

## MOLECULAR PHYLOGENETICS

## Phylogenetic relationships in Asphodelaceae (subfamily Alooideae) inferred from chloroplast DNA sequences (*rbcL*, *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 *matK* and *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 *Aloe* and *Haworthia*, are apparently not monophyletic. From a molecular point of view, *Haworthia* can be divided into two distinct groups that agree closely with the current subgeneric classification: a monophyletic group including species of subgenus *Haworthia*, and a second polyphyletic group with the subgenera *Hexangulares* and *Robustipedunculares*. This second clade includes *Poellnitzia*, *Astroloba*, *Gasteria* and even one *Haworthia*-like aloe (*Aloe aristata*). In the polyphyletic assemblage currently classified as *Aloe*, several smaller clades can be recognised, often reflecting morphological, chemical and geographical discontinuities. The tree aloes (sections *Aloidendron* and *Dracoaloe*) and climbing aloes (series *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. *Chortolirion* clusters with the grass-like aloes (section *Graminialoe* Reynolds, syn. *Leptaloe* Berger), *A. boylei* and *A. verecunda*, on a well-defined branch. The current taxonomic system clearly does not reflect the phylogenetic affinities and relationships amongst the succulent genera *Aloe*, *Astroloba*, *Chortolirion*, *Gasteria*, *Haworthia*, and *Poellnitzia*.

**KEYWORDS:** Asphodelaceae, ISSR, *matK*, phylogeny, *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, Aloid-eae, 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 *Lomatophyllum* Willd. (Rowley, 1996) and *Poellnitzia* Uitewaal, which have been regarded as synonymous with *Aloe* L. (Fig. 1) and *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-



Fig. 1. Representatives of the genus *Aloe* from South Africa. A, hybrid between *Aloe ferox* and *A. africana*; B, *A. merlotii*; C, *A. arborescens*. Photographs by Gideon Smith.

dence.

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 hemitropous 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 important 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), cultivar 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 orientated 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., subspecies and species give a specific banding profile that can be used to solve phylogenetic questions. In the present 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 previous study by Chase & al. (2000) had included only seven species of six succulent Alooideae genera. Therefore, the apparent 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 *Anthericum liliago* using the CTAB method (Doyle & Doyle, 1990) with minor modifications. Alterations were selective removal of the leaf epidermal layer and collection of about 0.5 cm<sup>2</sup> 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 conditions, 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 MgCl<sub>2</sub>, 0.1 mM of dGTP, dCTP, and dTTP, 0.075 mM dATP, 1 μCi [α-<sup>33</sup>P]-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 thermocycler; then at 72°C for 4 min, followed by 4°C for storage. PCR products were subjected to 0.2 mm 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)<sub>10</sub>, (CTGT)<sub>4</sub> and (AG)<sub>12</sub>, or in bands which were less well defined: i.e., (CT)<sub>8</sub>, (GTG)<sub>5</sub>, and (GGAT)<sub>4</sub>. The failure in amplification 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)<sub>4</sub> 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)<sub>4</sub> distinguishes all accessions 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 amplified by PCR using the primers *matK*-724F: 5'-CGC ACT ATG 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

**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.**

Taxon	Origin of plant samples	Bot. garden acc. number	Herbarium and voucher numbers	EMBL acc. number	
				<i>rbcL</i> gene	<i>matK</i> gene
<i>Aloe aristata</i> Haw.	BGH	-	HEID: IPMB 040301	AJ512319	AJ511407
<i>A. barberae</i> T.-Dyer	BGH	9483	HEID: IPMB 040302	AJ512294	AJ511371
<i>A. boylei</i> Baker	BGP	Smith 119	JRAU: <i>van Wyk 4113</i>	AJ512311	AJ511394
<i>A. bulbifera</i> H.Perr.	BGH	100725	HEID: IPMB 040303	AJ512305	AJ511385
<i>A. ciliaris</i> Haw. var. <i>ciliaris</i>	BGC	1182-70	HEID: IPMB 040304	AJ512287	AJ511379
<i>A. conifera</i> H.Perr.	BGH	72081	HEID: IPMB 040305	AJ512303	AJ511383
<i>A. deltoideodonta</i> Baker	BGH	-	HEID: IPMB 040306	AJ512304	AJ511384
<i>A. forbesii</i> Balf.	BGH	12610	HEID: IPMB 040307	AJ512308	AJ511389
<i>A. glauca</i> Mill.	BGH	3741	HEID: IPMB 040308	AJ512313	AJ511396
<i>A. inermis</i> Forssk.	BGH	-	HEID: IPMB 040309	AJ512288	AJ511387
<i>A. lineata</i> (Ait.) Haw.	BGC	0082-92	HEID: IPMB 040310	AJ511397	AJ511397
<i>A. pillansii</i> L. Guthrie	BGH	100718	HEID: IPMB 040311	AJ512292	AJ511369
<i>A. ramosissima</i> Pillans	BGH	-	HEID: IPMB 040312	AJ512293	AJ511370
<i>A. scobinifolia</i> Reynolds & P.R.O. Bally	BGH	-	HEID: IPMB 040313	AJ512307	AJ511388
<i>A. sinkatana</i> Reynolds	BGH	17353	HEID: IPMB 040314	AJ512306	AJ511386
<i>A. striata</i> Haw.	BGH	100403	HEID: IPMB 040315	AJ512310	AJ511392
<i>A. striata</i> Haw. subsp. <i>karasbergensis</i> (Pillans) Glen & D.S. Hardy	BGH	-	HEID: IPMB 040316	AJ512283	AJ511391
<i>A. vera</i> (L.) Burm.	BGH	100677	HEID: IPMB 040317	AJ512309	AJ511390
<i>A. verecunda</i> Pole Evans	BGP	-	JRAU: <i>van Wyk 4114</i>	AJ512312	AJ511395
<i>A. viguieri</i> H.Perr.	BGH	12724	HEID: IPMB 040318	AJ512302	AJ511382
<i>Anthericum liliago</i> L.	BGH	000493	-	AJ512331	AJ511426
<i>Asphodeline lutea</i> (L.) Reichb.	BGH	000495	HEID: IPMB 040319	AJ512277	AJ511416
<i>Asphodelus aestivus</i> Brot.	Tenerife, Canaries	-	HEID: IPMB 040320	AJ512314	AJ511415
<i>Astroloba congesta</i> (Salm-Dyck) Uitew.	BGW	49/72	HEID: IPMB 040321	AJ512279	AJ511413
<i>A. corrugata</i> N.L.Meyer & G.F. Smith	BGW	114/98	HEID: IPMB 040322	AJ512280	AJ511410
<i>A. foliolosa</i> (Haw.) Uitew.	BGW	157/94	HEID: IPMB 040323	AJ512278	AJ511412
× <i>Astroworthia bicarinata</i> (Haw.) G.D. Rowley	BGH	142433	HEID: IPMB 040324	AJ512321	AJ511409
<i>Bulbine frutescens</i> (L.) Willd.	collection Van Wyk	-	JRAU: <i>van Wyk 4115</i>	AJ512323	AJ511414
<i>Bulbinella nana</i> P.L. Perry	BGW	303/92	JRAU: <i>van Wyk 4116</i>	AJ512325	AJ511419
<i>Chortolirion angolense</i> (Bak.) Berger	BGH	-	HEID: IPMB 040325	AJ512284	AJ511393
<i>Eremurus himalaicus</i> Baker	BGH	-	HEID: IPMB 040326	AJ512291	AJ511417
<i>E. stenophyllus</i> (Boiss. & Buhse) Baker	BGH	-	-	AJ512324	AJ511418
<i>Gasteria batesiana</i> G.D. Rowley	BGW	56/93	JRAU: <i>van Wyk 4117</i>	AJ512285	AJ511399
<i>G. glomerata</i> Van Jaarsv.	BGW	95/93	HEID: IPMB 040327	AJ512281	AJ511398
<i>G. huttoniae</i> N.E.Br.	BGM	-	HEID: IPMB 040328	AJ512290	AJ511402
<i>G. maculata</i> (Thunb.) Haw.	BGM	-	HEID: IPMB 040329	AJ512282	AJ511401
<i>G. subnigricans</i> Haw.	BGM	-	HEID: IPMB 040330	AJ512289	AJ511400
<i>Haworthia angustifolia</i> Haw.	BGH	142428	HEID: IPMB 040331	AJ512295	AJ511372
<i>H. aristata</i> Haw.	BGH	142418	HEID: IPMB 040332	AJ512319	AJ511407
<i>H. attenuata</i> (Haw.) Haw. f. <i>britteniana</i> Poelln.	BGH	142441	HEID: IPMB 040333	AJ512315	AJ511403
<i>H. blackburniae</i> W.F.Barker var. <i>blackburniae</i>	BGW	116/71	JRAU: <i>van Wyk 4118</i>	AJ512300	AJ511378
<i>H. cooperi</i> Baker	BGJ	-	-	AJ512275	AJ511374
<i>H. cymbiformis</i> (Haw.) Duval var. <i>transiens</i> (Poelln.) M.B. Bayer	BGH	142414	HEID: IPMB 040334	AJ512296	AJ511373
<i>H. geraldii</i> C.L.Scott	BGH	142450	HEID: IPMB 040335	AJ512317	AJ511405
<i>H. glauca</i> Baker var. <i>herrei</i> (Poelln.) M.B. Bayer	BGH	142437	HEID: IPMB 040336	AJ512318	AJ511406
<i>H. icosiphylla</i> Baker	BGJ	981243	-	AJ512316	AJ511404
<i>H. kewensis</i> Poelln.	BGH	142449	HEID: IPMB 040337	AJ512320	AJ511408
<i>H. ryderiana</i> Poelln.	BGH	45928	HEID: IPMB 040338	AJ512299	AJ511377
<i>H. turgida</i> Haw. var. <i>turgida</i>	BGW	847/93	HEID: IPMB 040349	AJ512298	AJ511376
<i>Kniphofia galpinii</i> Baker	BGT	-	HEID: IPMB 040340	AJ512329	AJ511423
<i>K. praecox</i> Baker	collection Van Wyk	-	JRAU: <i>van Wyk 4119</i>	AJ512276	AJ511424
<i>Kniphofia triangularis</i> Kunth subsp. <i>triangularis</i>	BGT	-	HEID: IPMB 040341	AJ512328	AJ511422
<i>K. uvaria</i> (L.) Hook.	BGF	-	HEID: IPMB 040342	AJ512330	AJ511425
<i>Lomatophyllum macrum</i> (Haw.) Salm-Dyck	BGP	-	JRAU: <i>van Wyk 4120</i>	AJ512301	AJ511381
<i>L. occidentale</i> H. Perr.	BGP	10819	JRAU: <i>van Wyk 4121</i>	AJ512286	AJ511380
<i>Poellnitzia rubriflora</i> (L. Bol.) Uitew.	BGW	-	HEID: IPMB 040343	AJ512322	AJ511411
<i>Trachyandra involucrata</i> (Baker) Oberm.	BGW	48/86	JRAU: <i>van Wyk 4122</i>	AJ512326	AJ511420
<i>T. tortilis</i> (Baker) Oberm.	BGW	717/93	JRAU: <i>van Wyk 4123</i>	AJ512327	AJ511421

*Aloe ramosissima*  
*Aloe barbaeae*  
*Aloe pillansii*  
*Aloe ciliaris*  
*Chortolion angolense*  
*Aloe striata*  
*A. striata karasbergensis*  
*Aloe vera*  
*Aloe forbesii*  
*Aloe scobinifolia*  
*Aloe inermis*  
*Aloe sinkatana*  
*Aloe lineata*  
*Aloe glauca*  
*Aloe conifera*  
*Aloe bulbiflora*  
*Aloe deltoideodonta*  
*Aloe viguieri*  
*Lomatophyllum macrum*  
*Lomatophyllum occidentale*  
*Haworthia blackburniae*  
*Haworthia aristata*  
*Haworthia cooperi*  
*Haworthia ryderiana*  
*Haworthia turgida*  
*Haworthia cymbiformis*  
*Haworthia angustifolia*  
*Haworthia gerardi*  
*Haworthia glauca*  
*Haworthia tosinjylla*  
*Haworthia attenuata*  
*Astroloba congesta*  
*Astroloba corrugata*  
*Astroloba foliolosa*  
*Poellnitzia rubriflora*  
*Xastroworthia bicarinata*  
*Haworthia kewensis*  
*Aloe aristata*  
*Gasteria huttoniae*  
*Gasteria maculata*  
*Gasteria subnigricans*  
*Gasteria batesiana*  
*Gasteria glomerata*

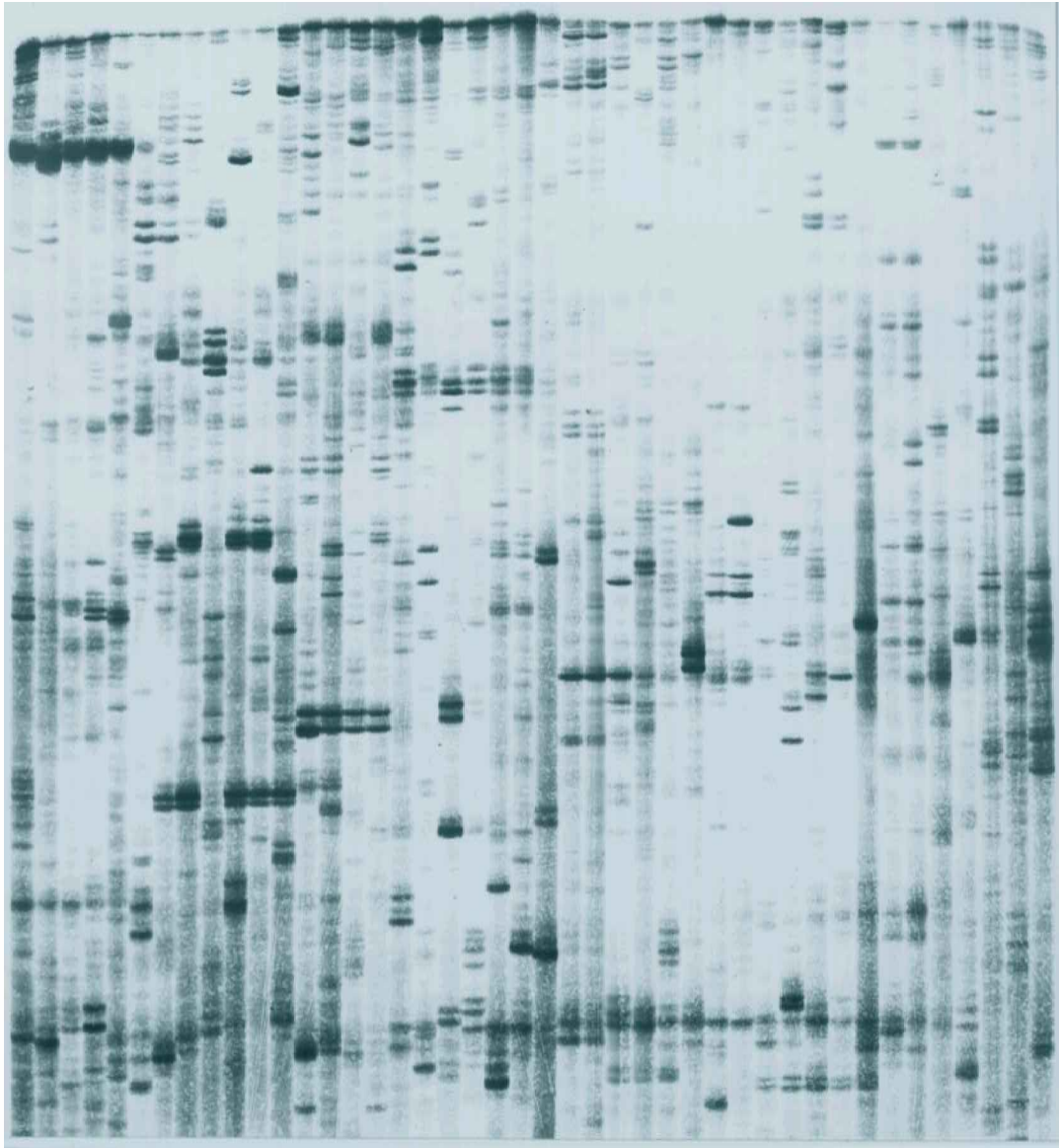


Fig. 2. Autoradiogram showing the Inter Simple Sequence Repeat (ISSR) patterns amplified by primer (GACA)<sub>4</sub> in Alooeidae. Intraspecific taxon names (see Materials & Methods) are omitted in the labelling.

contained 0.5–1 µg DNA, 5 µl 10× Taq buffer (500mM KCl, 100mM Tris-HCl, 1% Triton × 100, pH 9.0), 3 µl 25 mM MgCl<sub>2</sub>, 12.5 pmol primer, 1.5 µl dNTPs (10mM), 0.75 U Taq-Polymerase (Amersham-Pharmacia Biotech) and 1 µl 20 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

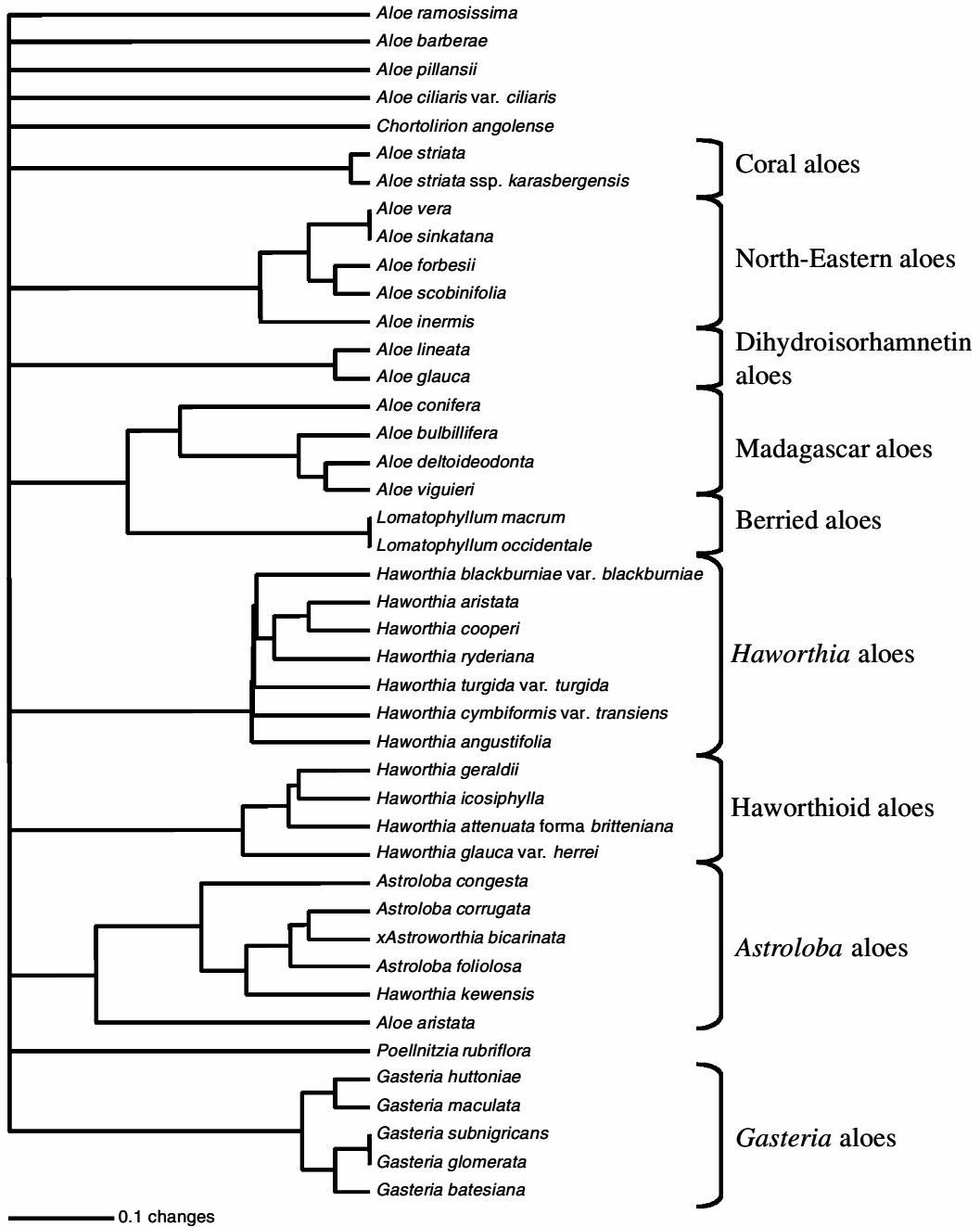


Fig. 3. UPGMA analysis (Nei-Li restriction site distance) of the ISSR data.

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 *matK* region was performed using the following Cy5 labelled forward primers: *matK*-AloeF4cy: 5'-GTA AGG ATT CAA ATG TTA GAG AAT T-3', *matK*-724Fcy: 5'-CGC ACT ATG TAT CAT TTG ATA AC-3', *matK*-F1/D1170Fcy: 5'-AKA ATT TAC GAT CAA TTC ATT CAA-3', *matK*-

F2/K1756Fcy: 5'-AGG ATC CAT ATA AAC CAA TTA TC-3', *MatF3cy*: 5'-GAA ATC TTT CTC ATT ATC ACA G-3', and the reverse primers *matK*-AloeR3cy: 5'-CGT AYT GTA CTT TTA TGT TTA CGA G-3', *matK*-R1/K1303Rcy: 5'-TRG AGAAAG AAT CGT AAT AAA TG-3', *AmatRcy*: 5'-GTA CAA AAT TTA GCT TTA GAC-3'. Sequencing of the *rbcL* gene was performed using the PCR primers *rbcL*-N (forward): 5'- ATG TCA CCA CAA ACA GAR ACK AAA GC-3', *rbcL*-R

(reverse): 5'-TAT CCA TTG CTG GGA ATT CAA ATT TG-3', and *rbcL*-1R (reverse): 5'-GGG TGC CCT AAA GTT CCT CC-3'. For *rbcL* sequencing the forward primers Leg3-cy: 5'-TGC GTT GGA GAG ACC GTT TC-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 *Asphodelus aestivus* and *Asphodeline lutea* sequencing of a part of the *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" branch 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  $-\ln L = 11034.51848$  was found.

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

## RESULTS

A combined dataset of *rbcL* and *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 *rbcL* and *trnL-F* DNA sequences. *Asphodelus* L. and *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 *Bulbinella* Kunth, *Trachyandra* Kunth, *Kniphofia* Moench and *Eremurus* M. Bieb. are not resolved unambiguously by *rbcL* and *matK* data. MP, NJ and ML show different placements of these genera, and the bootstrap supports are low. However, in the case of *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.

*Aloe pillansii*, *A. ramosissima*, and *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 *Aloidendron* Baker and *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,

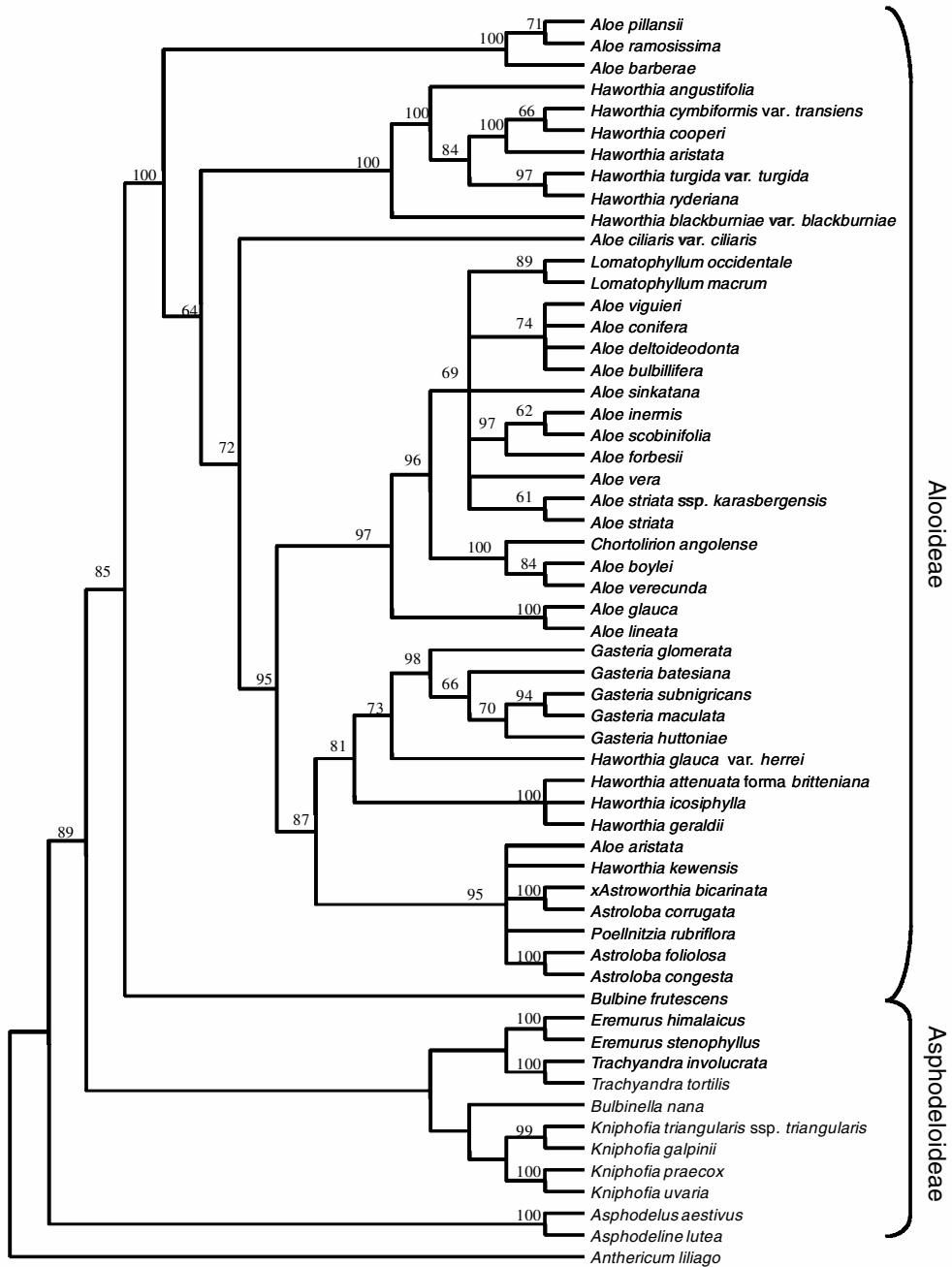


Fig. 4. Unweighted MP bootstrap cladogram (1000 replications, heuristic search, addseq = closest, branch swapping = TBR, maxtree = 100) from combined *rbcl* and *matK* sequence data. CI = 0.911, RI = 0.942, RC = 0.858, length = 1069. Bootstrap values < 50 % are omitted. Outgroup is *Anthericum liliago*.

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 Alooidae in MP, NJ and ML reconstructions (Figs. 4–6). The members of this group 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-



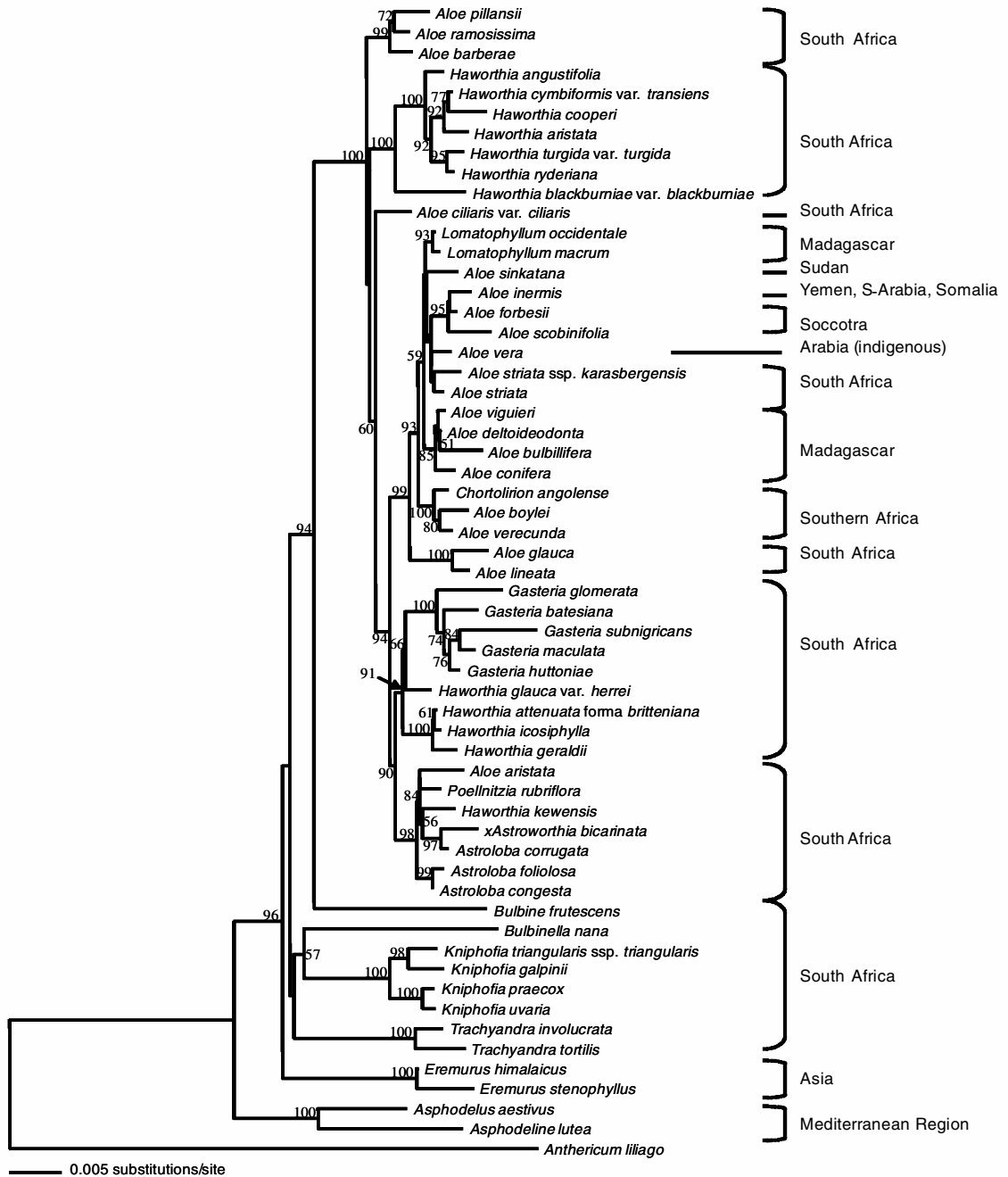


Fig. 5. Neighbour joining bootstrap tree. Phylogram resulting from nucleotide sequences of *rbcL* and *matK* sequence data. Tamura-Nei distance algorithm; number of bootstrap replicates: 1000; Bootstrap values >50 % are given at each node; bootstrap values <50 % are omitted. Outgroup *Anthericum liliago*. Distribution ranges of the groups are indicated.

continuity is also present in the flower morphology (Uitewaai, 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 sub-genus *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).

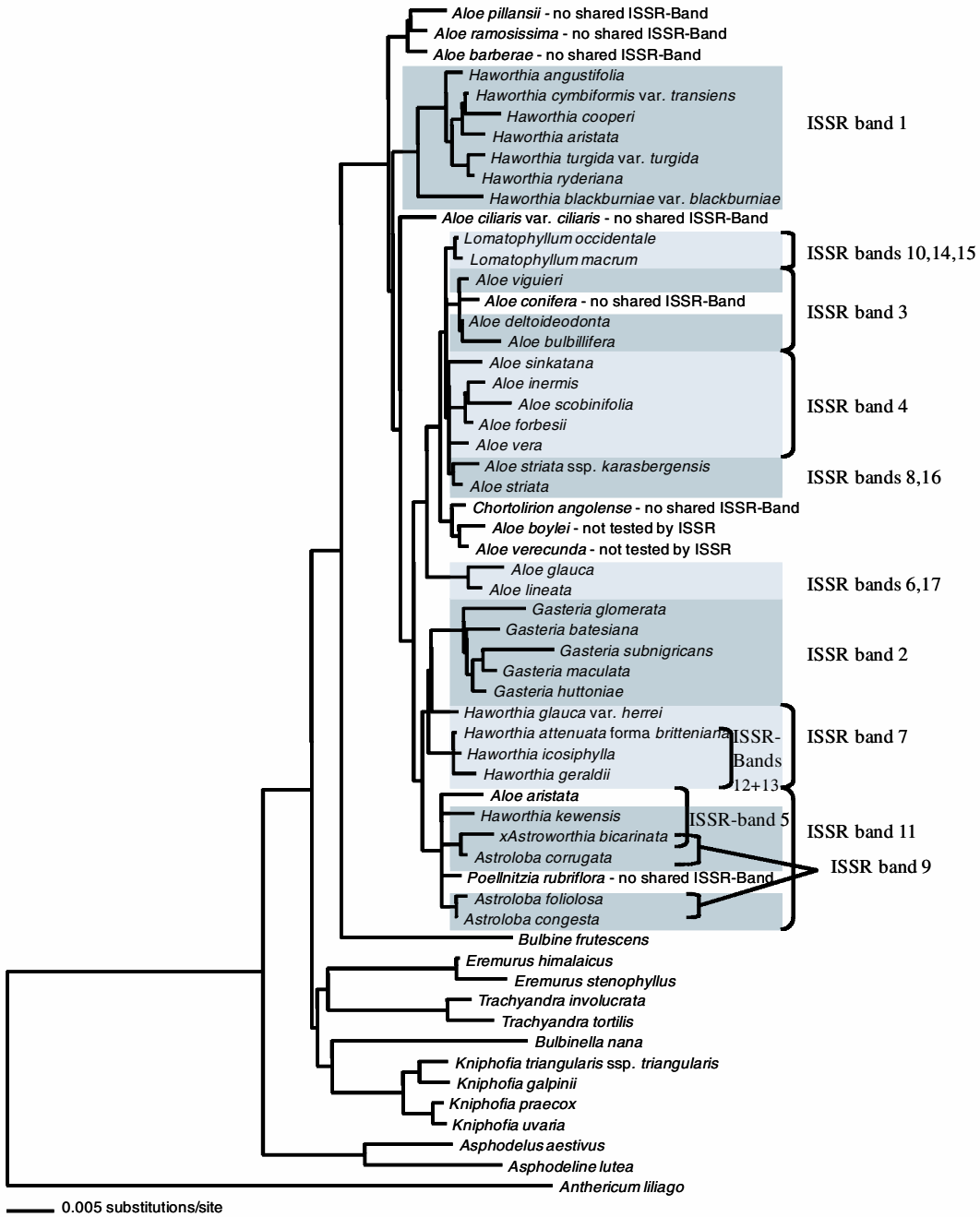


Fig. 6. Reconstruction of a phylogenetic tree from a combined *rbcL* and *matK* sequence dataset by maximum likelihood. A single tree of  $-ln L = 11034.51848$  was obtained. Results are presented as a phylogram. Branch lengths represent the genetic distance under the GTR+G+I model of substitution, and can be compared to the distance bar, equalling 0.005 substitutions per site. *Anthericum liliago* is presented as outgroup for presentation purposes only. Groups that share ISSR bands are superimposed on the phylogram.

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.**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
[1] <i>Anthriscum filago</i>	-	0.11539	0.12135	0.1434	0.12443	0.11434	0.11364	0.11596	0.11402	0.11469	0.11792	0.12292	0.1235	0.11797	0.11921	0.11681	0.1174	0.12137	0.12017	0.11911	0.11742	0.11343	0.11184	0.11192	0.11487	0.11596	0.11046	0.11205	0.11982	0.11728
[2] <i>Asphodelus aestivus</i>	0.04983	-	0.02909	0.04719	0.0613	0.04702	0.04443	0.05554	0.04452	0.04394	0.04744	0.05121	0.05698	0.0465	0.04994	0.04529	0.04849	0.05176	0.05173	0.05232	0.04975	0.04645	0.04427	0.04869	0.04527	0.04645	0.04523	0.04392	0.0516	0.05051
[3] <i>Asphodeline lutea</i>	0.05182	0.01405	-	0.05156	0.06429	0.05266	0.05092	0.05245	0.05267	0.05335	0.05625	0.06122	0.0638	0.05466	0.0575	0.05468	0.05791	0.06049	0.05799	0.06203	0.05726	0.05399	0.04828	0.05623	0.0528	0.05396	0.05019	0.05014	0.05847	0.05809
[4] <i>Eremurus himalaicus</i>	0.04877	0.01983	0.02366	-	0.04447	0.03468	0.03133	0.03418	0.03415	0.03671	0.0382	0.04585	0.04629	0.03864	0.04004	0.03932	0.03996	0.04583	0.04256	0.0414	0.03671	0.03417	0.03228	0.03885	0.03549	0.03541	0.03419	0.03542	0.03991	0.04009
[5] <i>Bulbinella nana</i>	0.05202	0.02559	0.03103	0.02055	-	0.04369	0.03784	0.05349	0.04182	0.04314	0.0466	0.04909	0.05136	0.04379	0.04721	0.04576	0.04833	0.05281	0.0497	0.05434	0.04895	0.04375	0.04385	0.0434	0.04061	0.04439	0.04061	0.04054	0.03912	0.03543
[6] <i>Trachyantra involucreata</i>	0.05515	0.02496	0.02894	0.01526	0.022	-	0.03126	0.04434	0.03209	0.03337	0.03549	0.04318	0.04488	0.03659	0.03863	0.03727	0.03792	0.04435	0.04047	0.04386	0.0398	0.03467	0.03535	0.03744	0.03279	0.03528	0.03215	0.03401	0.03912	0.03543
[7] <i>Knapifolia praecox</i>	0.05523	0.02533	0.03056	0.0199	0.02306	0.02243	-	0.03788	0.02736	0.02929	0.03278	0.03987	0.0403	0.03257	0.03594	0.03327	0.0339	0.03981	0.03585	0.03789	0.03518	0.03	0.03215	0.0281	0.03127	0.02806	0.02998	0.03584	0.03277	
[8] <i>Bulbine Frausesens</i>	0.05662	0.02489	0.03192	0.02072	0.02345	0.01841	0.02396	-	0.03138	0.03157	0.03627	0.04266	0.04446	0.03542	0.03688	0.03482	0.03609	0.0432	0.03934	0.04464	0.04122	0.03607	0.03611	0.03755	0.03352	0.03347	0.03227	0.03544	0.03861	0.03748
[9] <i>Aloe ciliaris</i> var. <i>ciliaris</i>	0.05306	0.01901	0.02133	0.01465	0.01747	0.01681	0.02134	0.01603	-	0.0135	0.01292	0.01934	0.02424	0.01478	0.01745	0.01479	0.01673	0.01865	0.0193	0.02518	0.02123	0.01412	0.01351	0.01552	0.01289	0.01542	0.01478	0.01285	0.02056	0.01933
[10] <i>Lomatophyllum occidentale</i>	0.05526	0.02238	0.02463	0.01682	0.01937	0.01874	0.02465	0.01798	0.03005	-	0.06443	0.01289	0.01442	0.00513	0.00774	0.00578	0.00771	0.01609	0.01225	0.0245	0.01929	0.01348	0.01412	0.01678	0.01094	0.01414	0.01546	0.01736	0.02053	0.02126
[11] <i>Aloe confusa</i>	0.05688	0.02436	0.02613	0.01851	0.02144	0.02076	0.02639	0.02069	0.00459	0.00151	-	0.01235	0.01649	0.00772	0.01038	0.00711	0.01032	0.0142	0.01488	0.02719	0.02197	0.01613	0.01614	0.01946	0.01486	0.01678	0.01746	0.01746	0.02322	0.02396
[12] <i>Aloe bulbiflora</i>	0.05575	0.02332	0.02513	0.01755	0.02048	0.02056	0.02536	0.01976	0.00381	0.00074	0.00074	-	0.0205	0.01099	0.01555	0.01098	0.01549	0.02194	0.02137	0.03106	0.02775	0.02254	0.02264	0.02595	0.02156	0.02324	0.02516	0.01807	0.0303	0.03089
[13] <i>Aloe scobinifolia</i>	0.05582	0.02262	0.02441	0.01678	0.01977	0.01983	0.02463	0.01904	0.00305	0	0.00147	0.00073	-	0.01315	0.01642	0.01251	0.01702	0.02557	0.02361	0.03662	0.03065	0.02481	0.02244	0.02614	0.0228	0.02475	0.02616	0.02811	0.02994	0.03137
[14] <i>Aloe vera</i>	0.05802	0.02414	0.02588	0.01909	0.02197	0.02204	0.02689	0.01983	0.00533	0.00224	0.00369	0.00293	0.0022	-	0.00581	0.00045	0.00772	0.01737	0.01417	0.02387	0.01928	0.01475	0.01476	0.01806	0.01351	0.01542	0.01738	0.01736	0.02245	0.02318
[15] <i>Aloe striata</i>	0.05624	0.02289	0.0252	0.01681	0.01994	0.01931	0.02465	0.01929	0.00305	0.00075	0.00223	0.00147	0.00073	0.00294	-	0.00581	0.01031	0.0207	0.01679	0.02918	0.02447	0.01746	0.01937	0.02206	0.01681	0.01871	0.02005	0.02261	0.02451	0.02564
ssp. <i>karasbergensis</i>																														
[16] <i>Aloe striata</i>	0.05577	0.0226	0.0244	0.01677	0.01976	0.01982	0.02462	0.01903	0.00305	0	0.00147	0.00073	-	0.00837	0.0174	0.01482	0.02777	0.02188	0.01543	0.01544	0.01937	0.01479	0.01669	0.0174	0.01866	0.02057	0.02386			
[17] <i>Chamaelirion angolense</i>	0.0549	0.02149	0.02375	0.01602	0.02	0.01937	0.02389	0.01861	0.00229	0.00149	0.00299	0.00221	0.00148	0.0037	0.00223	0.00148	-	0.01932	0.0161	0.02639	0.02252	0.0167	0.01735	0.02004	0.01546	0.01736	0.0187	0.02057	0.02377	0.02452
[18] <i>Aloe glauca</i>	0.05589	0.02278	0.02308	0.0184	0.0228	0.02289	0.02629	0.02135	0.00457	0.00299	0.00369	0.00367	0.00294	0.00514	0.00337	0.00294	0.00297	-	0.00903	0.03421	0.02897	0.02187	0.02187	0.02264	0.0219	0.02379	0.02447	0.02381	0.02953	0.03029
[19] <i>Aloe lineata</i>	0.05651	0.02187	0.02366	0.01754	0.02194	0.02205	0.02537	0.02049	0.00382	0.00224	0.00295	0.00292	0.00219	0.00439	0.00295	0.00219	0.00222	0.00073	-	0.03107	0.02511	0.01802	0.01997	0.02328	0.01804	0.01994	0.02126	0.02383	0.02701	0.02711
[20] <i>Gasteria glomerata</i>	0.0566	0.02305	0.02605	0.01923	0.02228	0.02163	0.0263	0.02017	0.00537	0.00374	0.00625	0.00445	0.00371	0.00594	0.00446	0.00371	0.00523	0.00446	-	0.01807	0.02303	0.01939	0.02724	0.02324	0.02386	0.02909	0.03032	0.03417	0.0324	
[21] <i>Gasteria bauciana</i>	0.05804	0.02491	0.02659	0.01833	0.02344	0.02357	0.02683	0.02208	0.00458	0.00372	0.00619	0.00439	0.00567	0.00587	0.00442	0.00366	0.00369	0.00515	0.0044	-	0.01673	0.01608	0.02263	0.01866	0.01992	0.02384	0.02573	0.02955	0.02929	
[22] <i>Haworthia attenuata</i>	0.05651	0.0234	0.02442	0.01753	0.02196	0.02202	0.02537	0.02054	0.0038	0.00224	0.00369	0.00293	0.0022	0.00439	0.00294	0.0022	0.00222	0.00367	0.00292	0.00293	-	0.0103	0.01616	0.0116	0.01414	0.01737	0.01863	0.02376	0.02193	
forma <i>britanniana</i>																														
[23] <i>Haworthia glauca</i>	0.05578	0.02268	0.02439	0.01677	0.02193	0.0213	0.02534	0.01984	0.00305	0.00222	0.00295	0.00219	0.00147	0.00366	0.00221	0.00147	0.00221	0.00294	0.00219	0.00297	0.00293	0.00073	-	0.0168	0.01224	0.0135	0.01804	0.01673	0.02312	0.02259
var. <i>herrei</i>																														
[24] <i>Aloe aristata</i>	0.05588	0.02565	0.0275	0.01993	0.02427	0.02436	0.02777	0.02276	0.00609	0.00449	0.00512	0.00439	0.00661	0.00518	0.00439	0.00446	0.00588	0.00513	0.00524	0.00516	0.00367	0.00294	-	0.00903	0.01223	0.01939	0.02067	0.02382	0.02266	
[25] <i>Poa liliacea rubriflora</i>	0.05729	0.02552	0.02735	0.01983	0.02414	0.02426	0.0276	0.0227	0.00609	0.00446	0.00659	0.00509	0.00437	0.00659	0.00515	0.00437	0.00443	0.00587	0.0051	0.0052	0.00512	0.00365	0.00364	0.00293	-	0.0077	0.01612	0.01738	0.02121	0.01936
[26] <i>Astrolaha fatulosa</i>	0.05579	0.02381	0.02608	0.01834	0.0223	0.02168	0.02612	0.02094	0.00457	0.00299	0.00451	0.00371	0.00298	0.00521	0.00374	0.00297	0.00298	0.00448	0.00372	0.00372	0.00372	0.00223	0.00223	0.0015	0.00148	-	0.01802	0.01928	0.0231	0.02192
[27] <i>Aloe barberae</i>	0.05351	0.02052	0.023	0.01532	0.01907	0.01914	0.02322	0.01761	0.00152	0.00299	0.00616	0.0044	0.00367	0.00587	0.00337	0.00367	0.00222	0.00515	0.0044	0.00523	0.00589	0.00444	0.00367	0.00661	0.00666	0.00448	-	0.01029	0.01995	0.01935
[28] <i>Aloe pillansii</i>	0.05512	0.02205	0.02454	0.01687	0.02059	0.02066	0.02479	0.01914	0.00304	0.00451	0.00664	0.00588	0.00514	0.00736	0.0052	0.00514	0.00373	0.00662	0.00588	0.00675	0.00739	0.00588	0.00515	0.00808	0.00809	0.006	0.00147	-	0.02249	0.02191
[29] <i>Haworthia turgida</i>	0.05238	0.02057	0.02306	0.01761	0.02061	0.01915	0.02251	0.01916	0.00458	0.00676	0.00817	0.00736	0.00663	0.00884	0.00744	0.00663	0.00597	0.00813	0.00736	0.00896	0.00885	0.00736	0.00664	0.00961	0.00957	0.00824	0.0059	0.00739	-	0.01996
var. <i>turgida</i>																														
[30] <i>Haworthia buckeri</i>	0.05657	0.02413	0.02589	0.01983	0.02342	0.02351	0.02																							

*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-anthraquinones) 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 *britteniana*, *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. bulbifera* 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 aloes.

## DISCUSSION

The overall pattern of relationships at familial and subfamily level has been the subject of several studies (e.g., Dahlgren & al., 1985; Chase & al., 2000) all confirming that: (1) the family Asphodelaceae is monophyletic, and (2) the subfamily Alooideae is a monophyletic group within a paraphyletic subfamily Asphodeloideae. What has emerged from this study is that molecular evidence is in strong conflict with the current classification system of the subfamily Alooideae. Since Chase & al. (2000) only used a few place markers (mostly one species for each genus), the remarkable lack of congruence between molecular groupings and current generic circumscriptions was not detected in their study. Both of the genes used for reconstruction of the phylogeny, *rbcL* and *matK*, are localized on the chloroplast genome and therefore do not evolve independently. Due to the fact that the chloroplast molecule is inherited maternally, the phylograms reflect the maternal lineages and are prone to be misleading because of chloroplast capture. To detect such deviations of the chloroplast tree from the organism tree, we used genomic fingerprinting (ISSR) to examine the nuclear relationships. Due to the size of the nucleom in comparison to the plastom and the chondriom, the origin of the ISSR bands is likely to be nuclear and can serve as control for the groups found with chloroplast DNA. In other words, even if the true complexity of the organisms is not depicted in the chloroplast DNA trees and ISSR patterns, nuclear as well as chloroplast DNA at least show large congruence of the inheritance patterns of their DNA. Based on the results obtained in the present investigation, a number of possi-

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).**

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

## 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 & 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 *Hexangularis* and related groups are monophyletic.

Current generic circumscriptions in the subfamily Aloioideae are delicately poised but workable. It would seem premature to dismantle the system piecemeal without providing a practical and comprehensive alternative. There is a danger that a reductionist approach to taxonomy in this case may lead to a naïve under-estimation of the real complexity of the problem. It may be prudent to reserve judgement until all the new patterns have become clearer. Our results demonstrate that there is at least partial congruence between morphological-chemical patterns and genetic patterns, so that future studies in molecular systematics are likely to reveal further important new insights into the complicated evolutionary history of Asphodelaceae.

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