CHAPTER 4 PHYLOGENETICS

4.1 Phylogeny and classification of the spotted aloes, *Aloe* section *Pictae* (Asphodelaceae).. 55

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Introduction

The genus *Aloe* L. is the largest and arguably the most iconic of 15 genera in Asphodelaceae (Asparagales). With increasing appreciation of the diversity in *Aloe*, the genus has expanded over the past two centuries to include over 500 accepted specific and infraspecific names and almost as many synonyms (Newton 2001). The first comprehensive infrageneric classification of *Aloe* (Berger, 1908) was expanded and revised by Reynolds (1950, 1966) in a two volume work, but remained incomplete as the second volume was compiled shortly before the author's death. Berger's (1908) and Reynolds' (1950, 1966) infrageneric classifications of *Aloe* have been lauded for their intuitive arrangement, but the extent to which they remain artificial has limited their usefulness. For instance, the apparently natural group comprising fire tolerant, barely succulent species of *Aloe* occurring in grasslands is broadly circumscribed in two sections (*Leptaloe* A. Berger; *Graminaloe* Reynolds), while, in contrast, the relatively few taxa that are arborescent in form are recognised in no fewer than four sections (*Dracaloe* A. Berger, *Aloidendron* A. Berger, *Kumara* (Medik.) Baker and *Sabaealoe* Berger). The proliferation of names and incomplete infrageneric classification have contributed to considerable taxonomic confusion over generic boundaries and species demarcation in *Aloe*.

The classification of *Aloe* is further frustrated by the use of three family names in the literature. The original family circumscription of *Aloe* and other succulent-leaved alooid genera, in Aloaceae (Batsch 1802), continues to be recognised (e.g. Brummit 1992; Carter 1994; Glen and Hardy 2000). The more inclusive Asphodelaceae, comprising genera in Alooideae and Asphodeloideae (Cronquist 1981), was not widely recognised until it was convincingly shown to be monophyletic (Smith and Van Wyk 1998; Chase et al. 2000; Treutlein et al. 2003a, 2003b). Asphodelaceae is among the "bracketed" families considered to be "acceptable monophyletic alternatives to the broader circumscription favoured" by the Angiosperm Phylogeny Group (APG II 2003). The latter places Asphodelaceae and Hemerocallidaceae in the formerly monotypic Australian family Xanthorrhoeaceae. Due to the absence of morphological synapomorphies to support an expanded Xanthorrhoeaceae (Devey et al. 2006), and since Asphodelaceae is both a monophyletic and widely accepted taxonomic unit, we consider it the most practical classification for *Aloe* at the family rank.

Comparative data have accumulated in recent decades, contributing to emerging views of the systematics of *Aloe* not reflected by the infrageneric arrangement, which is largely

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based on floral characters (Berger 1908; Reynolds 1950, 1966). For instance, patterns in seed morphology (Kamstra 1971), leaf surface sculpturing (Cutler 1982), pollen morphology (Steyn et al. 1998) and the presence of secondary metabolites such as microdontin (Viljoen et al. 1999) are not congruent with existing infrageneric groups. However, similarities in gross morphology and certain phytochemical characters such as the presence of flavonoids (Viljoen et al. 1998) and the composition of waxes in the cuticle (Herbin and Robins 1968) do correspond with infrageneric groups recognised by the present classification. Treutlein et al. (2003a) found no agreement between traditional infrageneric groups and the relationships suggested by *matK*, *rbcL* and genomic fingerprinting in *Aloe*; these authors questioned (with unintended irony) the usefulness of cryptic characters that have highlighted, rather than resolved, taxonomic questions in Asphodelaceae. Nonetheless, the consensus of these studies is that considerable comparative data are required to inform a stable taxonomic classification of *Aloe* that not only reflects evolutionary relationships, but has practical value for assisting in plant identification and predicting plant properties.

This chapter focuses on the poorly resolved section *Pictae* Salm-Dyck of *Aloe*, the so-called maculate or spotted aloes. The name refers to their patterned leaf surfaces, which bear pale green to white markings, often described as "H-shaped" by Reynolds (1950, 1966) and arranged in transverse bands, on at least one leaf surface. Plants are usually robust with rosulate, succulent leaves that exude yellow, reddish or purple sap on wounding, the leaf margins armed with sharp teeth (prickles). Like other acaulescent or short-stemmed species of *Aloe*, the inflorescence is usually a tall, dichotomously branched panicle, bearing conical to capitate racemes of pale cream, but usually pink to bright orange or red flowers. However, the bulbous base of the perianth tube and constriction above the ovary are very distinctive of maculate species. The status of these floral and leaf morphological characters as synapomorphies for section *Pictae*, however, is uncertain, since all these characters are known in other infrageneric groups in *Aloe*. For example, a constricted perianth is typical of section *Paniculatae* Salm-Dyck ex Kunth which includes the glaucous-leaved *A. striata* Haw., while patterned leaf surfaces are typical of series *Hereroenses* Reynolds, including *A. hereroensis* Engl. In fact, Berger (1908) thought the latter species both belonged in the maculate group, his series *Saponariae* A. Berger.

Innovations in the infrageneric arrangement of *Aloe* introduced by Reynolds (1950, 1966) had little influence on the recognition of at least a core group within section *Pictae*, in which about forty species are currently accepted. Section *Pictae* has not, however, escaped the inconsistencies and nomenclatural confusion that affect the infrageneric classification of

Aloe; taxonomic irregularities have resulted in an arrangement of maculate species with very limited practical value. Indeed, among the most readily identifiable maculate species are atypical ones such as *A. suffulta* Reynolds, possessing an unusual inflorescence, and *A. simii* Pole-Evans, with channelled and often immaculate leaves. We have undertaken comparative studies of potentially informative taxonomic characters, including leaf surface morphology and phytochemistry (unpubl. data) in the maculate group to gain better understanding of its systematic relationships. Here, we evaluate for the first time phylogenetic evidence, from nuclear and plastid DNA data, for resolving the circumscription and assessing the monophyletic status of section *Pictae*.

Materials and methods

Taxonomic sampling

DNA sequence data were compiled for 29 species of *Aloe* from throughout continental Africa that have been classified at some time in sections *Pictae*, *Maculatae* or series *Saponariae*. Previous molecular studies of *Aloe* have included few maculate species, to which we have added 33 new sequences. Plant material was collected from natural populations in South Africa and plants of wild provenance kept in glasshouses at the Royal Botanic Gardens, Kew. Voucher specimens were deposited at Kew (K) and the National Herbarium (PRE) in South Africa. Additional published sequence data were obtained from GenBank for 17 ingroup taxa representing thirteen other infrageneric groups in *Aloe*. *Gasteria* was defined as outgroup in all analyses. Species voucher information and GenBank accession numbers are presented in Table 4.1

Table 4.1 Plant material and sequence data investigated in the phylogenetic analysis of *Aloe* section *Pictae*

Taxon	Accession(s)[1]
Aloe affinis A.Berger	Grace 87 (K, PRE), South Africa;
A. arborescens Mill.	Noguchi & De-yuan AB090942; Treutlein et al. 2003b AY323723; Adams et al. 2000a AF234333
A. aristata Haw.	—, Treutlein et al. 2003a AJ511407, Treutlein et al. 2003b AY323651
A. barbertoniae Pole-Evans	Grace 85 (K, PRE)
A. branddraaiensis Groenew.	RBG 1957-14502 (K), South Africa
A. burgersfortensis Reynolds	Grace 89 (K, PRE), South Africa
A. capitata var. gneisicola H.Perrier	—, Treutlein et al. 2003b AY323720, Treutlein et al. 2003b AY323677
A. compressa var. compressa H.Perrier	—, Treutlein et al. 2003b AY323721, Treutlein et al. 2003b AY323678
A. dewetii Reynolds Grace 83	(K, PRE), South Africa
A. doei Lavranos	—, Treutlein et al. 2003b AY323724, Treutlein et al. 2003b AY323682
A. ellenbeckii A.Berger	RBG 1977-2441 (K), Kenya;
A. ellenbeckii A.Berger	RBG 1973-2107 (K), Kenya
A. forbesii Balf.f.	—, Treutlein et al. 2003a AJ511389, Adams et al. 2000a AF234342
A. fosteri Pillans	RBG 2003-1796 (K), South Africa
A. glauca Mill.	—, Treutlein et al. 2003a AJ511396, Adams et al. 2000a AF234344
A. grandidentata Salm-Dyck	RBG 1972-2520 (K), South Africa
A. greatheadii var. *davyana* (Schönland) Glen & D.S. Hardy	Grace 66 (K, PRE), South Africa
A. greatheadii var. *davyana* (Schönland) Glen & D.S.Hardy	Grace 67 (K, PRE), South Africa
A. greatheadii var. *davyana* (Schönland) Glen & D.S.Hardy	Grace 56 (K, PRE), South Africa
A. greatheadii Schönland	Grace 72 (K, PRE), South Africa
A. greenii Baker	Grace 74 (K, PRE), South Africa
A. humilis (L.) Mill.	—, Treutlein et al. 2003b AY323719, Treutlein et al. 2003b AY323675
A. immaculata Pillans	Grace 62 (K, PRE), South Africa
A. jucunda Reynolds	—, Treutlein et al. 2003b AY323718, Treutlein et al. 2003b AY323674

Table 4.1 *(continued)*

Taxon	Accession(s)
A. juvenna Brandham & S.Carter	—, Treutlein et al. 2003b AY323717, Treutlein et al. 2003b AY323673
A. lateritia var. *graminicola* (Reynolds) S.Carter	RBG 1973-2058 (K), Cult. Kenya
A. lettyae Reynolds	Grace 60 (K, PRE), South Africa
A. leptosiphon A.Berger	RBG 1967-16201 (K), Cult. Zambia
A. macrocarpa Tod.	RBG 1972-4103 (K), Cult. Ethiopia
A. maculata All.	Grace 82 (K, PRE), South Africa
A. maculata All.	RBG 1990-1902 (K), Cult. California, USA
A. monotropa I. Verd.	Grace 65 (K, PRE), South Africa
A. mudenensis Reynolds	RBG 1947-52506 (K), South Africa
A. prinslooi I. Verd. & D.S. Hardy	Grace 68 (K, PRE), South Africa
A. pruinosa Reynolds	Grace 69 (K, PRE), South Africa
A. scobinifolia Reynolds & P.R.O.Bally	—, Treutlein et al. 2003a AJ511388, Treutlein et al. 2003b AY323687
A. sinkatana Reynolds	—, Treutlein et al. 2003a AJ511386, Treutlein et al. 2003b AY323689
A. somaliensis var. *somaliensis* W.Watson	—, Treutlein et al. 2003b AY323716, Treutlein et al. 2003b AY323672
A. striata Haw.	—, Treutlein et al. 2003a AJ511392, Treutlein et al. 2003b AY323668
A. suffulta Reynolds	RBG 1961-56203 (K), Mozambique
A. suprafoliata Pole-Evans	—, Treutlein et al. 2003b AY323715, Treutlein et al. 2003b AY323676
A. swynnertonii Rendle	Grace 59 (K, PRE), South Africa
A. umfoloziensis Reynolds	Grace 73 (K, PRE), South Africa
A. vanbalenii Pillans	Grace 81 (K, PRE), South Africa
A. vanrooyenii G.F. Sm. & N.R. Crouch	Grace 70 (K, PRE), South Africa
A. vera L.	Chase et al. 2000 AJ290255 AJ290289, Treutlein et al. 2003b AY323726, Treutlein et al. 2003b AY323685
A. vogtsii Reynolds	Grace 57 (K, PRE), South Africa
A. zebrina Baker	Grace 63 (K, PRE), South Africa
Gasteria Duval	Chase et al. 2000 AJ290264 AJ290298, Treutlein et al. 2003b AJ511401, Treutlein et al. 2003b AY323655

[1]Listed in this order: *trnL–F* intron and spacer, *matK*, and ITS; — = sequence not obtained.

DNA sequencing

Total genomic DNA was extracted from silica-gel dried flowers or leaves (ca 0.3 g) or fresh leaf material (ca. 1.0 g) according to a protocol modified from those described by Doyle and Doyle (1987) and Saghai-Maroof et al. (1984). Aliquots of DNA were purified with the Nucleospin® Extract II minicolumn kit (Macherey-Nagel, Düren) using the binding buffer from Qiagen (Crawley). The remaining DNA was purified by caesium chloride-ethidium bromide density gradient (1.55 $g\mu l^{-1}$) followed by a dialysis procedure, and accessioned to the DNA bank at Kew (data.kew.org/dnabank).

The *matK* region was amplified using the XF and 5R primers (kew.org/barcoding/). Polymerase chain reactions (PCRs) were prepared in 20 µl volumes containing 5× GoTaq FlexiBuffer (supplied by the manufacturer), 2 µl of 0.04% bovine serum albumin (BSA), 1.25 mM $MgCl_2$, 0.4 µl dNTPs, 2 U GoTaq polymerase and 1 µl of each primer. The *trnL* intron and *trnL-F* spacer were amplified using the primer pairs c-d and e-f, respectively (Taberlet et al., 1991). PCRs in 25 µl volumes were prepared with 22.5 µl ReddyMix PCR Master Mix (Thermo Scientific) containing 2.5 mM $MgCl_2$, 0.5 µl of 0.04% BSA,0.5 µl of each primer and 1 µl template DNA. The internal transcribed spacers (ITS) ITS1 and ITS2 were amplified with the ITS4 and ITS5 primers of White et al. (1990). The same PCR protocol described for the *trnL-F* region was used, but using 1.5 mg $MgCl_2$ ReddyMix PCR Master Mix (Thermo Scientific) and 4% DMSO.

Thermal cycling was conducted with a GeneAmp PCR System 9700 (Applied Biosystems) using the following procedures. For *matK*, we used an initial denaturation at 94 °C for 2 min followed by 33 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 1 min and extension at 72 °C for 1.5 min, and a final extension of 4 min at 72 °C. For the *trnL-F* region, the initial denaturation at 94 °C for 2 min was followed by 28 cycles comprising denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min and extension at 72 °C for 1.5 min, and a final extension of 7 min at 72 °C. For the ITS region, the initial denaturation at 94 °C for 3 min was followed by 33 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 30 sec and extension at 72 °C for 1.5 min, and a final extension of 4 min at 72 °C. PCR products were purified with the Nucleospin® Extract II minicolumn kit (Macherey-Nagel, Düren) using the binding buffer from Qiagen (Crawley).

Cycle sequencing of the PCR products was performed with the same primer pairs used for amplification and the BigDye Terminator Cycle Sequencing kit (version 3.1; Applied Biosystems) in 10 µl reaction volumes. The products were purified on a Biomek NX S8 (Beckman Coulter) automated workstation according to the manufacturer's protocol. Sequences from the complementary strands of the amplified templates were recorded on a 3730 DNA Analyzer (Applied Biosystems/Hitachi). Electropherograms were edited and assembled using Sequencher 4.5 (Gene Codes Corporation) and aligned by eye in PAUP* 4.0b10 (Swofford, 2002).

Phylogenetic analyses

Phylogenetic reconstructions were obtained using maximum parsimony and Bayesian inference with *Gasteria* defined as outgroup. Maximum parsimony analyses were conducted in PAUP* 4.0b10 (Swofford 2002). All characters were treated as independent, unordered and equally weighted (Fitch parsimony; Fitch 1971). An analysis was performed using the heuristic search option with 1000 replicates of random taxon addition, tree bisection and reconnection (TBR) branch swapping, and no more than 10 trees were saved per replicate. The trees obtained from the first analysis were used as starting trees in a second analysis using the same parameters, and saving a maximum of 10000 trees. Support for the internal nodes was evaluated with bootstrap percentages (Felsenstein 1985) calculated by performing 1000 replicates with simple taxon addition, TBR branch swapping and saving no more than 10 trees per replicate. Clades with bootstrap percentages (BP) of 50–74% were described as weakly supported, 75–89% moderately supported and 90–100% strongly supported.

Trees determined by Bayesian inference were obtained in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit model of DNA substitution for each partition using the Akaike and Bayesian Information Criterion. Two parallel runs of four simultaneous chains of the Markov Chain Monte Carlo (MCMC) were executed for 5,000,000 generations, sampled every 1000 generations. All parameters were stationary after 500 000 generations; the 500 initial suboptimal trees were removed (burn-in) from the compilation of posterior probabilities (PP). A majority rule consensus tree was calculated from the remaining trees in PAUP* 4.0b10. Clades with posterior probabilities above 0.95 were considered strongly supported.

Results

The resulting aligned sequence matrix comprised 2267 characters, of which 13.6% were variable and potentially parsimony informative. The ITS region comprises 51 (7.5%) potentially parsimony informative characters, while the *trnL* intron, *trnL-F* spacer and *matK* have 8.4%, 4.4% and 3.4%, respectively (Table 4.2). Trees generated from parsimony analyses of each partition were congruent but lacked internal support (trees not shown), prompting us to conduct further analyses on the combined plastid and nuclear data partitions. Heuristic searches identified 4660 trees of 525 steps with a consistency index (CI) of 0.42 and retention index (RI) of 0.59 (excluding uninformative characters), and good bootstrap support for deeper nodes. A majority rule consensus tree calculated from trees generated by Bayesian inference produced a very similar topology (Fig. 4.1), with high posterior probabilities shown for the spine of the tree.

Representatives of *Aloe* were recovered in a strongly supported clade (PP 1.00; 100 BP) sister to *Gasteria* and a *Haworthia*-like species, *A. aristata* Haw. (Fig. 4.1). A well supported (PP 1.00; 99 BP) yet morphologically diverse southern African clade (*A. arborescens* Mill., *A. glauca* Mill., *A. humilis* (L.) Mill.) was sister to *A. suprafoliata* Pole-Evans, a southern African species bearing distinctive glaucous leaves with toothed margins. A large group (PP 0.94; 80 BP) comprising the remainder of the genus was sister to this. The first diverging lineages in this large clade comprised two short-stemmed species endemic to Madagascar (*A. capitata* Baker and *A. compressa* H. Perrier; PP 1.00, 86 BP), together sister to a polytomy (in the parsimony analysis) consisting of four lineages: (1) a clade comprising East African and Arabian species (PP 1.00; 59 BP); (2) a Horn of Africa clade, (PP 1.00; 73 BP), (3) a maculate group, which is convincingly supported by a high posterior probability (PP 1.00) and moderate bootstrap value (87 BP); (4) species of marginal status in section *Pictae*, *A. suffulta* Reynolds and *A. leptosiphon* A. Berger (PP 0.98; 56 BP). A southern African stemless species, *A. vanbalenii* Pillans, was unplaced in the strict consensus tree, but was associated, although not supported (PP 0.35), with the Horn of Africa group by Bayesian inference. The East Africa/Arabia clade comprised a group of three East African species (*A. forbesii* Balf. f., *A. scobinifolia* Reynolds & P. R. O. Bally, *A. sinkatana* Reynolds; 74 BP) sister to two Arabian species (*A. doei* Lavranos, *A. vera* L.) and the Horn of Africa clade (*A. jucunda* Reynolds, *A. juvenna* Brandham & S. Carter, and *A. somaliensis* W. Watson). The crown node of the maculate clade was a large polytomy comprising most species in this group with a few weakly to moderately supported species assemblages.

Table 4.2. Characteristics of the four partitions used in the phylogenetic analyses of *Aloe* section *Pictae*.

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	ITS	*trnL* intron	*trnL-F* spacer	*matK*	Combined
Aligned length (characters)	736	600	406	818	2560
Included characters	680	406	363	818	2267
Parsimony informative characters	7.5%	8.4%	4.4%	3.4%	5.7%
Variable characters	15.0%	23.2%	16.5%	11.2%	13.6%
Constant characters	85%	76.8%	83.5%	88.8%	84.6%

Discussion

Closely comparable trees generated by parsimony and Bayesian inference analyses yielded the first molecular evidence for the monophyletic status of section *Pictae* (Fig. 4.1). *Aloe* is a heterogeneous and possibly polyphyletic taxonomic entity; its boundaries with *Gasteria* and *Haworthia* are particularly unclear (Treutlein et al. 2003a). The original broad concept of *Aloe* circumscribed by Linneaus (1753) in *Species Plantarum* has since been segregated into seven morphologically recognisable and widely accepted genera (Smith and Van Wyk 1991), yet natural classifications of these genera and constituent species have remained unresolved. The suspected polyphyletic status of segregate genera such as *Haworthia* has even led to the Linnean concept of *Aloe* being reconsidered (Treutlein et al. 2003b). Morphologically, *Aloe* differs from *Gasteria* and *Haworthia* in its usually straight, tubular flowers, often spiny leaves, and species with tree-like woody growth. *Gasteria* has characteristic gasteriform (curved) flowers that are pendulous at anthesis, whereas flowers in *Haworthia* are bilabiate and whitish; prominent spines are absent from the leaves of species in both genera (Smith and Van Wyk 1998, Smith and Steyn 2004). We used published sequence data for *A. aristata* which was consistently resolved within *Haworthia* in studies by Treutlein et al. (2003b). While it was beyond the scope of the present study to test generic boundaries among alooid taxa, in this case the *Haworthia*-like *A. aristata* was shown to be more closely related to *Gasteria* (defined as outgroup in our analyses), and is a doubtful member of *Aloe.*

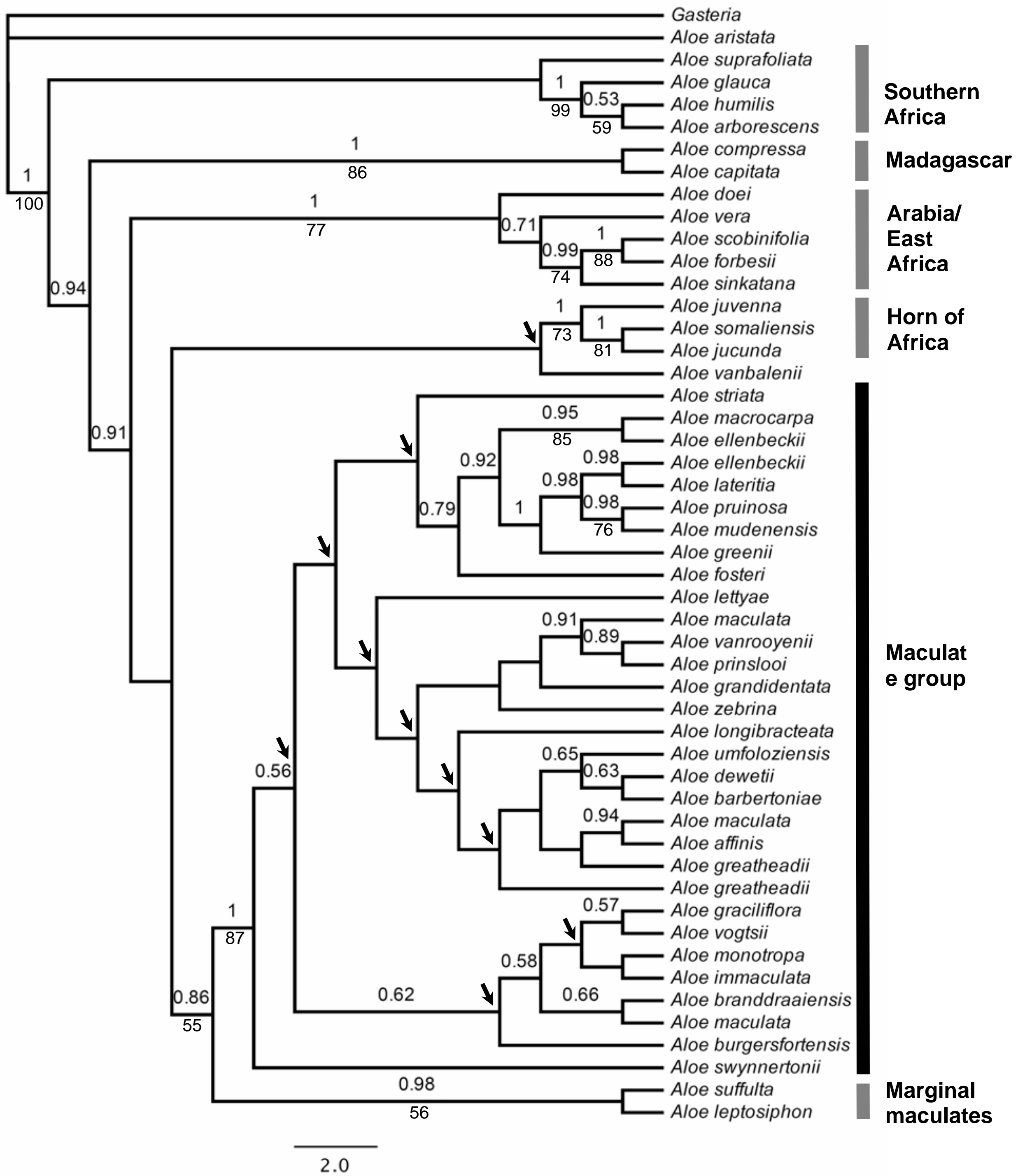

Figure 4.1 Topology from Bayesian analysis of nuclear and plastid data, with *Gasteria* as outgroup. Posterior probabilities >0.5 are shown above branches and bootstrap percentages >50 are shown below branches. Branches that collapsed in a strict consensus tree of 4660 equally parsimonious trees from the same data are indicated by arrows.

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Aloe section *Pictae* is generally regarded as a natural group, comprising an assemblage of poorly defined, closely related species with spotted leaf surfaces, and a perianth that is basally inflated and constricted above the level of the ovary (Groenewald 1941, Glen and Hardy 2000). Despite the questionable validity of these morphological apomorphies at the infrageneric rank, the traditionally core maculate group was recovered (PP 1.00) with moderate support (87 BP) in our phylogenetic reconstructions.

Evolutionary interpretation of these data would require a revised hypothesis for section *Pictae* to accommodate descendants from a common ancestor, in which *A. striata* and related species are included, and which excludes the marginal species *A. leptosiphon* and *A. suffulta.* Significantly, *A. leptosiphon* and *A. striata* are among only four accepted species that Reynolds (1950, 1966) excluded in his revision of Berger's (1908) classification of maculate species. In a parsimony tree inferred from ITS data, *A. striata* was sister to the well supported maculate group (98 BP), but the position lacked support (57 BP) (results not shown); cladistic analyses of additional genomic characters and species may add clarity. Morphologically, *A. striata* and close relatives in section *Paniculatae* Salm-Dyck ex Kunth (*A. buhrii* Lavranos, *A. karasbergensis* Pillans, *A. komaggasensis* Kritzinger & Van Jaarsv., *A. kouebokkeveldensis* van Jaarsv. & A. B. Low, and *A. reynoldsii* Letty) share the basal swelling and perianth constriction typical of section *Pictae.* Leaves of some members of the group (*A. buhrii*, *A. reynoldsii*) also have irregular white spots typical of maculate species. Phytochemical affinities have been noted between *A. striata* and southern African representatives of section *Pictae* (unpubl. data). However, the glaucous and conspicuously striate, sometimes leathery (e.g. *A. reynoldsii*) leaves with entire or minutely dentate leaf margins of *Paniculatae* differ markedly to the sharply toothed, robust leaves of section *Pictae.* Berger (1908) was the single author to adopt a broad circumscription of maculate species in *Aloe* that included section *Paniculatae.* Subsequent authors (e.g. Groenewald 1941; Reynolds 1950, 1966; Glen and Hardy 2000) followed the narrow concept of sections *Pictae* and *Paniculatae* originally circumscribed by Salm-Reifferscheid-Dyck (1837). Further molecular data are necessary to resolve the relationships between these two sections.

The status in section *Pictae* of the unusual species *A. leptosiphon* and *A. suffulta* may also be clarified on the basis of molecular evidence. In addition to convincing posterior probability value (PP 0.98), the two species comprise a well supported group sister to the maculate group in parsimony trees based on ITS data (79 BP) (results not shown), although confidence is notably lower (56 BP) in trees generated from combined plastid and nuclear

data. Berger (1908) included *A. leptosiphon* in his broad concept of the maculate section, presumably on account of the pale leaf surface markings, and in spite of its atypical floral morphology. The tenuous affiliation of *A. leptosiphon* with section *Pictae* is further illustrated by Reynolds' (1966) failure to associate *A. greenwayi* Reynolds, a species subsequently reduced to synonymy under *A. leptosiphon*, with the maculate section when he named it. In a similar case, the copiously spotted leaf surfaces of *A. suffulta* led Berger (1908) and Groenewald (1941) to include this species with southern African representatives of the maculate section. However, *A. suffulta* has since been classified, on the basis of floral characters, with other species of *Aloe* characterised by diagnostic trigonous indentations in the perianth above the ovary (series *Aethiopicae* A. Berger; section *Chabaudia* Glen and Hardy) (Reynolds 1950; Glen and Hardy 2000). It is clear that perianth characters have greater significance as synapomorphies for section *Pictae* than leaf markings.

The natural boundaries of section *Pictae* identified from phylogenetic reconstructions include maculate species from throughout southern and tropical Africa. With the exception of Berger's (1908) infrageneric treatment of *Aloe*, tropical and southern African maculate species have been dealt with separately, adding to taxonomic inconsistency evident in section *Pictae.* Seventy-two species names have been proposed for 39 species taxa currently accepted as representing valid taxa (Newton 2001) in section *Pictae*. Infraspecific ranks have been more conservatively used in the classification of maculate species than in other infrageneric groups in *Aloe* (varieties are recognised only in the southern African *A. greatheadii* Schönland and East African *A. lateritia* Engl.). However, it is likely that there are still more species names than good species in section *Pictae*, due to the treatment of variable, widespread taxa as poorly defined species, as well as the challenge of defining species undergoing hybridisation and active speciation (Reynolds 1966, Glen and Hardy 2000). Evidence for active speciation in *Aloe* has been recovered from chromosome termini, and in ITS variation (Adams et al. 2000a, 2000b). These factors may also explain low levels of support for terminal branches in the maculate clade, which pre-empt detailed analysis of relationships among species reduced to a polytomy in the clade.

Unambiguous phylogenetic signals were recovered for relationships among very few species in the maculate group, the remainder reduced to polytomies. *Aloe ellenbeckii* A. Berger collected near Marsabit in northern Kenya was convincingly associated (PP 0.95; 85 BP) with *A. macrocarpa* Tod., a maculate species occurring from the Horn of Africa region into West Africa, while a second accession of *A. ellenbeckii* collected near Nairobi in Kenya

was associated with *A. lateritia*, a species that Wabuyele (2006) reduced to synonymy under *A. macrocarpa* on the basis of morphometric, phytochemical and isozyme data. The KwaZulu-Natal endemics *A. mudenensis* Reynolds and *A. pruinosa* Reynolds were well resolved (PP 0.98; 76 BP) together. In a majority rule consensus tree, the closely related *A. greenii* Baker was sister to the KwaZulu-Natal assemblage in all of the most parsimonious trees, while the East African *A. lateritia* was associated with *A. ellenbeckii* in 70% of the most parsimonious trees. Other terminal groups recovered in the majority rule consensus included *A. affinis* A. Berger and *A. maculata* All. (100%), two southern African species which bear capitate or sub-capitate racemes; the morphologically distinct but geographically overlapping *A. dewetii* Reynolds and *A. umfoloziensis* Reynolds (100%); and the very restricted southern African *A. branddraaiensis* Groenew. and another accession of *A. maculata* (97%). The appearance of *A. maculata* and *A. greatheadii* at different positions in the topology of the parsimony trees may be explained by the heterogeneity of these species. Despite major phylogenetic patterns being recognised in this analysis, we anticipate that considerable additional data will be necessary to resolve the apparently complex species relationships in this section.

Ingroup species included in our study were recovered in geographically congruent groups representing southern Africa, Madagascar, southern Arabia, East Africa and the Horn of Africa. The absence of any species of *Aloe* with maculate leaves on Madagascar could indicate that *Aloe* species with leaf markings diversified in Africa after the dispersal event that lead to the diversification of Madagascan species (Holland 1978).

The absence of groups comprising species alike in habit or gross morphology is striking, and may be explained by convergent evolution. For instance, maculate leaves in *Aloe* may have arisen independently in section *Pictae* and other infrageneric groups of *Aloe*, presumably as an adaptive advantage to regulate photosynthetic capacity and for camouflage. On the other hand, it is not clear if the floral morphology restricted to section *Pictae* (and the segregate section *Paniculatae*) is associated with a primitive or derived pollination syndrome, or indeed a reversal. Insect pollination is speculated to be ancestral in *Aloe*, and is less common than bird pollination (Hargreaves et al. 2008). In section *Pictae*, the bulbous base and constricted perianth of flowers may constrain nectar thieving by birds (although in several maculate species we have observed external damage to the base of flowers caused by birds). In *A. greatheadii* var. *davyana* (Schönland) Glen and D.S. Hardy, the nectar is considerably more concentrated (approximately 20% w/w) than in other species of *Aloe* (Human and Nicolson 2008). Nectar accumulates in the bulbous base of the perianth

and moves by capillarity along a nectar duct in the corolla tube, to be presented as droplets at the mouth of the flower where it is foraged by bees (Nepi et al. 2006). *Aloe greatheadii* var. *davyana* is visited by bees for nectar and pollen during the winter flowering period and is greatly valued for apiculture in South Africa (Human and Nicolson 2008). Although among the most common visitors to the flowers of *Aloe*, however, bees are seldom the pollinators (Hargreaves et al. 2008); neither bees nor other insects and sunbirds known to visit *A. greatheadii* var. *davyana* have been positively identified as the pollinating agents. The significance of the distinctive floral morphology of section *Pictae*, and the direction of pollination syndromes in *Aloe*, require systematic study.

Phylogenetic reconstructions based on nuclear and plastid sequence data provided novel insights into the infrageneric status, and interspecific relationships, of *Aloe* section *Pictae.* We anticipate that further phylogenetic evidence will add considerable understanding of evolutionary relationships and taxonomic stability to help propose a revised infrageneric classification of *Aloe.* This evolutionary framework will also be essential to examine the biogeographical patterns and causes of speciation in this important genus of succulents.

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