



CHAPTER 2 MATERIALS AND METHODS

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2.1 Material

An overview of materials used during this research project is presented. Comparative studies undertaken during this research were primarily concerned with section *Pictae*, and effort was made to sample thoroughly within this taxonomic unit and associated species in the so-called maculate species complex. Species classified in other infrageneric groups within *Aloe* and neighbouring genera were included as outgroups. Material for experimental purposes was collected from plants of field provenance kept in glasshouses at the Royal Botanic Gardens, Kew, from natural populations in South Africa or from pressed herbarium specimens. Voucher specimens of field collections were deposited in the National Herbarium in South Africa (PRE) and the herbarium at Kew (K), where voucher specimens of *ex hort* material were deposited (Table 2.1). Localities (Figures 2.1 and 2.2) are given on a large scale due to the sensitivity of locality data for certain rare species threatened by plant collectors.

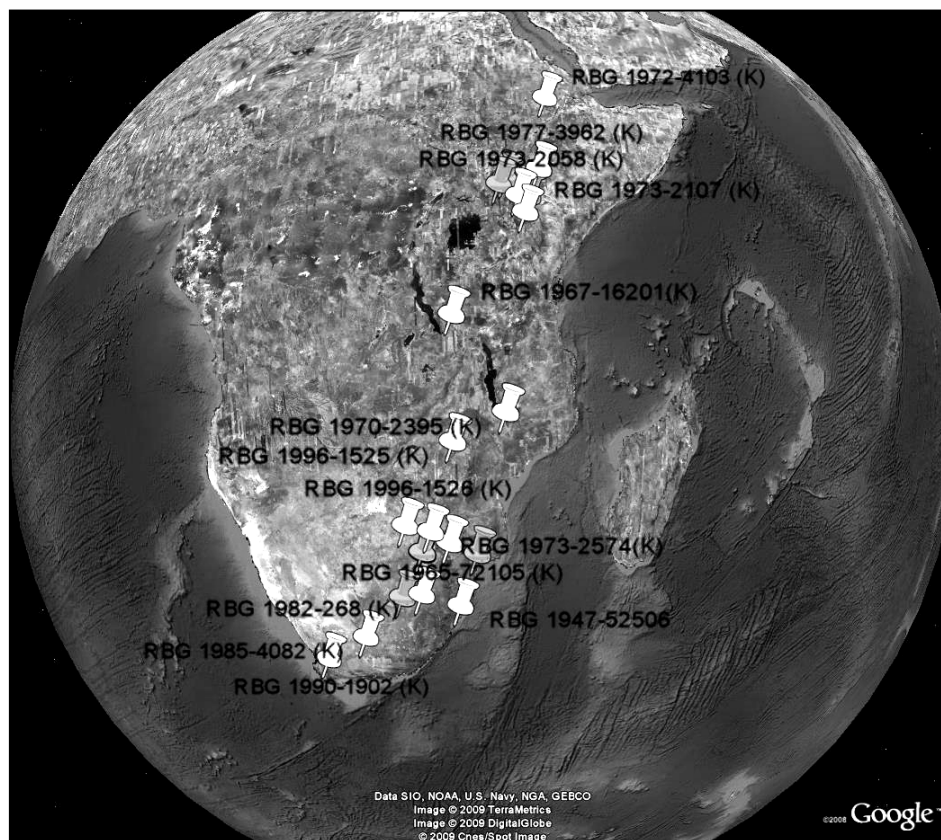


Figure 2.1 Approximate origin localities of plant material at Kew used in this study, labels are Kew Living Collection accession numbers (see Table 2.1).

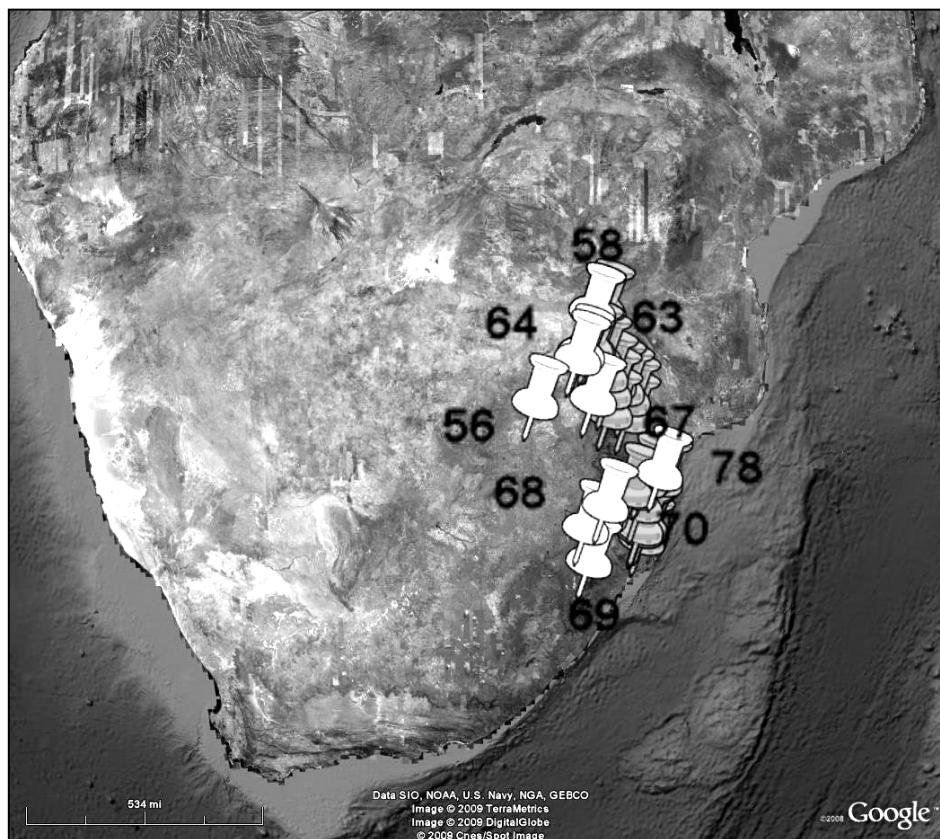


Figure 2.2 Approximate localities of plant material collected for this study, labels are Grace collector's numbers (see Table 2.1).

2.2 Methods

An overview of methods used during this research project is presented. Detailed methods used for comparative studies are described in respective Chapters.

Ethnobotany

The biocultural and economic value of *Aloe* L. was assessed using the literature as a surrogate for ethnobotanical field study (Chapter 3). Approximately 500 references were surveyed by searching library catalogues and information repositories, and by browsing library shelves. Most sources were located in the Library, Art and Archives collections of the Royal Botanic Gardens, Kew. Documented uses and useful properties of species of *Aloe* were recorded. Data were analysed and subjected to consensus analysis, where a mathematical formula is applied to quantify the relative importance of use categories.

Phylogenetics

DNA sequences were obtained for representatives of section *Pictae*, supplemented by published sequences available for ingroup and outgroup species. The plastid *trnL-F* intron and spacer, and *matK* gene and the nuclear ribosomal Internal Transcribed Spacer (ITS) were amplified and sequenced. Phylogenetic reconstructions from these data were obtained with statistical models for parsimony supported by bootstrap and consensus tests, as well as maximum likelihood using Bayesian inference (Chapter 4).

Phytochemistry

The leaf chemistry of species in section *Pictae* was surveyed using hyphenated chromatographic techniques (Chapter 5). Qualitative comparative data were analysed by quantifying the peak area of constituents shown on chromatograms from high performance liquid chromatography (HPLC). Mass spectrometry was used to identify known flavonoids, while nuclear magnetic resonance spectroscopy was used to characterise a novel anthrone C-glycoside.

Micromorphology

Leaf material of species in section *Pictae* were fixed and mounted appropriately for examination under scanning electron microscope (SEM) and light microscope. Digital micrographs of leaf surfaces and transverse sections were recorded (Chapter 6).

Table 2.1 Plant material used for comparative studies (synonyms recognised at time of collection shown in parentheses)

Taxon	Voucher and herbarium ¹	Collection locality ²
<i>Aloe affinis</i> A.Berger	Grace 87	Mac Mac Falls, South Africa
<i>A. amudatensis</i> Reynolds	RBG 1977-6734 (K)	Weiwei, Kenya
<i>A. barbertoniae</i> Pole-Evans	Grace 85	Barberton, South Africa
<i>A. branddraaiensis</i> Groenew.	RBG 1957-14502 (K)	South Africa
<i>A. burgersfortensis</i> Reynolds	RBG 1965-72105 (K)	Lydenburg, South Africa
<i>A. burgersfortensis</i> Reynolds	Grace 89	Burgersfort, South Africa
<i>A. chabaudii</i> Schönland	RBG 1996-1526(K)	Buffel's Drift, Zimbabwe
<i>A. dewetii</i> Reynolds	Grace 83	Alpha, South Africa
<i>A. ellenbeckii</i> A.Berger	RBG 1973-2107(K)	Nairobi, Kenya
<i>A. ellenbeckii</i> A.Berger	RBG 1977-2441 (K)	Marsabit, Kenya
<i>A. ellenbeckii</i> A.Berger (= <i>A. dumetorum</i>)	RBG 1977-3962 (K)	Marsabit, Kenya
<i>A. fosteri</i> Pillans	RBG 2003-1796 (K)	South Africa
<i>A. fosteri</i> Pillans	Grace 88	Ohrigstad, South Africa
<i>A. grandidentata</i> Salm-Dyck	RBG 1973-2520(K)	Orange Free State, South Africa
<i>A. greatheadii</i> Schönland	RBG 1996-1525 (K)	Harare, Zimbabwe
<i>A. greatheadii</i> var. <i>davyana</i> (Schönland) Glen & D.S. Hardy (= <i>A. graciliflora</i> Groenew.)	Grace 67	Tonteldoos, South Africa
<i>A. greatheadii</i> var. <i>davyana</i> (Schönland) Glen & D.S. Hardy (= <i>A. longibracteata</i> Pole-Evans)	Grace 66	Lydenburg, South Africa
<i>A. greatheadii</i> var. <i>davyana</i> (Schönland) Glen & D.S.Hardy	RBG 1965-12201 (K)	Pretoria, South Africa
<i>A. greatheadii</i> var. <i>davyana</i> (Schönland) Glen & D.S.Hardy (= <i>A. davyana</i>)	RBG 1973-2542 (K)	Pretoria, South Africa
<i>A. greatheadii</i> var. <i>greatheadii</i>	Grace 58	Louis Trichardt, South Africa
<i>A. greatheadii</i> var. <i>greatheadii</i>	Grace 61	Boyne, South Africa
<i>A. greenii</i> Baker	Grace 74	Eshowe, South Africa
<i>A. immaculata</i> Pillans	Grace 62	Chuniespoort, South Africa
<i>A. immaculata</i> Pillans	Grace 64	Chuniespoort, South Africa

Table 2.1 (continued)

Taxon	Voucher and herbarium ¹	Collection locality ²
<i>A. lateritia</i> var. <i>graminicola</i> (Reynolds) S.Carter	RBG 1973-2058 (K)	Thompson's Falls, Kenya
<i>A. lateritia</i> var. <i>graminicola</i> (Reynolds) S.Carter (= <i>A. lateritia</i> var. <i>solaiana</i>)	RBG 1973-2070 (K)	Nanyuki, Kenya
<i>A. leptosiphon</i> A.Berger (= <i>A. greenwayi</i>)	RBG 1967-16201 (K)	Abercorn, Zambia
<i>A. lettyae</i> Reynolds	Grace 60	Haenertsburg, South Africa
<i>A. macrocarpa</i> Tod.	RBG 1972-4103 (K)	Adamitulla, Ethiopia
<i>A. maculata</i> All.	Grace 82	Ngome, South Africa
<i>A. maculata</i> All.	Grace 84	Carolina, South Africa
<i>A. maculata</i> All. (= <i>A. saponaria</i> var. <i>ficksburgensis</i>)	RBG 1982-268 (K)	Ficksburg, South Africa
<i>A. maculata</i> All. (= <i>A. saponaria</i>)	RBG 1990-1902 (K)	Cape Province, South Africa
<i>A. monotropa</i> I.Verd.	Grace 65	Mmafeke, South Africa
<i>A. mudenensis</i> Reynolds	RBG 1947-52506 (K)	Natal, South Africa
<i>A. parvibracteata</i> Schönland	Grace 77	Jozini, South Africa
<i>A. parvibracteata</i> Schönland	Grace 78	Ingwavuma, South Africa
<i>A. parvibracteata</i> Schönland	Grace 79	Pongola, South Africa
<i>A. parvibracteata</i> Schönland	Grace 80	Pongola, South Africa
<i>A. petrophila</i> Pillans	RBG 1973-2501 (K)	Transvaal, South Africa
<i>A. prinslooii</i> I.Verd. & D.S.Hardy	Grace 68	Colenso, South Africa
<i>A. pruinosa</i> Reynolds	Grace 69	Ashburton, South Africa
<i>A. simii</i> Pole-Evans	Grace 86	White River, South Africa
<i>A. striata</i> Haw.	RBG 1985-4082 (K)	Karoo, South Africa
<i>A. suffulta</i> Reynolds	RBG 1961-56203 (K)	Mozambique
<i>A. swynnertonii</i> Rendle	RBG 1970-2395 (K)	Livingstone Falls, Malawi
<i>A. swynnertonii</i> Rendle	Grace 59	Thohoyandou, South Africa
<i>A. umfoloziensis</i> Reynolds	Grace 71	Eshowe, South Africa

Table 2.1 (continued)

Taxon	Voucher and herbarium ¹	Collection locality ²
<i>A. umfoloziensis</i> Reynolds	Grace 72	Eshowe, South Africa
<i>A. umfoloziensis</i> Reynolds	Grace 73	Eshowe, South Africa
<i>A. umfoloziensis</i> Reynolds	Grace 75	Eshowe, South Africa
<i>A. umfoloziensis</i> Reynolds	Grace 76	Eshowe, South Africa
<i>A. vanbalenii</i> Pillans	Grace 81	Nongoma, South Africa
<i>A. vanrooyenii</i> G.F.Sm. & N.R.Crouch	Grace 70	Muden, South Africa
<i>A. vogtsii</i> Reynolds	Grace 57	Louis Trichardt, South Africa
<i>A. wollastonii</i> Rendle (= <i>A. lateritia</i> var. <i>kitaliensis</i>)	RBG 1973-1982 (K)	Kitale, Kenya
<i>A. zebrina</i> Baker (= <i>A. ammophila</i> Reynolds)	Grace 63	Chuniespoort, South Africa
<i>A. zebrina</i> Baker (= <i>A. ammophila</i>)	RBG 1973-2574 (K)	Potgietersrus, South Africa

¹K, Herbarium at Kew, United Kingdom; PRE, National Herbarium, Pretoria, South Africa.

²Original locality data may reflect historical or vague geographical names