 792. 1940. – Type: South Africa, [Western Cape], Laingsburg, *Lockwood Hill 215* (GRA, holo.).

13. ***Aloe magnifica*** (Poelln.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia magnifica* Poelln., Repert. Spec. Nov. Regni Veg. 33:240. 1933. – Type: South Africa, [Western Cape], Riversdale, *Ferguson s.n.* (BOL, lecto., designated by Breuer & Metzing, 1997).

14. ***Aloe maraisii*** (Poelln.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia maraisii* Poelln, Repert. Spec. Nov. Regni Veg 38: 194. (1935). – Type: Unpublished photograph "*Haworthia Maraisii* v. P." (B, lecto., designated by Bayer, 1999).

15. ***Aloe marumiana*** (Uitewaal) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia marumiana* Uitewaal in Cact. & Vetpl. 6:33. 1940. – Type: South Africa, [Western Cape], *Ladismith ex hort. Stellenbosch sub No. 6610* (AMD, holo.).

16. ***Aloe mirabilis*** Haw. in Trans. Linn. Soc. London 7: 9. 1804. ≡ *Haworthia mirabilis* (Haw.) Haw., Syn. Pl. Succ.: 95. 1812. – Type: Curtis's Bot. Mag.: t. 1354. 1811. (epi., designated by Bayer, 1977); South Africa, [Western Cape], Skuitsberg, between Caledon and Greyton, *Bayer 2453* (NBG, epi., designated by Breuer & Metzing, 1997).

17. ***Aloe monticola*** (Fourc.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia monticola* Fourc., Trans. Roy. Soc. S. Afr. 21: 78. 1937. – Type: South Africa, George and Uniondale Districts, *Fourcade 2498* (K, holo.).

18. ***Aloe mucronata*** (Haw.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia mucronata* Haw., Suppl. Pl. Succ.: 50. 1819. – Type: Unpublished illustration "*Haworthia mucronata* Haw." (k, lecto., designated by Bayer 1999).

19. ***Aloe mutica*** (Haw.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia mutica* Haw., Saxifrag. Enum 2: 55. 1821. – type: Illustration in Excelsa 8: 50. 1978. (lecto., designated by Bayer 1978); South Africa, [Western Cape], Bredasdorp to Swellendam, NE of Soesrivier bridge, *Bayer s.n.* (NBG, epi., designated by Breuer & Mitzing, 1997).

20. ***Aloe nortieri*** (G.G.Sm.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia nortieri* G.G.Sm. in J. S. Afr. Bot. 12: 13. 1946. – Type: South Africa, [Western Cape], Doorn River between Clanwilliam and Vanrhynsdorp, *Smith 6115* (NBG, holo.; NBG, iso.).

21. ***Aloe outeniquensis*** (M.B.Bayer) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia outeniquensis* M.B.Bayer, Haworthia Revisited: 130. 1999. – Type: South Africa, [Western Cape], Moerasrivier, *Venter, Marx & Kent 94/61* (NBG, holo.).

22. ***Aloe parksiana*** (Poelln.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia parksiana* Poelln., Repert. Spec. Nov. Regni Veg. 41: 205. 1937. – Type: Illustration published in Desert Pl. Life 10: 48. 1938 "*Haw. Parksiana* v. P." (lecto., designated by Breuer & Metzing, 1997).

23. ***Aloe pilifera*** (Baker) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia pilifera* Baker, Saund. Ref. Bot. 4: t. 234. 1871. – Type: Illustration in Saund. Ref. Bot. 4: t. 234. 1871. (lecto., designated by Bayer, 1999).

=*Haworthia cooperi* Baker, Saund. Ref. Bot. 4: t. 233. 1871., non *Aloe cooperi* Baker (1874) – Type: South Africa, [Eastern Cape], without precise locality, *Cooper s.n.* (K, holo.).

24. ***Aloe pringlei*** (C.L.Scott) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia pringlei* C.L.Scott in Bradleya 12: 103. 1994. – Type: South Africa, [Eastern Cape], Adelaide Distr., *Scott s.n.* (PRE, holo.).

=*Haworthia decipiens* Poelln, Repert. Spec. Nov. Regni Veg. 28: 103. 1930., non *Aloe decipiens* Schult. & Schult. f. (1829) [=*Aloe nitida* Salm-Dyck] – Type: South Africa, [Eastern Cape], Zuurberg at Georgida, *Fourcade 4367* (BOL, neo., designated by Breuer & Metzing, 1997).

25. ***Aloe pubescens*** (M.B.Bayer) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia pubescens* M.B.Bayer in J.S. Afr. Bot. 38: 125. 1972. – Type: South Africa, [Western Cape], Sandberg Hills, 12 km SSE of Worcester, *Bayer 161* (NBG, holo.).

26. ***Aloe pulchella*** (M.B.Bayer) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia pulchella* M.B.Bayer in J. S. Afr. Bot. 39: 232. 1973. – Type: South Africa, [Western Cape], Avondrust, SE of Touwsrivier, *Bayer 162* (NBG, holo.).

27. ***Aloe pygmaea*** (Poelln.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia pygmaea* Poelln., Repert. Spec. Nov. Regni Veg. 27: 132. 1929. – Type: South Africa, [Western Cape], hills E of Great Brak River, *Fourcade 4759* (BOL, neo., designated by Breuer & Metzing, 1997).

28. ***Aloe reticulata*** Haw. in Trans. Linn. Soc. London 7: 9. 1804. ≡ *Haworthia reticulata* (Haw.) Haw., Syn. Pl. Succ.: 94. 1812. – Type: Illustration in Curtis's Bot. Mag.: t. 1314. 1810. (neo., designated by Bayer, 1972a); South Africa, [Western Cape], Ribbokkop, 19.2 km SW of Worcester, *Bayer 160* (NBG, holo.).

29. ***Aloe retusa*** L., Sp. Pl.: 322. 1753. ≡ *Haworthia retusa* (L.) Duval., Pl. Succ. Alencon.: 7. 1809. – Type: Illustration in Commelin, Horti Med. Amstelad. 2: t. 6. 1701. (lecto., designated by Scott, 1985); South Africa, [Western Cape], Blikbonnie, 2 km E of Riversdale, *Dekenah s.n.* (NBG, epi., designated by Breuer & Metzing, 1997).

30. ***Aloe semiviva*** (Poelln.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia bolusii* var. *semiviva* Poelln. in Repert Spec. Nov. Regni Veg. 44: 40. 1928. ≡ *Haworthia semiviva* (Poelln.) M.B.Bayer, Haworthia Handbook: 153. 1976. – Type: Illustration in Succulenta (Netherlands) 22: 25. 1940. "*Haw. Bolusii* var. *semiviva* v. P. Typ." (lecto., designated by Breuer & Metzing, 1997).

31. ***Aloe serrata*** (M.B.Bayer) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia serrata* M.B. Bayer in J. S. Afr. Bot. 39: 249. 1973. – Type: South Africa, [Western Cape], Oudekraalkop, 40 km SW of Heidelbereg, *Bayer 166* (NBG, holo.).

32. ***Aloe springbokvlaktensis*** (C.L.Scott) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia springbokvlaktensis* [as *springbokvlakensis*] C.L.Scott in J. S. Afr. Bot. 36:

287. 1970. – Type: South Africa, [Eastern Cape], Steytlerville, Farm Springbokvlakte, *Scott 245* (PRE, holo.).

33. ***Aloe stenophylla*** Schult. & Schult. f., Syst. Veg. 7(1): 641. 1829. – Type: Illustration in Salm-Dyck Monographia generum Aloes et Mesembryanthemi 1 sect. 13: fig. 2. 1836–1863. (neo, designated by Bayer, 1999); Epitype: South Africa, [Eastern Cape], Highlands, *Cooke s.n.* (NBG, epi, designated by Bayer, 1999).

=*Haworthia angustifolia* Haw. in Philos. Mag. J. 66: 283. 1825. non *Aloe angustifolia* Haw. 1819. [= *Aloe africana* Mill.] – Type: South Africa, [Eastern Cape], 34 km from Grahamstown to Alicedale, *Bruyns 1653* (NBG, neo, designated by Breuer & Metzing, 1997).

34. ***Aloe translucens*** Haw. in Trans. Linn. Soc. London: 10. 1804. ≡ *Haworthia translucens* (Haw.) Haw., Suppl. Pl. Succ.: 52. 1819. – Type: South Africa, [Eastern Cape], 2 km E of Hankey, *Bayer 4476* (NBG, neo, designated by Breuer & Metzing, 1997).

=*Haworthia gracilis* Poelln., Feddes. Repert. Spec. Nov. 27: 133. 1929. – Type: Illustration in B.; South Africa, [Eastern Cape], Helspoort, *Britten s.n.* (PRE, epi., designated by Bayer, 1999).

35. ***Aloe truncata*** (Schönland) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia truncata* Schönland in Trans. Roy. Soc. S. Afr. 1: 391. 1910. – Type: South Africa, [Western Cape], a farm 11.2 km from Oudtshoorn, *Britten s.n.* (K., holo.).

36. ***Aloe turgida*** (Haw.) Schult. & Schult. f., Syst. Veg. 7(1): 635. 1829. ≡ *Haworthia turgida* Haw., Suppl. Pl. Succ.: 52. 1819. – Type: South Africa, [Western Cape],

Swellendam, Breede River Bridge, *Bayer 2420* (NBG, neo., designated by Breuer & Metzing, 1997).

37. ***Aloe punctulata*** Boatwr. & J.C.Manning, **nom. nov.**, pro *Haworthia variegata* L.Bolus in J. Bot. Soc. S. Afr.: 137. 1929., non *Aloe variegata* L. (1753) – Type: South Africa, [Western Cape], Still Bay, Botterkloof, *Ferguson s.n.* (BOL, holo.).

38. ***Aloe venteri*** Boatwr. & J.C.Manning **nom. nov.** *pro*. *Haworthia maculata* (Poelln.) M.B.Bayer in Haworthia Handbook: 130. 1976. ≡ *Haworthia schuldtiana* var. *maculata* Poelln., in Repert. Spec. Bot. Regni Veg. 49: 25. 1940., non *Aloe maculata* All. 1773. – Type: South Africa, [Western Cape], Worcester, *Venter 6* (BOL, lecto., designated by Breuer & Metzing, 1997)

= *Haworthia intermedia* Poelln., Kakteenkunde 9: 133. 1937., non. *Aloe intermedia* Haw. 1804. [=*Aloe carinata* Mill.]. – Type: Unpublished photograph, "*Haworthia intermedia* v. P." (B, lecto., designated by Bayer, 1999); South Africa, [Western Cape], Buitenstekloof, *Bayer 4461* (NBG, epi., designated by Bayer, 1999).

39. ***Aloe vlokii*** (M.B.Bayer) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia vlokii* M.B.Bayer in Haworthia Revisited: 160. 1999. – Type: South Africa, [Western Cape], Swartberg Mountains, *Vlok sub Venter 91/2* (NBG, holo.).

40. ***Aloe wittebergensis*** (W.F.Barker) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia wittebergensis* W.F.Barker in J. S. Afr. Bot. 8: 245. 1942. – Type: South Africa, [Western Cape], Witteberg, *Pieterse s.n.* (NBG, holo.).

41. ***Aloe zantneriana*** (Poelln.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia zantneriana* Poelln. in Repert. Spec. Nov. Regni Veg. 41: 217. 1937. – Type: Illustration

in Desert Pl. Life 9: 90. 1937. "*Haworthia Zantneriana* v. P." (lecto., designated by Breuer & Metzing, 1997).

V. Section *Macrifoliae* (Haw.) Glen & D.S.Hardy, FSA 5 (1, 1): 92. 2000. Type: *Aloe ciliaris* Haw.

VI. Section *Aristatae* (A.Berger) Glen & D.S.Hardy, FSA 5 (1, 1): 31. 2000. Type: *Aloe aristata* Haw.

VII. Section *Poellnitzia* (Uitewaal) Boatwr. & J.C.Manning, **comb. et stat. nov.** ≡ *Poellnitzia* Uitewaal in Succulenta 22: 61. 1940. – Type: *Apicra rubriflora* L.Bolus [=*Poellnitzia rubriflora* (L.Bolus) Uitewaal].

1. ***Aloe rubriflora*** (L.Bolus) G.D.Rowley in Cactus & Succulent Journal of Great Britain 43: 2. 1981. ≡ *Apicra rubriflora* L. Bolus in Ann. Bolus Herb. 3: 13. 1920. – Type: South Africa [Western Cape], Bonnie Vale [Bonnievale], *Smith s.n. BOL45213* (BOL, holo.).

VIII. Section *Imbricatae* Salm-Dyck in Monographia generum Aloes et Mesembryanthemi 1: sect. 1. 1836–1863. Lectotype species, designated here: *Aloe imbricata* Haw. [=*Aloe spiralis* L.].

=Section *Foliolosae* Salm-Dyck in Monographia generum Aloes et Mesembryanthemi 1: sect. 2. 1836–1863, **syn. nov.** Lectotype species, here designated: *Aloe foliolosa* Haw.

=*Astroloba* Uitewaal in Succulenta 5: 53. 1947, **syn. nov.** Type: *Astroloba pentagona* (Ait.) Uitewaal [=*Aloe spiralis* L.].

1. ***Aloe bullulata*** Jacq., Fragm. Bot. 72. t. 109. ≡ *Astroloba bullulata* (Jacq.) Uitewaal in Succulenta 5: 53. 1947. – Type: Illustration in Fragm. Bot. 72. t. 109.

2. ***Aloe congesta*** Salm-Dyck in Monographia generum Aloes et Mesembryanthemi 1: sect. 2, fig. 1. 1836–1863. ≡ *Astroloba congesta* (Salm-Dyck) Uitewaal in Succulenta 5: 54. 1947. – Type: Illustration in Monographia generum Aloes et Mesembryanthemi 1: sect. 2, fig. 1. 1836–1863.

3. ***Aloe corrugata*** (N.L.Mey. & G.F.Sm.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Astroloba corrugata* N.L.Mey. & G.F.Sm. in Bothalia 28: 61. 1998. – Type: South Africa, Western Cape, 7.5 km W of Warmwaterberg turnoff on Montagu-Ladismith road, *Van Jaarsveld 13913* (PRE, holo.).

4. ***Aloe foliolosa*** Haw. Trans. Linn. Soc. London 7: 7. 1804. ≡ *Astroloba foliolosa* (Haw.) Uitewaal in Succulenta 5: 54. 1947. – Type: Illustration in Curtis’s Bot. Mag. t. 1352. 1811. [This illustration was drawn from Masson’s original introduction cultivated by Haworth and is accepted as the type in the absence of a specimen.].

5. ***Aloe herrei*** (Uitewaal) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Astroloba herrei* Uitewaal in Desert Pl. Life 20. 37. 1948. – Type: Uniondale, *Herre s.n.* “Stellenbosch 5703” (?STEU, holo.).

6. ***Aloe spiralis*** L., Sp. Pl. 1: 322. 1753. ≡ *Astroloba spiralis* (L.) Uitewaal in Succulenta 5: 53. 1947. Type: “*Aloe Afr. erecta rotundata etc. Comm.*” in Dillenius, Hort. Eltham. 1: 16, t. 13, f. 14. 1732. (lecto., designated by Wijnands, 1983).

IX. Section ***Margaritiferae*** Salm-Dyck in Monographia generum Aloes et Mesembryanthemi 1: sect. 6. 1836–1863. ≡ *Haworthia* section *Margaritiferae* (Salm-Dyck) Haw. in Handbuch der Succulenten Pflanzen: 710. 1954. Lectotype, here designated: *Aloe pumila* L. [=*Haworthia margaritifera* (L.) Haw.]

≡*Haworthia* subgenus *Robustipedunculatae* (Uitewaal) M.B.Bayer [as *Robustipedunculares* Uitewaal ex M.B.Bayer] in Haworthia Handbook: 14. 1976. *Haworthia* [unranked] *Robustipedunculatae* Uitewaal in Succulenta 51. 1947. Lectotype, designated by Bayer in Haworthia Handbook: 14. 1976: *Haworthia margaritifera* (L.) Haw. [= *Aloe pumila* L.].

= *Aloe* section *Albicantes* Salm-Dyck in Monographia generum Aloes et Mesembryanthemi 1: sect. 5. 1836–1863., **syn. nov.** – Type: *Aloe albicans* Haw. [=*Aloe marginata* Lam.] [Note: this section is monotypic].

1. ***Aloe granata*** (Willd.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Apicra granata* Willd. in Berl. Mag.: 269. 1811. – Type: not seen.

=*Haworthia minima* (Ait.) Haw., Syn. Pl. Succ.: 92. 1812. ≡ *Aloe margaritefera* var. *minima* Ait., Hort. Kew 1:468. 1789. – Type: Illustration in Dillenius, Hort. Eltham.: t. 16, f. 18. 1732 (lecto., designated by Scott, 1985).

2. ***Aloe kingiana*** (Poelln.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia kingiana* Poelln. In Feddes Repert. 41: 203. 1937. – Type: South Africa, [Western Cape], Great Brak, *Dekenah 201* (NBG, neo., designated by Breuer & Metzing, 1997.).

3**. *Aloe marginata*** Lam., encycl. 1: 89. 1783. ≡ *Haworthia marginata* (Lam.) Stearn in Cact. J. (Croydon) 7: 34. 1938. – Type: Illustration in Commelin, Praeludia Bot.: t. 30. 1703 (lecto., designated by Scott, 1985).

4. ***Aloe pumila*** L., Sp. Pl.: 322. 1753. ≡ *Aloe pumila* var. *margaritifera* L., Sp. Pl.: 322. 1753. ≡ *Aloe margaritifera* (L.) Burm. f., Prodr. Fl. Cap.: 10. 1768. ≡ *Haworthia margaritifera* (L.) Haw., Suppl. Pl. Succ.: 55. 1819. ≡ *Haworthia pumila* (L.) M.B.Bayer, Haworthia revisited: 214. 1999.– Type: Illustration in Commelin, Hort Med. Amstelad. Pl. Rar. 2: 19, t. 10. 1701 (lecto., designated by Scott, 1978). [Although Breuer and Metzing (1997) treated the species under the name *H. margaritifera*, Scott's (1978) lectotypification renders this name homotypic with *Aloe pumila* L., which as the autonym has statutory priority (Jarvis, 2007).].

X. Section *Parviflorae* Haw. in Trans. Linn. Soc. London 7: 6. 1804. ≡ *Aloe* subgenus *Parviflorae* (Haw.) Salm-Dyck in Monographia generum Aloes et Mesembryanthemi 1. 1836–1863. Lectotype species, here designated: *Aloe viscosa* L. [the first species listed by Haworth in his *A.* section *Parviflorae* is here chosen as lectotype]

≡*Haworthia* section *Hexangulares* Uitewaal in Succulenta 5: 51. 1947, **syn. nov.** ≡ *Haworthia* subgenus *Hexangulares* (Uitewaal) M.B.Bayer [as Uitewaal ex M.B.Bayer] in Haworthia Handbook: 14. 1976. – Lectotype species, designated by Bayer (1976): *Haworthia coarctata* Haw.

= *Aloe* section *Triquetrae* Salm-Dyck in Monographia generum Aloes et Mesembryanthemi 1: sect. 3. 1836–1863., **syn. nov.** –Lectotype species, here designated:

Aloe cordifolia (Haw.) Schult. & Schult.f. [=*Aloe viscosa* L.] [note: as there is no nominate species in this section, the first listed species is chosen as lectotype].

= *Aloe* section *Tortuosae* Salm-Dyck in Monographia generum Aloes et Mesembryanthemi 1: sect. 4. 1836–1863., **syn. nov.** – Lectotype species, here designated: *Aloe tortuosa* Haw. [= *Aloe viscosa* L.]

= *Aloe* section *Luridae* Salm-Dyck in Monographia generum Aloes et Mesembryanthemi 1: sect. 7. 1836–1863., **syn. nov.** – Lectotype species, here designated: *Aloe scabra* (Haw.) Schult. & Schult.f. [Note: As there is no nominate species in this section, the first listed species is chosen as lectotype].

= *Aloe* section *Tessellatae* Salm-Dyck in Monographia generum Aloes et Mesembryanthemi 1: sect. 8. 1836–1863., **syn. nov.** – Lectotype species, here designated: *Aloe tessellata* (Haw.) Schult. & Schult.f. [=*Aloe venosa* Lam.].

1. ***Aloe attenuata*** Haw., Trans. Linn. Soc. 7: 11. 1804. ≡ *Haworthia attenuata* (Haw.) Haw., Syn. Pl. Succ.: 92. 1812. – South Africa, [Eastern Cape], Sandland, 20 km E of Paternsie, *Perry 660* (NBG, neo., designated by Breuer & Metzing, 1997).

2. ***Aloe brucei*** Boatwr. & J.C.Manning, **nom. nov.** pro *Haworthia pungens* M.B.Bayer in Haworthia Revisited: 188. 1999. non *Aloe pungens* A.Berger (1908). – Type: South Africa, [Eastern Cape], Joubertina, *Bruyns 7090* (BOL, holo.).

3. ***Aloe bruynsii*** (M.B.Bayer) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia bruynsii* M.B.Bayer in J.S. Afr. Bot. 47: 789. 1981. – Type: South Africa, [Eastern Cape], SE of Steytlerville, *Rossouw 456* (NBG, holo.).

4. ***Aloe coarctata*** (Haw.) Schult. & Schult. f., Syst. Veg. ed 15, v. 7(1): 647. 1829. ≡ *Haworthia coarctata* Haw., Phil. Mag. 44: 301. 1824. – Type: South Africa, [Eastern Cape], 16 km from Grahamstown to Bathurst, *Smith 7092* (NBG, neo., designated by Breuer & Metzing, 1997).

5. ***Aloe fasciata*** (Willd.) Salm-Dyck in Schult. & Schult. f., Syst. Veg. ed 15, v. 7(2): 1713. 1830. ≡ *Apicra fasciata* Willd. In Ges. Naturf. Freunde Berlin Mag. Neuesten Entdeck. Gesammten Naturk. 5: 270. 1811. ≡ *Haworthia fasciata* (Willd.) Haw., Saxifrag. Enum. 2: 54. 1821. – Type: South Africa, [Eastern Cape], Hankey, *Stayner s.n.* (NBG, neo., designated by Breuer & Metzing, 1997).

6. ***Aloe glabrata*** Salm-Dyck, Hort. Dyck.: 325. 1834. ≡ *Haworthia glabrata* (Salm-Dyck) Baker, J. Linn. Soc., Bot. 18: 206. 1880. – Type: Illustration in Salm-Dyck, Monographia generum Aloes et Mesembryanthemi 1 sect. 3: fig. 7 [sect. 6, fig. 13] (neo., designated by Smith & Greyling, 1990).

7. ***Aloe glauca*** (Baker) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia glauca* Baker, J. Linn. Soc. Bot. 18: 203. 1880. –Type: South Africa, [Eastern Cape], Cape, without precise locality, *Cooper s.n.* (K., holo.).

8. ***Aloe koelmaniorum*** (Oberm. & Hardy) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia koelmaniorum* [as *koelmaniora*] Oberm. & Hardy, Fl. Pl. Afr.: t1502. 1967. – Type: South Africa, [Mpumalanga], Groblersdal, *Hardy & Mauve 2267* (PRE, holo.).

9. ***Aloe limifolia*** (Marloth) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia limifolia* Marloth, Trans. Roy. Soc. S. Afr. 1: 409. 1910. – Type: Mozambique, W of Delagoa Bay, *Marloth 4678* (PRE, holo.).

10. ***Aloe longiana*** (Poelln.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia longiana* Poelln. in Repert. Spec. Nov. Regni Veg. 41: 203. 1937. – Type: unpublished photographic icon, "*Haworthia Longiana* v. P." (B, lecto. designated by Breuer & Metzing, 1997).

11. ***Aloe nigra*** (Haw.) Schult & Schult. f., Syst. Veg. ed 15, v. 7(1): 647. 1829. ≡ *Apicra nigra* Haw. in Philos. Mag. J 64: 302. 1824. ≡ *Haworthia nigra* (Haw.) Baker in J. Linn. Soc., Bot 18: 203. 1880. –Type: South Africa, [Eastern Cape], Campherspoort [Kamferspoort], *Barker 5099* (NBG, neo., designated by Breuer & Metzing, 1997).

12. ***Aloe reinwardtii*** Salm-Dyck in Observ. Bot. Horto Dyck: 37. 1821. ≡ *Haworthia reinwardtii* (Salm-Dyck) Haw., Saxifrag. Enum. 2: 53. 1821. – Type: Illustration in Salm-Dyck, Monographia generum Aloes et Mesembryanthemi 1 sect. 6: fig. 12. 1836–1863. (neo, designated by Scott, 1981a); South Africa, [Eastern Cape], near top of hill above Ncera River Bridge, *Smith 3563* (NBG, epi., designated by Breuer & Metzing, 1997).

13. ***Aloe scabra*** (Haw.) Schult. & Schult. f., Syst. Veg. ed 15, v. 7(1): 644. 1829. ≡ *Haworthia scabra* Haw., Suppl. Pl. Succ.: 58. 1819. – Type: South Africa, without precise locality, illustration in Cact. Succ. J. (Los Angeles) 52: 274. 1980. (lecto., designated by Scott, 1980).

14. ***Aloe sordida*** (Haw.) Schult. & Schult. f., Syst. Veg. ed 15, v. 7(1): 644. 1829. ≡ *Haworthia sordida* Haw. in Saxifrag. Enum. 2: 51. 1821. – Type: South Africa, without locality, illustration in Salm-Dyck, Monographia generum Aloes et Mesembryanthemi 1: sect. 7: fig. 1. 1836–1863. (neo, designated by Scott, 1985).

15. ***Aloe venosa*** Lam., Encycl. 1: 89. 1873. ≡ *Haworthia venosa* (Lam.) Haw., Saxifrag. Enum.: 51. 1821. – Type: South Africa, without locality, illustration in Commelin, Praeludia Bot.: t. 29. 1703. (lecto., designated by Scott, 1978); South Africa, [Western Cape], Swellendam, W of Breede River Bridge, *Bayer 168* (NBG, epi., designated by Breuer & Metzing, 1997).

16. ***Aloe viscosa*** L., Sp. Pl.: 322. 1753. ≡ *Haworthia viscosa* (L.) Haw., Syn. Pl. Succ.: 90. 1812. – Type: Illustration in Commelin, Praeludia Bot.: t. 31. 1703. (lecto., designated by Scott, 1981b); South Africa, [Western Cape], Calitzdorp, Blackburn Valley, *Barker 5073* (NBG, epi., designated by Breuer & Metzing, 1997).

XI. Section *Curviflorae* Haw. in Trans. Linn. Soc. Lond. 7: 12. 1804. – Lectotype species, here designated: *Aloe verrucosa* Mill. [=*Aloe carinata* Mill.] [Note: the species is selected as lectotype being the first listed in Haworth's section].

=*Gasteria* Duval, Plantae succulentae in Horto Alenconio: 6. 1809. ≡ *Aloe* section *Gasteria* [as *Gasteriae*] (Duval) Salm-Dyck, Monographia generum Aloes et Mesembryanthemi 1: sect. 29. 1836–1863., **syn. nov.** – Lectotype, designated by Maire in Flore de l'Afrique du nord 5: 71. 1958.: *Gasteria angustifolia* (Ait.) Duval. [= *Aloe disticha* L. non Thunb.].

=*Gasteria* section *Breviflorae* Haw., Rev. Pl. Succ.: 47. 1821. *nom. nud.*

=*Gasteria* section *Longiflorae* Haw., Rev. Pl. Succ.: 47. 1821. *nom. nud.*

=*Gasteria* section *Mediocres* Haw., Rev. Pl. Succ.: 47. 1821. *nom. nud.*

1. **Series *Bifariae*** Haw. in Trans. Linn. Soc. London 7: 12. 1804. Lectotype species, here designated: *Aloe verrucosa* Mill. [=*Aloe carinata* Mill.] [note: the species is selected as lectotype being the first listed in Haworth's series and displaying the characteristics of the series.].

1.1 ***Aloe acinacifolia*** J.Jacq., Ecloge pl. rar.: 49. 1811–1816. ≡ *Gasteria acinacifolia* (J.Jacq.) Haw., Suppl. Pl. succ.: 49. 1819. – Type: South Africa, without precise locality, illustration in J.Jacq., Ecloge pl. rar. t. 31. 1811–1816.

1.2. ***Aloe armstrongii*** (Schönland) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Gasteria armstrongii* Schönland in Records of the Albany Museum 2: 258. 1912. – Type: South Africa, without precise locality, *Drège 566* (GRA, holo.).

1.3. ***Aloe batesiana*** (G.D.Rowley) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Gasteria batesiana* G.D.Rowley in National Cactus & Succulent Journal 10: 32. 1955. – Type: South Africa, without locality, *Rowley s.n.* (RNG, neo., designated by Van Jaarsveld, 1992).

1.4. ***Aloe carinata*** Mill., Gard. Dict. ed. 8. 1768. ≡ *Gasteria carinata* (Mill.) Duval in Plantae succulentae in Horto Alenconio: 6. 1809. – Type: South Africa, without precise locality, illustration in Commelin, Hort. Med. Amst. t. 9. 1701., (lecto. designated by Wijnands, 1983).

1.5. ***Aloe croucheri*** Hook. f. in Curtis's Bot. Mag. 95: t. 5812. 1869. ≡ *Gasteria croucheri* (Hook. f.) Baker in J. Linn. Soc., Bot. 18: 196. 1880. – Type: South Africa, without precise locality, *Cooper s.n.* (K, holo.).

1.6. ***Aloe ellaphieae*** (Van Jaarsv.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Gasteria ellapieae* Van Jaarsv. in Journal of the Cactus & Succulent Society (US) 63: 3. 1991. – Type: South Africa [Eastern Cape], 20 km W of Patensie, *Van Jaarsveld et al. 9904* (NBG, holo.).

1.7. ***Aloe fuscopunctata*** (Baker) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Gasteria fuscopunctata* Baker in J. Linn. Soc. Bot. 18: 195. 1880. – Type: South Africa, "C. B Spei", *Cooper s.n.* (?K). [Note: selection of this name is based on the synonymy given in Van Jaarsveld (2007). In an earlier treatment (Van Jaarsveld, 1994) this species was treated under the synonymy of *G. acinacifolia*].

=*Gasteria excelsa* Baker in J. Linn. Soc. Bot. 18: 195. 1880. non *Aloe excelsa* A.Berger (1906) – Type: South Africa [Eastern Cape], Chalumna River, *Cooper s.n*. (K, holo.).

1.8. ***Aloe nitida*** Salm-Dyck, Catalogue raisonne des especes d'Aloes: 13. 1817. ≡ *Gasteria nitida* (Salm-Dyck) Haw. in Philosophical Mag. 2: 359. 1827. –Type: South Africa, without locality, illustration in Salm-Dyck, Monographia generum Aloes et Mesembryanthemi 1 sect. 29: fig. 17. 1836–1863 (neo., designated by Van Jaarsveld, 1992).

1.9. ***Aloe polita*** (Van Jaarsv.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Gasteria polita* Van Jaarsv. in Cactus & Succulent Journal (US) 73: 127–130. 2001. – Type: South Africa [Western Cape], Whisky Creek, Plettenberg Bay, *Van Jaarsveld & Kok 13742* (NBG, holo.).

1.10. ***Aloe pulchra*** (Ait.) Jacq., Pl. Rar. Hort. Schon.: t. 419. 1805 "1804". ≡ *Aloe maculata* var. *pulchra* Ait. in Hort. Kew.: 469. 1789. ≡ *Gasteria pulchra* (Ait.) Haw., Synopsis Plantarum Succulentarum: 86. 1812. – Type: South Africa, without precise locality, illustration in Miller, Fig. Pl.: t. 292. 1759 (lectotype designated by Van Jaarsveld, 1992).

1.11. ***Aloe thunbergii*** (N.E.Br.) Boatwr. & J.C.Manning, **comb.nov.** ≡ *Gasteria thunbergii* N.E.Br. in Bothalia 1: 140. 1923. – Type: South Africa, without precise locality, *Thunberg s.n. UPS-THUNB 8595* (UPS-THUNB, holo.).

1.12. ***Aloe tukhelensis*** (Van Jaarsv.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Gasteria tukhelensis* Van Jaarsv. in Bothalia 35: 164. 2005. –Type: South Africa, KwaZulu-Natal, Ngubevu, *Van Jaarsveld et al. 17996* (NBG, holo.).

1.13. ***Aloe vanjaarsveldii*** Boatwr. & J.C.Manning, **nom. nov.** pro *Gasteria glauca* Van Jaarsv. in Cactus & Succulent Journal (US) 70: 65. 1998, non *Aloe glauca* Mill. (1768) – Type: South Africa [Eastern Cape], Kouga River, E of Guernakop, *Van Jaarsveld & Welsh 14670* (PRE, holo.).

1.14. ***Aloe vlokii*** (Van Jaarsv.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Gasteria vlokii* Van Jaarsv. in Cactus and Succulent Journal (US) 59: 170. 1987. –Type: South Africa [Western Cape], Waboomsberg, *Vlok 880* (NBG, holo.).

2. **Series *Pictae*** Haw. in Trans Linn. Soc. London 7: 14. 1804. Type: *Aloe obliqua* Haw., nom. illegit. [=*Gasteria bicolor* Haw.]

2.1. ***Aloe baylissiana*** (Rauh.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Gasteria baulissiana* Rauh. in J. S.Afr. Bot. 43: 187. 1977. – Type: South Africa [Eastern Cape], Farm Oudekraal, S of Somerset-East, *Bayliss 2796* (HEID, holo.; PRE, iso.).

2.2. ***Aloe bicolor*** (Haw.) Schult. & Schult. f., Syst. Veg. ed 15, v. 7: 682. 1829. ≡ *Gasteria bicolor* Haw. in Philosophical Mag. 57: 275. 1826. – Type: South Africa, without locality, illustration in Salm-Dyck, Monographia generum Aloes & Mesembryanthemi 1 sect. 29: fig. 5. 1836–1863 (neo., designated by Van Jaarsveld, 1992).

2.3. ***Aloe brachyphylla*** Salm-Dyck in Monographia generum Aloes & Mesembryanthemi 1 sect. 29: fig. 8. 1836–1863. ≡ *Gasteria brachyphylla* (Saml-Dyck) Van Jaarsv. in Aloe 29:19. 1992. – Type: South Africa, without locality, illustration in Saml-Dyck, Monographia generum Aloes & Mesembryanthemi: t. 8. 1836–1863 (lecto., designated by Van Jaarsveld, 1992).

2.4. ***Aloe disticha*** L. Sp. Pl.: 321. 1753. ≡ *Gasteria disticha* (L.) Haw. in Philosophical Mag. 2: 352. 1927. –Type: South Africa, without precise locality, illustration in Commelin, Hort. Med. Amst. t. 8. 1701 (lecto., designated by Wijnands, 1983).

2.5. ***Aloe doreeniae*** (Van Jaarsv. & A.E.van Wyk) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Gasteria doreeniae* Van Jaarsv. & A.E.van Wyk in Aloe 41: 81. 2004. – Type: South Africa, Eastern Cape, Swartwaterspoort, W of Riebeeck East, *Quart 448* (NBG, holo.).

2.6. ***Aloe glomerata*** (Van Jaarsv.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Gasteria glomerata* Van Jaarsv. in Bradleya 9: 100. 1991. – Type: South Africa, [Eastern Cape], Kouga Dam, 25 km W of Patensie, *Van Jaarsveld & Sardien 11054* (NBG, holo.).

2.7. ***Aloe neliana*** (Poelln.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Gasteria neliana* Poelln. in Feddes Rep. 27: 139. 1930. non *Aloe pillansii* L.Guthrie (1928)–Type: South Africa, Clanwilliam, *Poellnitz 559* (holo., supposedly in STE, specimen not found).

=*Gasteria pillansii* Kensit in Trans. Roy. Soc. S. A. 1: 163. 1909. – Type: South Africa [Western Cape], Clanwilliam, *Pillans 833* (BOL, holo.; PRE, iso.).

2.8. ***Aloe rawlinsonii*** (Oberm.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Gasteria rawlinsonii* Oberm., Flow. Pl. Afr. 43: t. 1701. 1976. – Type: South Africa [Eastern Cape], Willowmore Distr., Baviaanskloof, *Rawlinson s.n. PRE34421* (PRE, holo.).

2.9. ***Aloe retusa*** (Van Jaarsv.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Gasteria carinata* var. *retusa* Van Jaarsv. in Aloe 29: 15. 1992. ≡ *Gasteria retusa* (Van Jaarsv) Van Jaarsv. in Aloe 44: 100. 2007. – Type: South Africa, [Western Cape], Orange Grove near De Wet, *Van Jaarsveld & Steyner 4656* (NBG, holo.).

XII. et seq. Remaining sections of *Aloe* s.s. (including *Lomatophyllum*, *Chortolirion*), following Glen & Hardy (2000) and Reynolds (1966, 1969).

Section *Chortolirion* (Berger) Boatwr. & J.C.Manning, **stat. et sect. nov.** ≡ *Chortolirion* Berger in Pflanxenr. 4, 38, 3, 2: 723. 1908., syn. nov. – Type: *Chortolirion angolense* (Bak.) Berger [=*Aloe tenuifolia* (Engl.) Boatwr. & J.C.Manning]

1. ***Aloe tenuifolia*** (Engl.) Boatwr. & J.C.Manning, **comb. nov.** ≡ *Haworthia tenuifolium* Engl. In Bot. Jahrb. 10: 2, t. 1. 1889. – Type: South Africa, [Northern Cape], near Kuruman, *Marloth 1049* (B, holo.; PRE, iso.).

=*Haworthia angolensis* Bak. in Trans. Linn. Soc. Bot. 1: 263. 1878. ≡ *Chortolirion angolense* (Bak.) Berger Berger in Pflanxenr. 4, 38, 3, 2: 723. 1908., non *Aloe angolensis* Bak. – Type: Angola, Huilla, *Welwitsch 3756* (BM, holo.).