Phylogenetic relationships in Asphodelaceae (subfamily Alooideae) inferred from chloroplast DNA sequences (\textit{rbcL}, \textit{matK}) and from genomic fingerprinting (ISSR)

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Two independent lines of molecular evidence have been studied to explore phylogenetic relationships in the family Asphodelaceae. Genomic fingerprinting by ISSR (Inter Simple Sequence Repeats) analysis was compared to sequence data of the chloroplast genes \textit{matK} and \textit{rbcL}. Molecular data indicate that some long-established taxonomic concepts would have to be re-evaluated. The subfamily Asphodeloideae clusters as a sister group to a distinctly monophyletic Alooideae. However, several Alooideae genera, including \textit{Aloe} and \textit{Haworthia}, are apparently not monophyletic. From a molecular point of view, \textit{Haworthia} can be divided into two distinct groups that agree closely with the current subgeneric classification: a monophyletic group including species of subgenus \textit{Haworthia}, and a second polyphyletic group with the subgenera \textit{Hexangulares} and \textit{Robustipedunculares}. This second clade includes \textit{Poellnitzia}, \textit{Astroloba}, \textit{Gasteria} and even one \textit{Haworthia}-like aloe (\textit{Aloe aristata}). In the polyphyletic assemblage currently classified as \textit{Aloe}, several smaller clades can be recognised, often reflecting morphological, chemical and geographical discontinuities. The tree aloes (sections \textit{Aloidendron} and \textit{Dracoaloe}) and climbing aloes (series \textit{Macrifoliae}) appear to have separated early in Alooideae, while other groups (e.g., the flavonoid-containing group and a Madagascan group) are embedded within and amongst other genera. \textit{Chortolirion} clusters with the grass-like aloes (section \textit{Graminialoe} Reynolds, syn. \textit{Leptaloe} Berger), \textit{A. boylei} and \textit{A. verecunda}, on a well-defined branch. The current taxonomic system clearly does not reflect the phylogenetic affinities and relationships amongst the succulent genera \textit{Aloe}, \textit{Astroloba}, \textit{Chortolirion}, \textit{Gasteria}, \textit{Haworthia}, and \textit{Poellnitzia}.

KEYWORDS: Asphodelaceae, ISSR, \textit{matK}, phylogeny, \textit{rbcL}.

INTRODUCTION

Asphodelaceae are a medium-sized petaloid monocot family in Asparagales (Smith & Van Wyk, 1998). It consists of two more or less well-defined subfamilies, Alooideae and Asphodeloideae, the latter with a predominantly Eurasian distribution, but with significant outliers in Africa, Australia and New Zealand. Most species are non-succulent. The other subfamily, Alooideae, has a distinctive southern African centre of radiation, with outliers in Saudi Arabia, Madagascar and some on the Mascarene islands off the African east coast. It consists mainly of rosulate leaf succulents. Asphodelaceae as circumscribed here comprise 13 genera including \textit{Lomatophyllum} Willd. (Rowley, 1996) and \textit{Poellnitzia} Uitewaal, which have been regarded as synonymous with \textit{Aloe} L. (Fig. 1) and \textit{Astroloba} Uitewaal, respectively (Manning & Smith, 2000). Asphodelaceae have a significant present-day centre of diversity in southern Africa, with at least 10 genera represented in the region (Smith & Meyer, 2000). The remaining three genera have predominantly Eurasian distributional ranges (Wendelbo, 1964; Tuzlaci, 1987; Díaz Lifante & Valdés, 1996).

Despite various attempts to provide a stable classification system for the two subfamilies of Asphodelaceae based on vegetative and reproductive features (Smith & Van Wyk, 1991, 1998), the interrelationships amongst genera, especially in Alooideae, are still unresolved. Even the circumscription of some genera is still seriously questioned (Smith & al., 1995; Manning & Smith, 2000). A recent molecular study by Chase & al. (2000) confirmed the monophyly of Alooideae and the paraphyly of Asphodeloideae as was suggested by Van Wyk & al. (1995) on the basis of morphological and chemical evi-
The monophyly of the family Asphodelaceae has been convincingly demonstrated based on the presence of arillate seeds (Dahlgren & al., 1985). The aril initiates from the distal part of the funicle during the early development of the ovule primordium (Steyn & Smith, 1998) and through a process of annular invagination gradually takes on the appearance of a third integument around the ovule. Likewise, genera of the subfamily Alooideae (Aloe and its succulent-leaved relatives) share a number of convincing apomorphies. These include: consistently hemitropic ovules (Steyn & Smith, 1998), a distinctly bimodal karyotype consisting of four long and three short chromosomes (Brandham, 1983; Smith, 1991), the presence of a parenchymatous, cap-like inner bundle sheath at the phloem poles (Beaumont & al., 1985; Smith & Van Wyk, 1992), and the presence of anthrone-C-glycosides in the leaves and 1-methyl-8-hydroxyanthraquinones in the roots (Van Wyk & al., 1995).

The taxonomy of at least Alooideae started off in a conservative way with Linnaeus (1753) who included all known species in a single genus, Aloe. Some 50 years later, Haworth (1804) split the genus into three distinct subunits, Grandiflorae (current generic concept of Aloe), Curviflorae (current generic concept of Gasteria) and Parviflorae (current generic concept of Haworthia and Astroloba). Shortly thereafter, the proliferation of generic names started, with Aloe being atomised into numerous smaller, “natural” genera. This eventually resulted in 29 genus names for the 800-odd species known today. However, only five genera are presently widely recognised in Alooideae, namely Aloe, Astroloba, Chortolirion, Gasteria and Haworthia.

In Asphodelaceae the investigation of cryptic characters, other than morphological ones, has compounded classification efforts rather than providing clarity (see, for example, Steyn & al., 1998, on palynology). Furthermore, a comprehensive survey of chemical characters in
Aloe has provided some new ideas about intra- and inter-
relationships, but also showed evidence of reticulation,
suggesting that hybridisation may have played an impor-
tant role in the evolution of the group (Viljoen, 1999).

Sequence analysis of chloroplast and nuclear genes has
become a powerful tool to understand phylogenetic and
phylogeographic relationships in plants (Soltis & al.,
1992, 1998). Additionally, the ISSR method has recently
been added to the growing list of molecular tools. ISSR
analysis is useful for testing genomic instability (Leroy
& al., 2000), genetic diversity (Kantety & al., 1995), cul-
tivar identification (Charters & al., 1996), molecular
mapping (Ratnaparkhe & al., 1998), in forensic DNA
profiling (Kumar & al., 2001) in plants, as well as for
sexing in birds (Wink & al., 1998) or detecting hybrids in
birds and reptiles (Wink & al., 2000). This PCR-based
method uses primers annealing to microsatellite repeats
to amplify the regions between adjacent, inversely orien-
tated SSRs, if they are close enough to allow exponential
multiplication. The method targets inversions, insertions,
deletions, and mutational events of microsatellites at
multiple loci in the genome. Individuals of the same
species usually show few to no differences between their
ISSR patterns, whereas closely related taxa, i.e., sub-
species and species give a specific banding profile that
can be used to solve phylogenetic questions. In the pres-
ent study, we used ISSR fingerprinting to corroborate the
groups resulting from chloroplast DNA sequences with a
predominantly nuclear marker, and to detect potential
chloroplast introgression.

In this study molecular data (sequence data of matK
and rbcL, and genomic ISSR analyses) of 12 genera of
Asphodelaceae (except Jodrellia Baijnath, a segregate of
Bulbine) were analysed. Results indicate that the genera
Aloe and Haworthia as traditionally circumscribed and currently widely accepted in the subfamily Alooideae appear to be polyphyletic. A lack of congruence between the molecular/genetic patterns and the current classification system had not been detected.

### MATERIAL AND METHODS

**Sample origin and sequence database accession numbers.** — Origin of samples is documented in Table 1. DNA has been deposited with EMBL Genbank (accession numbers AJ511369-AJ511450). DNA was isolated from fresh leaves of 55 taxa of the family Asphodelaceae and from Anthriscum liliago using the CTAB method (Doyle & Doyle, 1990) with minor mod-
ifications. Alterations were selective removal of the leaf epidermal layer and collection of about 0.5 cm² of the underlying green parenchyma tissue. The mucilaginous,
transparent inner pulp of the leaves was avoided. Author
citations of all species names are included in the list
below and are not repeated elsewhere.

**ISSR-PCR.** — Amplification, electrophoresis con-
ditions, and detection of ISSR-amplification products
were as follows. For amplification, 15 ng of total DNA
was used as template, plus 3 pmol primer 5'-GAC AGA
CAG ACA GAC A-3', 1.5 mM MgCl₂, 0.1 mM of dGTP,
dCTP, and dTTP, 0.075 mM dATP. 1 µCi [α-³²P]-dATP, 1.25 µl of 10× amplification buffer (100 mM Tris-HCl,
PH 8.5, 500 mM KCl, 5% Triton X-100) and 0.4 units Taq
polymerase (Amersham Pharmacia Biotech) in a
total volume of 12.5 µl. After an initial denaturation
(120s at 94°C), 33 cycles of 60s at 94°C, 120s at 55°C,
and 120s at 72°C were performed on a Biometra thermo-
cycler; then at 72°C for 4 min, followed by 4°C for stor-
age. PCR products were subjected to 0.2 mm denaturing
denaturing polyacrylamide gels at 65W for 3h (size 45 ×
30 cm). After drying, the gel was exposed to Kodak Hyperfilm
for two days and developed. The reaction was performed
several times to ensure reproducibility of the pattern.
The film was scanned and synapomorphic bands were
marked. Phylogenetic analysis was conducted using Nei-
Li restriction-site distance with UPGMA tree-building
method in PAUP 4.0b10 (Swofford, 2002).

Some of the microsatellite repeat primers examined
resulted in no amplification, i.e., (CA)₁₀, (CTG₇₄), and
(AG)₁₂, or in bands which were less well defined: i.e.,
(C₇₈), (GTG)₅, and (GGAT)₄. The failure in amplifica-
tion using these primers may be due to the absence or
sequence alteration of target repeats of these types in the
genomes of Alooideae. Banding patterns obtained by the
tetranucleotide primer (GACA)₄ are presented in Fig. 2,
indicating the presence of microsatellites of this type in
all taxa of the subfamily Alooideae sampled.

The ISSR primer (GACA)₄ distinguishes all acces-
sions of the alooids in producing a characteristic banding
profile for each taxon. Alterations of the ISSR-pattern
were detected as gains and losses of individual bands.
Faint bands that could not be unequivocally scored as
present or absent, or bands showing somewhat altered
electrophoretic mobility were not taken into account,
even if they might bear some phylogenetic information.
Most prominent shared banding patterns are marked by
white boxes (Fig. 2). These and other countable bands
were scored in a 1/0 matrix of 100 characters. UPGMA
analysis resulted in the tree given in Fig. 3. ISSR profiles
proved to be reproducible in several replicates.

**PCR and DNA sequencing.** — *MatK* was ampli-
fied by PCR using the primers *matK*-724F: 5'- CGC ACT
TAT CAT TTG ATA AC -3' (forward) and *matK*-2303R: 5'- CAT TTA GAA AAT CTA AGA ATG AAT C
-3' (reverse). PCR conditions: a final volume of 50 µL

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Table 1. Origin of plant samples, accession numbers of botanical gardens (BGC: Botanical Garden Puerto de la Cruz, Tenerife, Canary Islands; BGF: Freiburg Botanical Garden, Germany; BGH: Heidelberg Botanical Garden, Germany; BGJ: Botanical Garden Jena, Germany; BGM: Marburg Botanical Garden, Germany; BGP: Pretoria National Botanical Garden, Pretoria, South Africa; BGT: Tübingen Botanical Garden, Germany; BGW: Karoo Desert National Botanical Garden, Worcester, South Africa). Voucher specimens and/or photographs are on deposit in the herbaria JRAU and HEID.  

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<tr>
<th>Taxon</th>
<th>Origin of plant samples</th>
<th>Bot. garden acc. number</th>
<th>Herbarium and voucher numbers</th>
<th>EMBL acc. number rbcL gene</th>
<th>matK gene</th>
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Voucher specimens and/or photographs are on deposit in the herbaria JRAU and HEID.
contained 0.5–1 µg DNA, 5 µl 10× Tag buffer (50mM KCl, 10mM Tris-HCl, 1.5% Triton X-100, pH 9.0), 3 µl 25 mM MgCl₂, 12.5 pmol primer, 1.5 µl dNTPs (10mM), 0.75 U Taq-Polymerase (Amersham-Pharmacia Biotech), 0.5 µl 10 mg/ml BSA. PCR cycle: 2 min at 94 °C, then 30 cycles with 45 sec at 94 °C, 90 sec at 70 °C and 90 sec at 45°C, and finally 5 min at 72 °C. PCR products were further amplified by Cycle Sequencing using the "ThermoSequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP" (Amersham Life Science), and finally 5 min at 94 °C, and finally 5 min at 72 °C. PCR products were further amplified by Cycle Sequencing using the "ThermoSequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP" (Amersham Life Science).
Pharmacia Biotech) according to the protocol of the manufacturer. Products were sequenced with an automatic sequencer ALFexpress II (Amersham-Pharmacia Biotech). Cycle sequencing of the \textit{matK} region was performed using the following Cy5 labelled forward primers: \textit{matK}-AloeF4cy: 5'\text{-}GTA AGG ATT CAA A TG TTA GAG AAT T-3', \textit{matK}-724Fcy: 5'\text{-}CGC ACT A TG TA T CA T TTG A TA AC-3', \textit{matK}-F1/D1170Fcy: 5'\text{-}AKA A TT TAC GA T CAA TTC A TT CAA-3', \textit{matK}-F2/K1756Fcy: 5'\text{-}AGG A TC CAT ATA AAC CAA TTA TC-3', MatF3cy: 5'\text{-}GAA ATC TTC TCT ATT ATC ACA G-3', and the reverse primers \textit{matK}-AloeR3cy: 5'\text{-}CGTAYT GTA CTT TTA TGT TTA CGA G-3', \textit{matK}-R1/K1303Rcy: 5'\text{-}TRG AGAAAG AA T CGT AA T AAA TG-3'. 

Sequencing of the \textit{rbcL} gene was performed using the PCR primers \textit{rbcL}-N (forward): 5'- TAT GAC ATG TTA GAG AAT T-3', an the reverse primers \textit{rbcL}-R (reverse): 5'- AGA AGG ATC GCT CAA ACA TAA-3', \textit{matK}-F1/D1170Fcy: 5'\text{-}AKA ATT TAC GAT CAA TTC ATC AAT ACA-3', \textit{matK}-F2/K1756Fcy: 5'\text{-}AGG A TC CAT ATA AAC CAA TTA TC-3', MatF3cy: 5'\text{-}GAA ATC TTC TCT ATT ATC ACA G-3', and the reverse primers \textit{matK}-AloeR3cy: 5'\text{-}CGTAYT GTA CTT TTA TGT TTA CGA G-3', \textit{matK}-R1/K1303Rcy: 5'\text{-}TRG AGAAAG AA T CGT AA T AAA TG-3'. 

Sequencing of the \textit{rbcL} gene was performed using the PCR primers \textit{rbcL}-N (forward): 5'- TAT GAC ATG TTA GAG AAT T-3', an the reverse primers \textit{rbcL}-R (reverse): 5'- AGA AGG ATC GCT CAA ACA TAA-3', \textit{matK}-F1/D1170Fcy: 5'\text{-}AKA ATT TAC GAT CAA TTC ATC AAT ACA-3', \textit{matK}-F2/K1756Fcy: 5'\text{-}AGG A TC CAT ATA AAC CAA TTA TC-3', MatF3cy: 5'\text{-}GAA ATC TTC TCT ATT ATC ACA G-3', and the reverse primers \textit{matK}-AloeR3cy: 5'\text{-}CGTAYT GTA CTT TTA TGT TTA CGA G-3', \textit{matK}-R1/K1303Rcy: 5'\text{-}TRG AGAAAG AA T CGT AA T AAA TG-3'. 

Sequencing of the \textit{rbcL} gene was performed using the PCR primers \textit{rbcL}-N (forward): 5'- TAT GAC ATG TTA GAG AAT T-3', an the reverse primers \textit{rbcL}-R (reverse): 5'- AGA AGG ATC GCT CAA ACA TAA-3', \textit{matK}-F1/D1170Fcy: 5'\text{-}AKA ATT TAC GAT CAA TTC ATC AAT ACA-3', \textit{matK}-F2/K1756Fcy: 5'\text{-}AGG A TC CAT ATA AAC CAA TTA TC-3', MatF3cy: 5'\text{-}GAA ATC TTC TCT ATT ATC ACA G-3', and the reverse primers \textit{matK}-AloeR3cy: 5'\text{-}CGTAYT GTA CTT TTA TGT TTA CGA G-3', \textit{matK}-R1/K1303Rcy: 5'\text{-}TRG AGAAAG AA T CGT AA T AAA TG-3'. Sequence of the \textit{rbcL} gene was performed using the PCR primers \textit{rbcL}-N (forward): 5'- TAT GAC ATG TTA GAG AAT T-3', an the reverse primers \textit{rbcL}-R (reverse): 5'- AGA AGG ATC GCT CAA ACA TAA-3'.
(reverse): 5′- TAT CCA TTG CTG GGA ATT CAA A TT TG-3′, and \textit{rbcL}-1R (reverse): 5′- GGG TGC CCT AAA GTT TC-3′. For \textit{rbcL} sequencing the forward primers Leg3-cy: 5′-TGC GTT GGA GAG ACC GTT TT-3′, and Leg4-cy: 5′- ACT TTA GGY TTT GTT GAT TT-3′, and the reverse primers Leg2-cy: 5′- ATT CGC AAA TCT TCC AGA CG-3′ and Leg7-cy: 5′- TTC GCA TGT ACC CGC AGT AGC A-3′ were used. In \textit{Asphodelus aestivus} and \textit{Asphodeline lutea} sequencing of a part of the \textit{rbcL} gene (base position 385–685) failed due to alteration of the binding site of sequencing primer Leg7-cy. In these two cases the 300 missing nucleotides were taken from published sequences in the EMBL nucleotide sequence database of the same species (accession numbers Z73682 and Z73681).

Sequences were aligned manually or with use of CLUSTAL V (1.6) (gap-penalty 10).Aligned sequences were analysed using the phylogeny program versions PAUP 3.1.1 and PAUP 4.0b10 (Swofford, 2002). Molecular phylogenies were reconstructed using unweighted Maximum Parsimony (MP), Maximum Likelihood (ML), and Tamura-Nei distance with Neighbour Joining algorithm (NJ). For MP, the addition sequence option “closest” and the “TBR” swapping option were applied. Both NJ and MP analyses were bootstrapped. The ML search was conducted under the GTR+G+I model in a heuristic search manner. Branch swapping using the tree bisection and reconnection swapping algorithm was done on a starting tree built under the parsimony criterion. Estimated substitution rate matrix: AC = 0.767026; AG = 1.735156; AT = 0.303820; CG = 0.820893; CT = 1.705676; GT = 1.000000. Gamma shape parameter = 1.24163. Assumed proportion of invariable sites = 0.425622. A single best tree with Likelihood -ln L = 11034.5184 was found.

Among Asphodelaceae, 141 \textit{rbcL} and 435 \textit{matK} positions were variable, and 78 \textit{rbcL} and 225 \textit{matK} positions were parsimony informative. Among Alooideae, 49 \textit{rbcL} and 286 \textit{matK} positions were variable, and 22 \textit{rbcL} and 118 \textit{matK} parsimony informative. This indicates a 2.9- to 5.8-fold increase of variability in \textit{matK} compared to \textit{rbcL}. Average \textit{matK} and \textit{rbcL} \(p\)-distances were calculated (Table 2). The maximum pairwise \(p\)-distances found in Asphodelaceae were 3.19% for \textit{rbcL} (\textit{Asphodeline lutea} / \textit{Bulbine frutescens}) and 6.56% (\textit{Asphodeline lutea} / \textit{Gasteria subnigricans}) for \textit{matK}.

### RESULTS

A combined dataset of \textit{rbcL} and \textit{matK} sequences was used to reconstruct MP, NJ and ML trees for 57 species and 12 genera (Figs. 4–6) and a \(p\)-distance matrix of representative taxa (Table 2). The ISSR banding pattern was scored as a 1/0 matrix, analyzed with UPGMA (Fig. 3) and mapped on the ML tree (Fig. 6). The topology of MP, NJ and ML trees is almost congruent and most clades are supported by high bootstrap values (Figs. 4, 5). Due to the existence of many autapomorphies, the ISSR pattern reveals great complexity. Since some subjectivity is known to be associated with the ISSR method, a complete gel view is shown in Fig. 2, which allows an independent interpretation. The majority of the few synapomorphies found agree well with the chloroplast groups. Therefore, some conclusions can already be drawn from the present analysis that must still be considered as preliminary as only a limited number of species within each clade has been sampled and analysed.

The MP, NJ and ML reconstructions show that Asphodeloideae are paraphyletic. This corroborates a preliminary cladistic analysis of Van Wyk & al. (1995), in which morphological and chemical characters were used to demonstrate that there are no known synapomorphies for the subfamily. This finding also agrees with the study of Chase & al. (2000) using \textit{rbcL} and \textit{trnL-F} DNA sequences. \textit{Asphodelus} L. and \textit{Asphodeline} Rchb. form a monophyletic group that is invariably separated as sister branch to all other genera of Asphodelaceae (MP: 89%; NJ: 96% bootstrap support) in all methods of phylogenetic reconstruction used. The interrelationships amongst \textit{Bulbinella} Kunth, \textit{Trachyandra} Kunth, \textit{Kniphofia} Moench and \textit{Eremurus} M. Bieb. are not resolved unambiguously by \textit{rbcL} and \textit{matK} data. MP, NJ and ML show different placements of these genera, and the bootstrap supports are low. However, in the case of \textit{Bulbine} Wolf the relationship is less ambiguous (MP: 85%; NJ: 94% bootstrap support), and the genus appears to be immediately ancestral to Alooideae.

As expected, the subfamily Alooideae is undoubtedly monophyletic. This agrees with several apomorphies mentioned above (the funicular aril, bimodal karyotype and presence of anthrone-C-glycosides in the leaves and 1-methyl-8-hydroxyanthraquinones in the roots; Van Wyk & al., 1995). The subfamily was also found to be monophyletic by Chase & al. (2000), who included only a few representatives for the Alooideae group. Surprisingly, several unconventional groupings emerged within the subfamily. These groupings are quite stable, regardless of the different methods of phylogenetic reconstruction that were used.

\textit{Aloe pillansii}, \textit{A. ramosissima}, and \textit{A. barberae} are weakly supported as the earliest branching sister group of the alooids (MP: 64%, NJ: 41% bootstrap support). The cluster of these tree aloes (sections \textit{Aloidendron} Baker and \textit{Dracoaloe} Baker) is supported by 100% (MP) and 99% (NJ) bootstrap support. No common ISSR marker could be found that was shared with other aloes. Therefore, the tree aloes may indeed be a distinct group,
as suggested by Viljoen (1999), and branched earlier in the natural history of the alooids than previously thought.

A homogeneous clade of *Haworthia* species was found as sister group to the rest of Alooideae in MP, NJ and ML reconstructions (Figs. 4–6). The members of this clade are characterised by a unique ISSR Band 1 complex (Fig. 2). These species belong to the subgenus *Haworthia*, the largest subgenus within the genus *Haworthia*. Uitewaal (1947) divided *Haworthia* into two main units (*Triangulares* and *Hexangulares*), the former including the subgenus *Haworthia* and subgenus *Robustipedunculares*. This division is strongly supported by the combined *rbcL* and *matK* sequences and the ISSR band patterns. It is interesting to note that a distinct dis-

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**Fig. 4.** Unweighted MP bootstrap cladogram (1000 replications, heuristic search, addseq = closest, branch swapping = TBR, maxtree = 100) from combined *rbcL* and *matK* sequence data. CI = 0.911, RI = 0.942, RC = 0.858, length = 1069. Bootstrap values < 50 % are omitted. Outgroup is *Anthericum liliago*. 

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continuity is also present in the flower morphology (Uitewaal, 1947) and the nectar sugar composition of these two groups (Smith & al., 2002). Other members of the genus *Haworthia* cluster in a distinctly more heterogeneous clade that includes also *Gasteria, Aloe aristata, Haworthia* and *Poellnitzia* (see below). The species of *Haworthia* included in this clade all belong to the subgenus *Hexangulares*, with the single exception of *H. geraldii* (a distinctive retusoid member of the typical subgenus).

The climber *Aloe ciliaris* is also positioned at the base of the tree (MP: 72%, NJ: 60% bootstrap support).
It has been suggested (Holland, 1978) that the climbing aloes (series Macrifoliae Haw.) represent an ancient, weakly succulent, forest-margin lineage from which other aloes evolved during the aridification of the African continent.

Gasteria is clearly defined as a group in both rbcL and matK and ISSR (Band 2). This genus is monophyletic, the monophyly being supported by a bootstrap support of 98% (MP) and 100% (NJ). There are several morphological and chemical autapomorphies (van Jaarsveld & al., 1994).

Aloe aristata clusters together with Haworthia...
Table 2. Relative pairwise genetic matK (below diagonal) and rbcL (above diagonal) p-distances of selected taxa of Asphodelaceae. A value of 1.0 equals 100% distance.

<table>
<thead>
<tr>
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</table>

**Note:** The table shows the pairwise genetic distances (p-distances) for various taxa within the Asphodelaceae family. The values are rounded to 2 decimal places. The distances range from 0.1339 to 1.0000, representing 0% to 100% similarity, respectively.
kewensis, Astroloba congesta, Astroloba foliosa, ×Astroworthia bicarinata, Astroloba corrugata and Poellnitzia rubriflora, but there is no single ISSR band supporting this cluster: ISSR band 11 is not present in A. aristata and Poellnitzia, but the affinity of A. aristata to this group is visible in ISSR Band 5, present in A. aristata, H. kewensis and ×A. bicarinata. ISSR band 9 is a common genetic element of A. foliolosa, ×A. bicarinata, and A. corrugata. The vegetative morphology of the dwarf A. aristata is strongly reminiscent of Haworthia, and its root metabolites (anthraquinones and pre-anthaquinones) conform to the pattern found in Haworthia, Poellnitzia and Astroloba (Van Wyk, unpubl.). Indeed, the genus Poellnitzia has recently been transferred to Astroloba (Manning & Smith, 2000). Similarly, Aloe variegata has a root chemistry and general morphology similar to that of Gasteria, and it will be interesting to include the small section Serrulatae Salm Dyck (three species) to which it belongs in future studies. The result demonstrates the close relationship between Astroloba and members of Haworthia subgenera Hexangulares and Robustipedunculares (the reputed garden hybrid H. kewensis belongs to the former, and ×A. bicarinata is generally accepted to be an intergeneric hybrid between a member of Robustipedunculares and an Astroloba; ISSR band 9 seems to reflect the link with Astroloba).

The rbcL and matK-Group Haworthia attenuata forma brittianiana, H. geraldii and H. icosiphylla is supported by ISSR Bands 12 and 13. ISSR Band 7 is present in the three former species and H. glauca var. herrei. With the exception of H. geraldii, all these species belong to subgenus Hexangulares.

Aloe glauca and A. lineata are clustered in the rbcL and matK-trees and share the ISSR Bands 6 and 17. It is interesting to note that these two species belong to a small group of only eight species that exude the flavanone naringenin and the dihydroflavonoid dihydroisorhamnetin from the leaves instead of the usual anthrone-C-glycosides (Viljoen & al., 1998).

Lomatophyllum occidentale and L. macrum (both nowadays included in the genus Aloe) have ISSR bands 10, 14 and 15 in common and are grouped on rbcL and matK data by 89% (MP) and 93% (NJ) bootstrap support. There have been suggestions that Lomatophyllum may not be monophyletic (Schill, 1973; Rowley, 1996), and it may be interesting to test this hypothesis using more samples.

Aloe forbesii, A. inermis, A. scobinifolia, A. sinkatana, and A. vera are not unequivocally grouped in rbcL and matK, but have ISSR band 4 in common. These five species are all from northeastern Africa and Arabia.

Aloe viguieri, A. conifera, A. deltoideodonta, and A. bulbillifera form a monophyletic cluster in rbcL and matK. The group has a bootstrap value of 74% (MP) and 85% (NJ) and is further supported by ISSR-band 3, except A. conifera, which lacks the marker. All the species are from Madagascar.

Aloe striata and A. striata subsp. karasbergensis form a monophyletic sister group (MP: 61%; NJ: 49% bootstrap support) closely allied to Lomatophyllum, the Malagassy (A. viguieri / conifera / deltoideodonta / bulbillifera) and the East African and Arabian (A. inermis / forbesii / scobinifolia / barbadensis) groups.

Chortolirion angolense and the grass-like species of Aloe (MP: 100 %; NJ: 100 % bootstrap support) are the sister group of the Malagassy, East African and Arabian, and A. striata groups. According to ISSR, no affinity between Chortolirion and any of the other groups can be seen. Whereas Chortolirion is closely similar to Haworthia in terms of its flower morphology, rbcL and matK results support its close affinity to the grass-like aloe.
ble scenarios are evident.

**Scenario 1. A gene tree only?** — The cladogram and ISSR bands (Figs. 2–6) may reflect little more than gene trees. The same is currently true if floral characters are preferentially weighted in favour of other evidence, e.g., chemical characters. Indeed, it is a feature of the subfamily Alooideae that each character chosen as a basis for classification and circumscription in the group will result in a different and often conflicting system. In *Aloe*, for example, classification based on floral morphological patterns conflicts with groups erected on the basis of chemical characters (Viljoen, 1999).

**Scenario 2. Splitter’s approach.** — It would appear to be possible to create a large number of smaller, monophyletic genera within Alooideae (Figs. 3–5). However, such an approach would be counter-productive in terms of nomenclatural stability. Furthermore, the lack of clarity on relationships among these units, and consequently the hierarchical rank at which they warrant recognition, creates uncertainty about the delimitation of genera. A large number of species remains to be sequenced and grouped, so that a final placement of all taxa is not yet possible.

**Scenario 3. Retaining the status quo.** — This approach supports acceptance of the current classification system (Smith & Van Wyk, 1998, but with *Lomatophyllum* included in *Aloe* and *Poellnitzia* included in *Astroloba*), implying that the system may not reflect phylogenetic reality, but merely provides a workable framework for identification and communication. However, the further the status quo departs from reality, the more difficult it will be to integrate practice and theory.

**Scenario 4. Lumper’s approach.** — A further possibility is to regard Alooideae as a super-genus, *Aloe*, with a multitude of infrageneric units (i.e., subgenera, sections, etc.). This approach may reflect the true evolutionary history of the group more accurately, but may not be acceptable from a practical perspective. Hierarchical classification systems depend on the assumption of divergent evolution. However, if phylogeny is reticulate, as is most likely the case in Alooideae, then a simple dichotomous hierarchy may not be achievable.

Based on these considerations, complete sampling of succulent Asphodelaceae may be needed prior to revision of the taxonomy. Nevertheless, we propose an informal taxonomic grouping based on the present results that could be tested in future studies and refined (Table 3).

### Table 3. Informal taxonomic groupings found in ISSR, *matK* and *rbcL* MP trees (Figs. 3, 4).

<table>
<thead>
<tr>
<th>Genetic group</th>
<th>Clade in <em>matK</em> and <em>rbcL</em> tree</th>
<th>Clade in ISSR tree</th>
<th>Relevant taxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tree aloes</td>
<td>present</td>
<td>absent</td>
<td><em>Aloe pillansii</em>, <em>A. ramosissima</em>, <em>A. barberae</em></td>
</tr>
<tr>
<td><em>Haworthia</em> aloes</td>
<td>present</td>
<td>present</td>
<td><em>Haworthia angustifolia</em>, <em>H. cooperi</em>, <em>H. cymbiformis var. transiens</em>, <em>H. aristata</em>, <em>H. turgida var. turgida</em>, <em>H. ryderiana</em>, <em>H. blackburniae var. blackburniae</em></td>
</tr>
<tr>
<td>Climbing aloes</td>
<td><em>A. ciliaris</em> the only taxon examined</td>
<td><em>A. ciliaris</em> the only taxon examined</td>
<td><em>Aloe ciliaris var. ciliaris</em></td>
</tr>
<tr>
<td>Berried aloes</td>
<td>present</td>
<td>present</td>
<td><em>Lomatophyllum macrum</em>, <em>L. occidentale</em></td>
</tr>
<tr>
<td>Madagascar aloes</td>
<td>present</td>
<td>present; <em>A. conifera</em> not supported to be in the group</td>
<td><em>Aloe viguieri</em>, <em>A. conifera</em>, <em>A. deltoideodonta</em>, <em>A. bulbillifera</em></td>
</tr>
<tr>
<td>Northeastern African aloes</td>
<td>present; <em>A. vera</em> and <em>A. sinkatana</em> ambiguously grouped</td>
<td>present</td>
<td><em>Aloe sinkatana</em>, <em>A. mermis</em>, <em>A. scobinifolia</em>, <em>A. forbesii</em>, <em>A. vera</em></td>
</tr>
<tr>
<td>Coral aloes</td>
<td>present</td>
<td>present</td>
<td><em>Aloe striata</em>, <em>A. striata ssp. karasbergenis</em></td>
</tr>
<tr>
<td>Grass aloes</td>
<td>present</td>
<td>present</td>
<td><em>Chortolirion angolense</em>, <em>Aloe boylei</em>, <em>A. verecunda</em></td>
</tr>
<tr>
<td>Dihydroisorhamnetinaleos</td>
<td>present</td>
<td>present</td>
<td><em>Aloe lineata</em>, <em>A. glauca</em></td>
</tr>
<tr>
<td><em>Gasteria</em> aloes</td>
<td>present</td>
<td>present</td>
<td><em>Gasteria glomerata</em>, <em>G. batesiana</em>, <em>G. subnigrican</em>, <em>G. maculata</em>, <em>G. huttoniae</em></td>
</tr>
<tr>
<td>Haworthioid aloes</td>
<td>present; <em>H. glauca</em> var. <em>herrei</em> is closer to <em>Gasteria</em></td>
<td>present</td>
<td><em>Haworthia attenuata var. britteniana</em>, <em>H. geraldii</em>, <em>H. icosiphylla</em>, <em>H. glauca var. herrei</em></td>
</tr>
<tr>
<td><em>Astroloba</em> aloes</td>
<td>present</td>
<td>present</td>
<td><em>Poellnitzia</em> not supported to be in the group</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Aloe aristata</em>, <em>Haworthia kewensis</em>, <em>Astroworthia bicarinata</em>, <em>Astroloba corrugata</em>, <em>Poellnitzia rubriflora</em>, <em>Astroloba foliolosa</em>, <em>Astroloba congesta</em></td>
</tr>
</tbody>
</table>
CONCLUSION

Of the scenarios sketched above, we propose retention of the status quo, pending a complete sampling of all species. The reason is obvious: it will lead to the interim retention of a stable taxonomy and nomenclature, even though it may not adequately reflect phylogeny.

One aspect of the results, namely the splitting of *Haworthia* into two genera, would currently seem to be a particularly undesirable step because of the pending rearrangement and rank changes foreseen in all groups. The variation detectable in molecular results has been alluded to in a detailed study of the nectar sugar composition of the subgenera of *Haworthia* and related genera (Smith et al., 2002), but this aspect has not been developed further since it would seriously complicate the taxonomy of the group and lead to an inordinate number of name changes. *Haworthia* subgenus *Haworthia* appears to be a monophyletic group, but there is as yet no convincing evidence that *Haworthia* subgenus *Hexangulares* and related genera are monophyletic.

Current generic circumscriptions in the subfamily Alooideae are delicately poised and related groups are monophyletic. Current generic circumscriptions in the subfamily Alooideae are delicately poised and related groups are monophyletic. Current generic circumscriptions in the subfamily Alooideae are delicately poised and related groups are monophyletic. Current generic circumscriptions in the subfamily Alooideae are delicately poised and related groups are monophyletic.

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LITERATURE CITED


