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A chemotaxonomic and morphological appraisal of *Aloe* series Purpurascentes, *Aloe* section Anguialoe and their hybrid, *Aloe broomii*

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Abstract

Evidence is presented to suggest the hybrid origin of *Aloe broomii*, with the one putative parent belonging to *Aloe* series *Purpurascentes* and the other a member of *Aloe* series *Anguialoe*. A chemotaxonomic and morphological assessment is presented for both infrageneric groups and their hypothesised hybrid. Four of the species belonging to the series *Purpurascentes* display a characteristic leaf exudate profile containing the chemotaxonomic marker microstigmin. *Aloe gariepensis* and *A. succotrina* lack the diagnostic leaf exudate compounds. The distinct morphological apomorphies for *Aloe* section *Anguialoe* are supported on the chemical level reinforcing the monophyly of this group. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *Aloe*; Aloaceae; *Purpurascentes*; *Anguialoe*; *Aloe broomi*; Microstigmin; Chemotaxonomy

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1. Introduction

Aloe series *Purpurascentes* Salm-Dyck comprises five species, *A. microstigma* Salm-Dyck, *A. framesii* L. Bolus, *A. gariepensis* Pillans, *A. khamiesensis* Pillans and *A. succotrina* All. (Reynolds, 1950). The species are characterised by their spotted leaves (hence speckled aloes) and mostly produce an unbranched inflorescence. *Aloe pictifolia* was described by Hardy (1976) and initially it was erroneously suggested to

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bear a taxonomic relationship with *Aloe* series *Echinatae* Salm-Dyck, especially to *A. krapohliana* Marloth (Van Jaarsveld, 1993). Laubscher and Swart (1977), however, suggests a more plausible taxonomic alliance between *A. pictifolia* and *A. microstigma* stating that it is almost impossible to distinguish between juvenile plants of these two species. Reynolds (1950) included five species in *Aloe* section *Anguialoe* Reynolds; *A. alooides* (Bolus) ven Druten (syn. *A. recurvifolia*), *A. castanea* Schönland, *A. dolomitica* Groenewald, *A. spicata* L. f. (syn. *A. sessiliflora*) and *A. vryheidensis* Groenewald. (Some taxonomists consider *A. dolomitica* to fall within the variation described for *A. vryheidensis*. For a complete listing of synonyms for the group under discussion see Glen and Hardy, 2000.) Reynolds believed that this group is sufficiently well defined by the sessile campanulate flowers and the distinct inflorescence to warrant sectional status in the taxonomic hierarchy of *Aloe* classification. In 1968 Leach described *A. tauri* from the southern part of Zimbabwe (Leach, 1968) and suggested a close affinity with *A. spicata*. Lastly, Reynolds (1950) unites *A. broomii* Schönland, *A. longistyla* Baker and *A. peglerae* Schönland in an artificial assemblage, series *Longistylae* Berger. In 1973 Lavranos described the distinctive *A. chlorantha* from Fraserburg, postulating a taxonomic kinship with *A. broomii*.

Rauwald and Beil (1993) discussed the chemotaxonomic value of 5-hydroxyaloin A in the genus *Aloe* while Reynolds (1985) reported the TLC patterns for various taxa included in this paper. A chemotaxonomic survey of 380 species in the genus *Aloe* (Viljoen, 1999) shows that the leaf exudate compounds are useful characters at the infrageneric level of classification (Viljoen, 1999, Viljoen and Van Wyk, 2000, 1999, Viljoen et al. 2001, 1999, 1998, 1996). This paper forms part of a series of publications illustrating the value of chemotaxonomic data to explore possible relationships between taxa in this large genus of ca. 420 species where no satisfactory classification system yet exists.

2. Material and methods

Leaf exudate was collected in situ and at the National Botanical Institute, Pretoria (NBI). Locality details for species included in the discussion are listed in Table 1.

The exudate was investigated with HPLC. Samples were dissolved in methanol and passed through C_{18} cartridges to remove substances of high retention time. These purified samples were dissolved in methanol–water (1:1) and injected into the HPLC system. Operating conditions were as follows: A Phenomenex IB-Sil column was used (C_{18} reverse phase, $5\,\mu m$ particle size, $250\,mm \times 4.6\,mm$ internal diameter; flow rate $1\,ml\,min^{-1}$; $20\,\mu l$ sample loop). The solvent system comprised a 30–60% linear gradient of methanol in water over 25 min, 3 min isocratic, 100% in 2 min, 4 min isocratic. Detection was by diode array detector, using two channels (A set at $275 \pm 70\,nm$; B set at $365 \pm 40\,nm$). Compounds were identified by comparison of the retention times and UV/VIS spectra with reference samples. Authentic reference samples were available through previous studies.

Table 1

The major leaf compounds in *Aloe* section *Anguialoe*, series *Purpurascentes* and related species. 1 = aloesin, 2 = 7-*O*-methylaloesin, 3 = caffeoyl chromone, 4 = caffeoyl chromone, 5 = 5-hydroxyaloin A, 6 = caffeoyl chromone, 7 = aloin B, 8 = homonataloin B, 9 = 6′-*O*-coumaroylaloesin, 10 = aloin A, 11 = microstigmin, 12 = homonataloin A, 13 = cinnamoyl chromone, 14 = cinnamoyl chromone, 15 = cinnamoyl chromone, 16 = cinnamoyl chromone, 17 = unidentified anthrone, 18 = cinnamoyl chromone

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	Retention time →	5.1	7	10.7	17.2	19.4	20.6	23.3	23.6	23.7	24.7	26.3	26.4	29.9	30.3	32	33.3	33.7	33.9
A. alooides (Bolus) van Druten	The Bonnet	+	−	−	−	−	+	+	−	+	+	−	−	−	+	+	−	−	−
A. castanea Schönland	Three Rondawels	+	−	−	−	−	+	+	−	+	+	−	−	−	−	+	−	−	−
	Elandslaagte	+	−	−	−	−	+	+	−	+	+	−	−	−	−	−	−	−	−
A. dolomitica Groenewald	Wolkeberge	+	−	−	−	−	+	+	−	+	+	−	−	−	+	−	−	−	−
A. spicata Linné (fil.)	Bourke's Luck	+	−	−	−	−	+	+	−	+	+	−	−	−	+	+	−	−	−
A. tauri L.C. Leach	WE 547	+	−	−	−	−	−	+	−	+	+	−	−	−	+	−	−	−	−
A. vryheidensis Groenewald	Louwsberg	+	−	−	−	−	+	+	−	+	+	−	−	−	+	+	−	−	−
A. framesii L. Bolus	ex hort NBI	+	+	−	−	+	−	−	−	−	−	+	−	−	−	−	−	+	+
A. gariepensis Pillans	Keimoes	+	+	−	−	−	−	−	+	−	−	−	+	+	−	−	−	−	−
A. khamiesensis Pillans	Skynshoogte Pass	+	−	−	−	+	−	−	−	−	−	+	−	−	−	−	+	+	+
A. microstigma Salm-Dyck	Robertson	+	+	−	+	+	−	−	−	−	−	+	−	−	−	−	+	+	+
	Cradock	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
	Calitzdorp	+	−	+	+	−	−	−	−	−	−	+	−	+	−	−	−	−	+
A. pictifolia D.S. Hardy	Patensie	+	−	+	−	+	−	−	−	−	−	+	−	−	−	−	−	+	+
A. succotrina[a] Allioni	Table Mountain	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
A. broomii Schönland	Springfontein	+	−	−	+	+	−	+	−	−	+	−	−	+	−	−	−	−	+
	Graaff-Reinet	−	−	−	+	+	−	−	−	−	−	+	−	−	−	−	−	−	+
	Whitlesea	+	+	−	+	+	−	−	−	−	−	+	−	+	−	−	−	−	+
A. chlorantha Lavranos	Fraserburg	+	−	−	−	+	−	+	−	−	+	−	−	+	−	−	−	−	−
A. longistyla[b] Baker	Calitzdorp	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
The other ca. 360 species of *Aloe*	(Viljoen, 1999)	±	±	−	−	±	−	±	±	−	±	−	±	±	−	−	−	−	−

[a] *Aloe succotrina* contains a series of 7-hydroxyaloin derivatives not found in any other species of *Aloe* (Sigler and Rauwald, 1994a, 1994b).

[b] *Aloe longistyla* contains a series of 5-hydroxyaloin A derivatives not found in any other species of *Aloe* (Viljoen, 1999).

3. Results and discussion

3.1. The leaf exudate chemistry

Each of the three groups; series *Purpurascentes*, section *Anguialoe* and the *Aloe broomii* – *A. chlorantha* alliance produce a diagnostic exudate profile with a degree of chemical similarity between the three groups. Table 1 shows the presence of the leaf exudate compounds for the species. Almost all species produce the chromone aloesin and it has been suggested through results generated in a broader chemotaxonomic study (Viljoen, 1999) that this chromone probably originated early in the evolution of the genus as it is the most widely distributed leaf exudate compound and therefore of no obvious chemotaxonomic value.

The chemotaxonomic value of the important chemical markers are discussed in relation to Fig. 1. All the species in section *Anguialoe* are characterised by a diagnostic chemical profile. Most species produce a caffeoyl chromone (compound 6 in Table 1) with a retention time of 20.6, which is usually present in trace amounts. Eluting between the aloin isomers is 6′-*O*-coumaroylaloesin, a second characteristic chromone with a coumaroyl ester group (compound 9 in Table 1). This chemotaxonomic marker is always present in species in the *Anguialoe* group and has recently been isolated from *A. castanea* (Van Heerden et al., 2000). The last of the characteristic "chromone-trio" is a late eluting cinnamoyl chromone (compound 15 in Table 1). The characteristic HPLC profiles for members of the *Anguialoe* are shown in Fig. 2. The leaf exudate of *Aloe broomii* was investigated by Holzapfel et al.

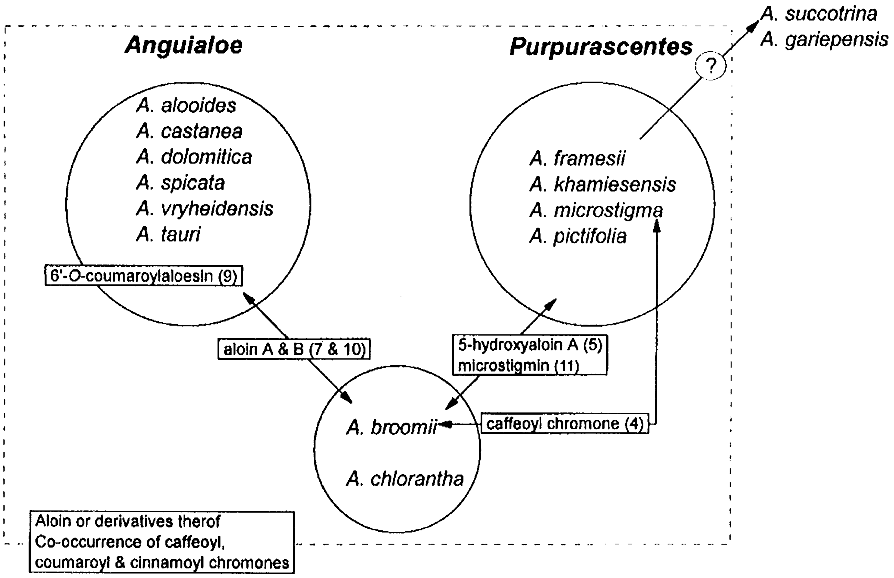

Fig. 1. Diagrammatic representation of chemotaxonomic markers in *Aloe* section *Anguialoe*, series *Purpurascentes* and *A. broomii*. Numbers in parenthesis refer to compounds in Table 1.

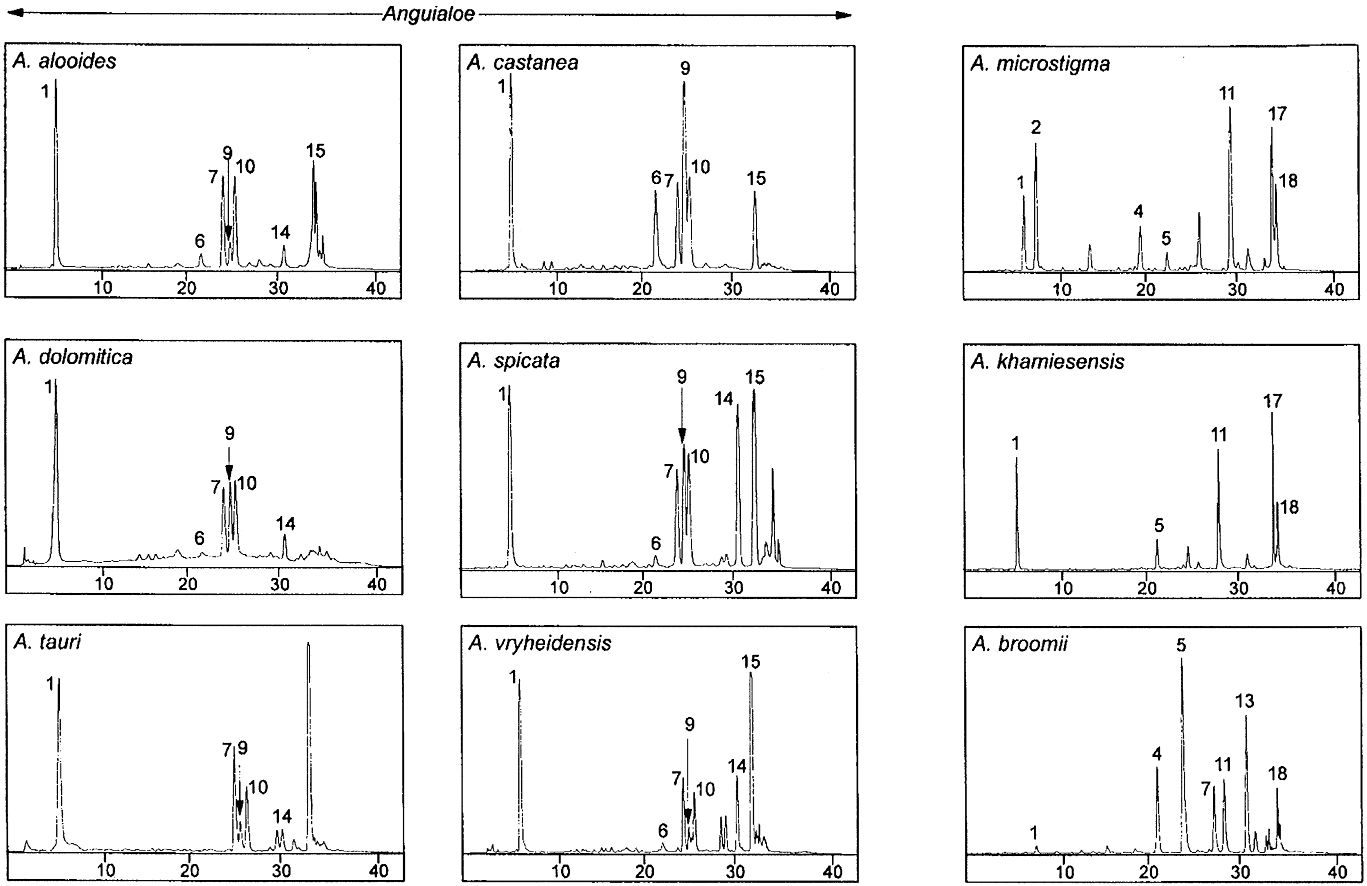

Fig. 2. HPLC chromatograms of the six species in the *Anguialoe* group showing the remarkable chemical similarity. The HPLC profiles for two species from *Aloe* series *Purpurascentes* (*A. microstigma* & *A. khamiesensis*) and *Aloe broomii* indicate the presence of the marker compound, microstigmin (11).

(1997) and two novel chromones were isolated from this species. The chromone eluting at 17.29 min is (E)-2-acetonyl-8-(2′-*O*-caffeoyl-β-D-glucopyranosyl-7-methoxy-5-methyl chromone (compound 4 in Table 1). This chromone is present in all three samples of *A. broomii*. Trace amounts of this chromone was also found in two of the three samples of *A. microstigma*. The second chromone isolated from *A. broomii* is (E)-2-acetonyl-8-(2′-*O*-cinnamoyl-β-D-glucopyranosyl-7-methoxy-5-methyl chromone (compound 13 in Table 1). *Aloe broomii* shows variation with reference to the anthrone composition. One sample from Springfontein contained 5-hydroxyaloin A together with aloin A and B while two samples from different localities contained microstigmin in co-occurrence with the anthrone 5-hydroxyaloin A. *Aloe chlorantha* also contains 5-hydroxyaloin A together with aloin A and B resembling the anthrone composition of *A. broomii* (Springfontein). The leaf exudate of the species belonging to series *Purpurascentes* is extremely complex. The chemotaxonomic marker, microstigmin (compound 11 in Table 1), was isolated by Dagne et al. (1997) and is present in *A. microstigma*, *A. pictifolia*, *A. framesii*, *A. khamiesensis* and in two of the three populations of *A. broomii*. It is interesting to note that this compound always occurs in the absence of the anthrone isomers aloin A and B. A selection of the HPLC profiles for the microstigmin-producing aloes is shown in Fig. 2. The distribution of chemical compounds shown in Table 1 clearly shows the importance of extensive sampling of plant populations. Through extensive sampling procedures various patterns emerge from the data, establishing a chemotaxonomic affinity between the taxa.

3.2. Morphological characters

The salient morphological characters believed to be of diagnostic value are tabulated in Table 2. Many groups defined by Reynolds (1950) are heterogeneous assemblages of various morphological characters. The taxa discussed here also represent almost the entire morphological variation in *Aloe*. Within a convincingly natural clade such as the *Anguialoe*, plants could be distinctly caulescent and erect (e.g. *A. castanea* and *A. alooides*) or acaulescent (e.g. *A. vryheidensis* and *A. tauri*). The leaves vary from strongly re-curved (*A. alooides*) to erect (*A. castanea*). The leaves are always immaculate. All species in the *Anguialoe* are characterised by a simple inflorescence with an erect, cylindrical, densely multiflowered raceme bearing cylindric–campanulate sessile flowers of which the stamens and stigma are exserted. This character is only shared with a small number of species from Madagascar, which do not seem to be related to the African species.

The species in series *Purpurascentes* are mostly very shortly caulescent except for *A. khamiesensis* which develops an erect stem up to 1.5 m tall. The leaves are variable in maculation. At some localities the leaves of *A. framesii* are copiously spotted while at other localities the leaves are immaculate. The inflorescence character is remarkably uniform in this group of species. All species but two produce an unbranched inflorescence with *A. khamiesensis* and *A. framesii* forming a panicle. The flowers are distinctly pedicellate, cylindrical trigonous in shape and the stamens and stigma are not exserted.

Table 2
Salient morphological characters in *Aloe* section *Anguialoe*, series *Purpurascentes* and related species

	Caulescence	Habit	Leaf orientation	Leaf maculation	Inflorescence	Bracts (length)	Pedicel	Perianth
A. alooides	Caulescent, erect	Solitary	Recurved	Immaculate	Simple	5–7 mm	Flowers sessile	Cylindric-campanulate
A. castanea	Caulescent, erect	Solitary or arborescent	Erect	Immaculate	Simple	12 mm	Sub-sessile	Cylindric-campanulate
A. dolomitica	Caulescent, erect	Solitary	Erect, slightly incurved	Immaculate	Simple	8 mm	Flowers sessile	Cylindric-campanulate
A. spicata	Caulescent, erect	Solitary or shrubby	Spreading/recurved	Immaculate	Simple	8 mm	Flowers sessile	Cylindric-campanulate
A. vryheidensis	Acaulescent, shortly procumbent	Solitary	Erect	Immaculate	Simple	15 mm	Flowers sessile	Cylindric-campanulate
A. tauri	acaulescent	Solitary	Recurved	Immaculate	Simple	12 mm	Flowers sessile	Cylindric-campanulate
A. framesii	Long procumbent stem	Dense groups	Spreading/incurved	Varies from spotted to unspotted	2 - Branched, rarely simple	2/3 length of pedicel	Pedicellate 30 mm	Cylindrical trigonous
A. khamiesensis	Stem erect, up to 1.5 m	Solitary	Erect, apex recurved	Obscurely lineate and spotted	Panicle	1/2 length of pedicel	Pedicellate 25 mm	Cylindrical trigonous
A. microstigma	Shortly procumbent	Solitary or in small groups	Erect	Variable, mostly copiously spotted	Simple	1/2 length of pedicel	Pedicellate 30 mm	Cylindrical, slightly ventricose
A. pictifolia	Shortly procumbent	Pendent	Deflexed	Copiously spotted	Simple	2/1 length of pedicel	Pedicellate 12 mm	Cylindrical trigonous
A. broomii	Short or long procumbent stem (1 m)	Mostly solitary	Erect	Obscurely lineate	Simple	Longer than perianth 30 mm	Sub-sessile	Cylindrical, slightly curved
A. chlorantha	Acaulescent or short procumbent stem	Solitary or splitting into 10 rosettes	Erect	Striate and often spotted	Simple, rarely forked	As long as perianth 12–20 mm	Shortly pedicellate	Ventricose

The leaves of *A. broomii* and *A. chlorantha* are often obscurely lineate, a character shared with *A. khamiesensis* of the *Purpurascentes*. The imbricate bracts of *A. broomii* and *A. chlorantha* are characteristic, being as long or longer than the perianth. The flowers of *A. broomii* and *A. chlorantha* are shortly pedicellate.

3.3. Taxonomic relationships

When superimposing the chemical and morphological patterns on the taxonomic arrangement of the species, interesting congruencies and incongruencies are noted. *Aloe* section *Anguialoe* is an example where the present grouping of the species (based on morphology only) is completely congruent with the chemical data. Irrespective of the specific taxonomy of this group, the distinct morphological coherence between the taxa in this group is reflected in the diagnostic and consistent leaf exudate profile as shown in Fig. 2. What could be debated, however, is if this chemical and morphological distinction warrants sectional status.

Of the six species included in *Aloe* series *Purpurascentes* (including *A. pictifolia*), four produce an identical exudate profile with 5-hydroxyaloin A and the recently described anthrone microstigmin, as chemotaxonomic marker compounds. *Aloe succotrina*, the type species of the *Purpurascentes* group produces leaf exudate very different from the other members of the *Purpurascentes* mentioned above. Siegler and Rauwald (1994a, b) indicated the presence of 7-hydroxyaloin derivatives in this species, and the survey by Viljoen (1999) has not detected the same combination of exudate anthrones in any other species. It has been demonstrated in Viljoen (1999) that 7-hydroxyaloin and derivatives thereof form readily when crossing an aloin and homonataloin producing species implicating that *A. succotrina*, like *A. broomii* could possibly be a product of hybridization of which the parents are not obvious in the case of *A. succotrina* (from a chemical and morphological perspective). The second chemical outlier included in series *Purpurascentes* is *A. gariepensis*, which follows a distribution along the western tributary of the Orange River (alternatively known as the Gariep River). This species is included by Reynolds (1950) in the series *Purpurascentes* with the following comments: "*A. gariepensis* is the only species in the series having long cylindric–acuminate racemes 35–50 cm long, and bracts much longer than the pedicels". In all the other species in this group the pedicels are long with the bracts always shorter than the pedicel. The leaves of *A. gariepensis* are always distinctly lineate and varies from spotted to unspotted. This plant also varies with respect to inflorescence character with the raceme being uni- or bicoloured. The leaf exudate chemistry is completely different to any other species in the series *Purpurascentes* as it produces the anthrone isomers homonataloin A and B together with cinnamoyl chromones. The anthrone produced by all species in this complex (as illustrated in Fig. 1) is aloin or derivatives thereof.

Reynolds (1950) places *A. broomii* in the heterogeneous series *Longistylae* together with *A. peglerae* and *A. longistyla*. In his description of *A. chlorantha* Lavranos (1973) suggests a taxonomic relationship between his new species and *A. broomii*. Viljoen and Van Wyk (1999) illustrated that *A. peglerae* could possibly be related to other aloeresin E and F containing species in the *Aloe* series *Mitriformes*-group, while *A. longistyla* is both morphologically and chemically anomalous. This species produces a series of unidentified anthrone derivatives resembling 5-hydroxyaloin A in UV absorbance characters. Considering the spottedness of the leaves and the presence of 5-hydroxyaloin derivatives which are both characteristic of the *Purpurascentes* one could imagine, perhaps, some ancient alliance with this group. More interesting and convincing is the taxonomic position of *A. broomii* which is best reflected by the distribution map (Fig. 3). It is here suggested that *A. broomii* is an ancient hybrid between the *Anguialoe* and the *Purpurascentes*. With respect to morphological characters it shares with the *Anguialoe* the sessile flowers and the distinct single inflorescence up to 1.5 m tall. This resemblance is supported on the chemical level as some populations produce the same anthrone found in the *Anguialoe*, aloin A and B. *Aloe broomii* also produces various caffeoyl, cinnamoyl and coumaroyl chromones. This co-occurrence of chromone esters in a single species is characteristic of the *Anguialoe* and *Purpurascentes*. With the *Purpurascentes, A. broomii* shares the spotted leaves (reduced in *A. broomii*) and obscure leaf lineation. More convincing are the chemical resemblances which includes 5-hydroxyaloin A and microstigmin which only occurs in the four other species of *Aloe* shown in Fig. 1. The occurrence of leaf exudate compounds in *Aloe broomii* is of special interest. Three populations were investigated; plants from the first population (Springfontein) contain aloin A and B, the major anthrone of the *Anguialoe*. The second (Graaff-Reinet) and third (Whitlesea) populations contained microstigmin

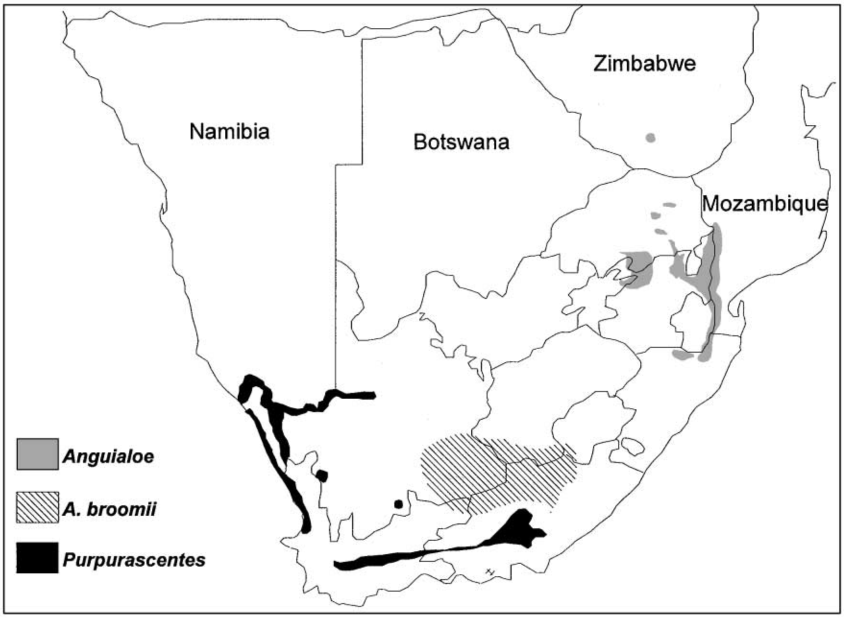

Fig. 3. Distribution of *Aloe* section *Anguialoe* (northeastern South Africa*),* *A. broomii* (central South Africa) and series *Purpurascentes* in the western parts of the country.

and 5-hydroxyaloin A (in the absence of aloin A and B), the marker compounds of the *Purpurascentes*. This is increasing support for the hybrid origin of *A. broomii* showing the leaf exudate to resemble that of any of the two putative parents. The last species, *A. chlorantha* is indisputably closely related to *A. broomii*. It is noteworthy that in the description of the species, the author Lavranos (1973) makes the following interesting statements:

> ...the peduncle is covered from the base up, by numerous, large sterile bracts which, like the floral ones, are rather fleshy. This character is met with in other South African species of *Aloe*, notably *A. glauca*, *A. pratensis*, *A. microstigma*, *A. comosa* etc. Mr Stayner suggests a certain resemblance between *A. chlorantha* and the decumbent form of *A. microstigma*. If flowers and inflorescence be a guide, a much closer relationship may exist between our species and *A. comosa*.

It is encouraging that independent of the chemical results the pattern of reason and suggestions of affinity as discussed here have been put forward by aloe authorities who by their field observation also suggest a possible relationship between the species illustrated in Fig. 1.

4. Conclusions

The leaf exudate composition of *Aloe* section *Anguialoe* supports the morphological delineation of this well-defined taxonomic group. All six species are characterised by 6-*O*-coumaroylaloesin, which has not been detected in any other species of *Aloe*. Four species in *Aloe* series *Purpurascentes* accumulate the anthrone microstigmin. *Aloe succotrina* and *A. gariepensis*, also members of *Aloe* series *Purpurascentes sensu stricto* does not accumulate this chemotaxonomic marker questioning their inclusion in this infrageneric group. Chemical, morphological and distribution data suggest that *Aloe broomii* is possibly an ancient hybrid between the *Anguialoe* and *Purpurascentes*. Chemically, it shares the distinct exudate anthrone, microstigmin, with the *Purpurascentes*. The chromone composition of *A. broomii* is also unique as it contains cinnamoyl, caffeoyl, and coumaroyl chromones. Besides *A. broomii*, this combination of chromone esters is only found in the *Anguialoe* and *Purpurascentes*. Morphologically *A. broomii* resembles members of the *Anguialoe* in the tall, dense, multiflowered inflorescence and the sub-sessile flowers. The possible hybrid origin and taxonomic affinities of *A. broomii* have eluded several taxonomists and even Reynolds himself. It is only through chemical evidence that the probable taxonomic affiliation of *A. broomii* has become apparent.

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