The chemotaxonomic value of two cinnamoyl chromones, aloeresin E and F, in *Aloe (Aloaceae)*

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Summary

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A chemotaxonomic study of the genus *Aloe* indicates a remarkable quantitative and qualitative similarity in leaf exudate composition between 12 species. The diagnostic leaf exudate profile found in all representatives of this group – a combination of homonataloin A and B with either or both of two recently described cinnamoyl chromones, aloeresin E and F, together with various coumaroyl chromones – may serve as a "fingerprint". Taxonomic assessment and cladistic analysis of both morphological and chemical data support the transfer of *A. pearsonii,* previously misplaced in *A.* ser. *Macrifoliae,* to *A.* ser. *Mitriformes,* and are consistent with the broadening of the circumscription of the latter to include 5 anomalous species: *A. angelica, A. yavellana, A. peglerae, A. melanacantha,* and *A. erinacea.*

Introduction

As part of a comprehensive chemotaxonomic study involving a detailed analysis, by high pressure liquid chromatography (HPLC), of 380 species of the genus *Aloe,* we wish to report here the unique leaf exudate composition of all 7 species belonging to *Aloe* ser. *Mitriformes* and its almost identical recurrence in 5 other species not previously associated with that series. Leaf exudate chemistry in itself is not a unifying taxonomic character, but when considered in conjunction with morphological characters it may lead to interesting new relationship hypotheses in a genus where no satisfactory infrageneric classification is available so far. Clear examples of lack of agreement between morphology and leaf exudate chemistry are provided by *A.* ser. *Asperifoliae* (Viljoen & al., 1996) and by the taxonomic distribution of plicataloside (Viljoen & al., 1999). Literature data and results from a broader study on *Aloe* (practically all species producing leaf exudate have now been studied) indicate that constructing a natural phylogeny for *Aloe* is a daunting challenge. The leaf compounds seem to be conservative characters of considerable taxonomic value, as is clearly demonstrated for the group discussed below.

Material and methods

Leaf material, of which the details of provenance are shown in Table 1, was collected either in the wild or at the National Botanical Institute, Pretoria (NBI), the Johannesburg Botanical Gardens (JBG), and the Royal Botanic Gardens, Kew (RBG). The identity of all species was carefully verified, but it was not considered practical to collect voucher specimens. The leaf exudate was analysed, except that for species that are poor producers of exudate *(Aloe* ser. *Macrifoliae)* the whole leaf was dried, ground, and extracted with methanol. This method of extraction resulted in a higher yield of extractable leaf phenolics (see Viljoen & al., 1998). The methanol extracts and exudates were investigated using thin layer chromatography (TLC)

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Table 1. Species studied, with accession numbers or locality data. – The content in major anthrones and chromones is indicated as follows: + = 1-9 %, ++ = 10-19 %, +++ = > 20 %, ± = present or absent, – = absent. The structure of the chemical compounds is given in Fig. 2 (1 = aloesin; 2 = aloeresin A; 3 = aloeresin E; 4 = aloeresin F; 5 = homonataloin B; 6 = homonataloin A; 7 = nataloin B; 8 = nataloin A).

Aloe	accession No. / locality data	1	2	3	4	5	6	7	8
angelica Pole-Evans	Waterpoort (22°55'S, 28°38'E)	+	++	+++	++	+	+++	–	–
–	Waterpoort	+	++	++	++	+	+++	–	–
–	Waterpoort	+	++	+++	++	+	+++	–	–
arenicola Reynolds	JBG s.n.	++	+++	–	+++	++	++	–	–
–	NBI 1283/92	++	+++	–	++	+	++	–	–
comptonii Reynolds	NBI 29356	++	–	+	+++	++	++	–	–
–	Perdepoort (33°43'S, 22°25'E)	++	+	–	+++	++	++	–	–
dabenorisana Van Jaarsv.	Pellaberg (28°55'S, 18°37'E)	+	–	++	+++	+	++	–	–
distans Haw.	Saldanha (33°02'S, 17°57'E)	+	++	++	+++	+	++	–	–
erinacea D. S. Hardy	NBI 13426	+	++	+++	+	–	–	+	+
–	NBI 24391	+	++	+++	+	–	–	+	+
melanacantha A. Berger	JBG 835440	+	–	+	+++	–	–	–	–
meyeri Van Jaarsv.	Rosyntjieberg (28°28'S, 17°12'E)	+	–	+	+++	+	++	–	–
mitriformis Mill.	Du Toitskloof (33°44'S, 19°03'E)	++	+	++	+++	+	++	–	–
–	Nieuwoudtville (31°22'S, 19°06'E)	++	++	–	+++	+	++	–	–
–	Kogmanskloof (33°49'S, 20°03'E)	++	+++	+	+++	+	++	–	–
pearsonii Schönland	Helskloof (28°20'S, 17°01'E)	++	–	+++	++	+	++	–	–
–	NBI 29382	+	–	+++	++	+	++	–	–
peglerae Schönland	Kingskloof (26°01'S, 27°40'E)	+	++	++	++	++	++	–	–
–	Scheerpoort (25°50'S, 27°46'E)	+	–	++	++	++	++	–	–
yavellana Reynolds	RBG 19744192	+++	–	+	++	++	++	–	–
–	ex horto L. Newton	++	–	+	++	++	++	–	–
Other (368 *Aloe* species analysed thus far)		±	±	–	–	±	±	±	±

and 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: Phenomenex IB-Sil column (C_{18}, 5 µm particle size, 250 mm × 4.6 mm internal diameter; flow rate 1 ml min^{-1}; 20 µl sample loop); solvent system a 30 % to 60 % linear gradient of methanol in water over 25 min, 3 min isocratic, 100 % in 2 min, 4 min isocratic; detection by diode array detector, using two channels (A set at 275 ± 70 nm; B set at 365 ± 40 nm). TLC was carried out on silica gel (Merck) plates using ethyl acetate : methanol : water (100 : 16.5 : 13.5) as eluent. Compounds were identified by comparison (the R_f values, visibility/colour under ultraviolet (UV) light at 254 nm and 366 nm, retention times, UV/visible spectra) with reference samples. The structural elucidation of the two chromones, aloeresin E and F, is reported elsewhere (Van Heerden & al., 1996).

The cladistic analysis was done with the HENNIG 86 package, using the "i.e." option to ensure that the shortest possible tree or trees were found. *A.* ser. *Macrifoliae* was selected as outgroup, based on previous suggestions of a possible phylogenetic relationship between this series and *A.* ser. *Mitriformes* (Reynolds, 1950; Van Jaarsveld, 1982; Van Wyk & al., 1995).

Results and discussion

A remarkable discovery was that only 12 out of a total of 380 species of the genus *Aloe* contain two unique cinnamoyl chromones, aloeresin E and F. These compounds were recently described by Van Heerden & al. (1996), but the chemotaxonomic implications of this interesting new chemical evidence have not yet been evaluated. T. Reynolds (1985, 1986, 1990) did include representatives of this group in a TLC screening of the genus and indicated the presence of homonataloin, but the chemotaxonomic utility of the data was not assessed. The 12 species containing aloeresin E and F, usually in combination with homonataloin, are listed in Table 1, and a selection of HPLC profiles showing the similarity between the species is presented in Fig. 1.

The value of this chemotaxonomic evidence is that it provides, for the first time, a plausible indication of relationship for five anomalous species which were previously but uneasily accommodated in the largely artificial infrageneric groups recognised in *Aloe*. These are *A. peglerae, A. melanacantha, A. erinacea, A. angelica,*

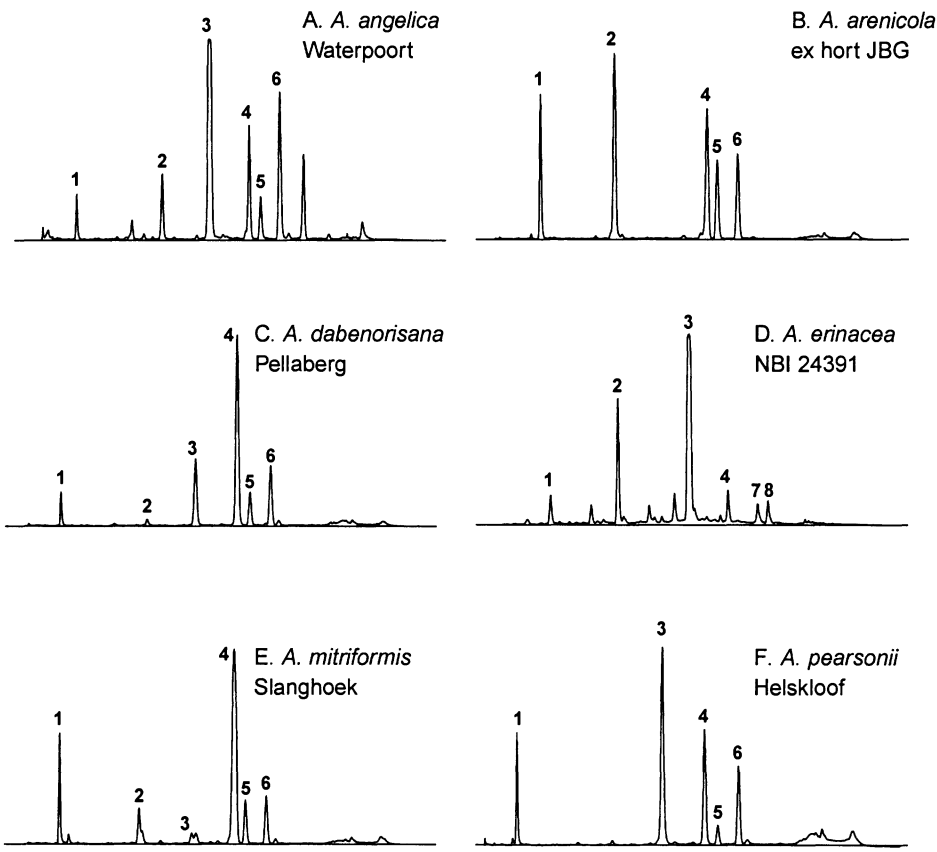

Fig. 1. Chromatograms of various species, showing the presence of the major anthrones and chromones. The peak numbers correlate to the structures in Fig. 2 and compounds in Table 1.

and *A. yavellana*. The presence of aloeresin E and F in these species can be either an evolutionary coincidence (a "convergence" in modern terminology) or else it must be interpreted as a shared derived characteristic. In the discussion below we wish to show that no convincing morphological characters are in conflict with our proposal of a newly defined taxonomic group, the "aloeresin E and F group", within *Aloe* .

The present taxonomic arrangement and accepted affinities between the taxa are diagrammatically represented in Fig. 3. The obvious morphological uniformity of *Aloe* ser. *Mitriformes* is supported by an equally uniform chemical composition of leaf exudate, as shown in Fig. 1 for some of the species. In his taxonomic treatment of the genus *Aloe*, G. W. Reynolds (1950) circumscribed *A.* ser. *Mitriformes* to include *A. mitriformis, A. arenicola, A. comptonii,* and *A. distans*. Since the work of Reynolds three additions have been made: *A. meyeri* (Van Jaarsveld, 1981), *A. dabenorisana* (Van Jaarsveld, 1982), and *A. pearsonii,* previously misplaced in *A.* ser. *Macrifoliae* (Venter & Beukes, 1982). Most members of this group are characterised by a procumbent stem with foliate apical section. The pedicel is slender, longer than or as long as the perianth. The distribution of the species generally coincides with the winter rainfall region of South Africa. The close morphological similarity between the species of the "*Mitriformes* group" has often been highlighted, with various authors querying the specific status of the taxa defined by G. W. Reynolds (1950). Marais (1980), for example, shows that the morphological characters used to distinguish between *A. comptonii, A. mitriformis,* and *A. distans* are ambiguous. The two more recently discovered species, *A. meyeri* and *A. dabenorisana,* have a pendulous habit and subclavate perianths (Van Jaarsveld, 1982). The similarity between the HPLC profiles of *A. dabenorisana* and *A. mitriformis* in Fig. 1 is obvious. The chemical evidence presented here (Fig. 1) gives convincing support for the now widely accepted transfer of *A. pearsonii* from *A.* ser. *Macrifoliae* to *A.* ser. *Mitriformes*. Genuine members of the *"Macrifoliae"* produce little or no exudate, and their leaf extracts contain flavonoids such as isovitexin and the anthrone isomers aloin A and B (Viljoen & al., 1998).

The five anomalous species exhibiting the *"Mitriformes"* leaf exudate chemistry (Fig. 1, Table 1) all have some morphological characteristics apt to support their

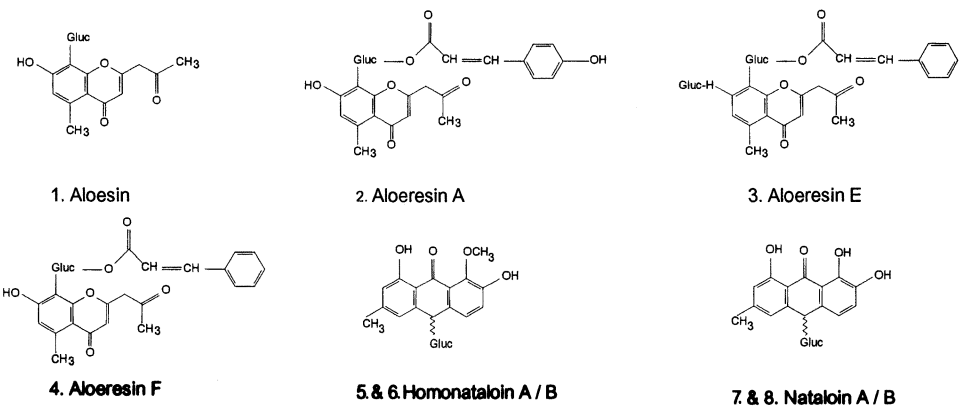

Fig. 2. Chemical structures relating to the HPLC peaks in Fig. 1 and compounds listed in Table 1.

inclusion in an "aloeresin E and F group". The Magaliesberg endemic *Aloe peglerae* is currently placed in *A.* ser. *Longistylae* (Fig. 3), a clearly unnatural group, both from a morphological and leaf chemical point of view. It shows chemical similarity with two other species, *A. erinacea* and *A. melanacantha,* placed in *A.* ser. *Echinatae* together with *A. krapohliana* Pole-Evans and *A. humilis* Mill. *A. krapohliana* is chemically identical with representatives of *A.* ser. *Latebracteatae* (Viljoen & Van Wyk, 1998; Viljoen, 1999), while *A. humilis* is a flavonoid-producing species (Viljoen & al., 1998). The protoloque of *A. erinacea* (Hardy, 1971) suggested a relationship with *A. melanacantha,* and Rowley (1980) indeed considered *A. erinacea* as a mere geographical form of *A. melanacantha,* but Rossouw (1980) demonstrated that the distinctness in floral characteristics warrants specific status for both taxa. No taxonomic alignment with other *Aloe* species has yet been suggested for *A. erinacea* and *A. melanacantha.* We think that *A. peglerae* is their closest morphological relative, as it agrees in dark pungent thorns on the leaf margin and keel, a single inflorescence, large bracts, and an acaulescent to very shortly caulescent habit. The exserted stamens, somewhat ventricose perianth and short pedicel of *A. peglerae,* albeit contrasting with the flower features of *A. erinacea* and *A. melanacantha,* do not permit to suggest other, perhaps more convincing relationships.

G. W. Reynolds (1950) placed *Aloe angelica* in *A.* sect. *Pachydendron* with the following comments "*A. angelica* does not fit well into any series or section. It is a very distinctive species with its much branched inflorescence of bicoloured capitate

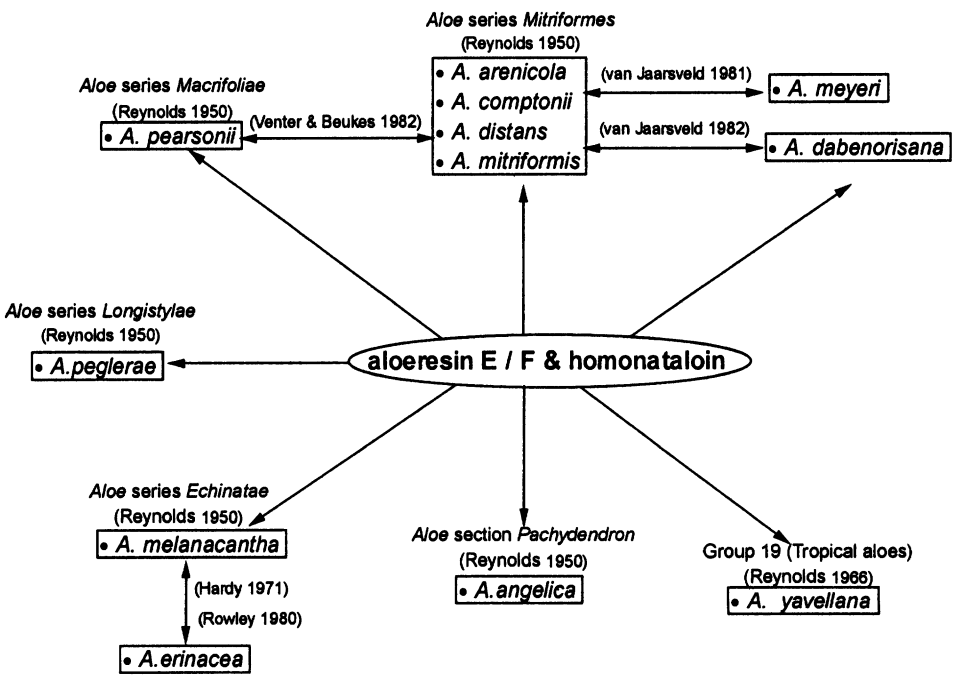

Fig. 3. Taxonomic affiliation between species containing aloeresin E and/or F, and usually also homonataloin.

racemes." A branched panicle of capitate racemes is however found in some members of *A.* ser. *Mitriformes,* e.g. *A. mitriformis.* The HPLC profiles of *A. angelica* and *A. mitriformis* (Fig. 1) also show close similarity. One may speculate that the development of a tall, erect stem in *A. angelica* reflects a survival strategy, to project above the dense bush of the Soutpansberg where this species grows.

Aloe yavellana presents an equally interesting situation. This species belongs in "group 19" of G. W. Reynolds (1966), whose system of classification is obviously based on criteria of utility rather than phylogenetic relationship. In the protoloque of *A. yavellana,* G. W. Reynolds (1966) states: "another peculiarity of *A. yavellana* is that it forms fairly compact shrubs when stems are erect and not exceeding 1 m in length, but with development stems topple over and form sprawling shrubs with stems 2-3 m long, especially on steep slopes, with the foliate portion ascending". This description also applies to many of the species in *A.* ser. *Mitriformes* and, together with the resemblances in the inflorescence structure and leaf exudate chemistry, suggests an affiliation of *A. yavellana* with the "*Mitriformes* group". A chemotaxonomic survey of the genus *Aloe* (Viljoen, 1999) has shown many examples of "chemical relationship" between species in South Africa and eastern Africa, a link that has previously been reported in broader floristic analyses by De Winter (1971) and Newton (1980).

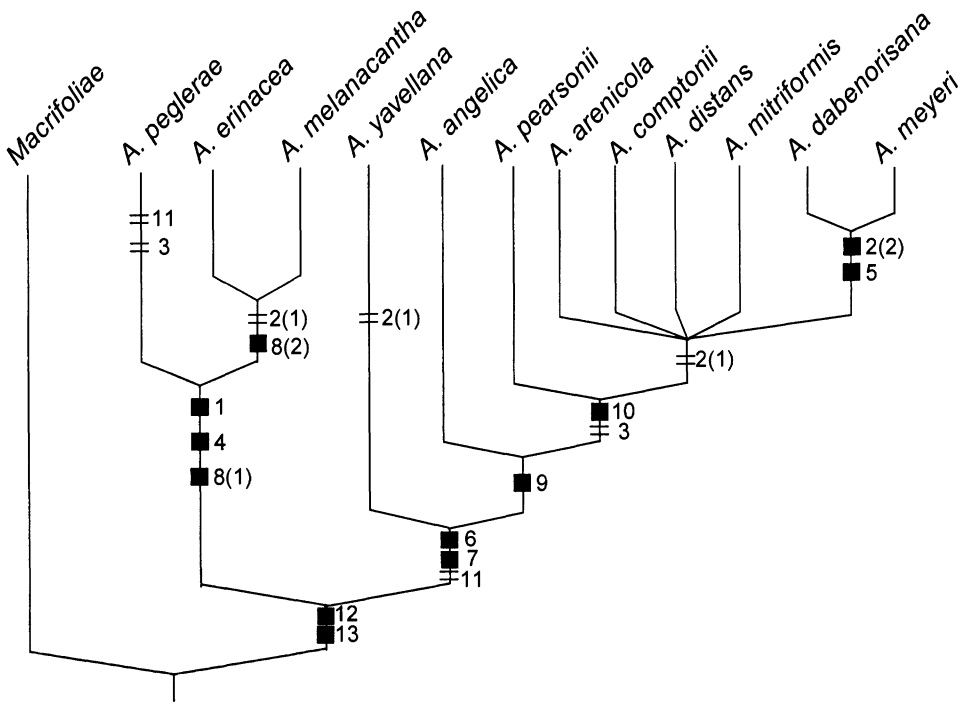

Fig. 4. Cladogram of phylogenetic relationships in *Aloe* ser. *Mitriformes* and related species, based on the data presented in Table 2. A partially resolved cladogram, with 19 steps and a consistency index of 78, was obtained using the "i.e." command in HENNIG 86.

Table 2. Characters and polarisation of morphological (1-10) and chemical (11-13) character states. – 1, habit (distinctly caulescent = 0, ± acaulescent = 1); 2, stem (erect = 0, procumbent = 1, pendulous = 2); 3, leaf shape (narrow triangular to oblong = 0, broadly triangular = 1); 4, thorns (white, blunt and harmless = 0, black and pungent = 1); 5, inflorescence habit (erect = 0, pendulous-recurved = 1); 6, inflorescence (simple to few-branched = 1, paniculate = 1); 7, raceme (cylindrical = 0, capitate to subcapitate = 1); 8, bract length (< 10 mm = 0, ± 15 mm = 1, < 18 mm = 2); 9, pedicels (shorter than perianth = 0, ± as long as perianth = 1); 10, flower shape (relatively broad = 0, narrow, slender = 1); 11, homonataloin (absent = 0, present = 1); 12, aloeresin E and/or F (absent = 0, present = 1); 13, flavones (present = 0, absent = 1).

Characters:	1	2	3	4	5	6	7	8	9	10	11	12	13
A. ser. *Macrifoliae*	0	0	0	0	0	0	0	0	0	0	0	0	0
A. angelica	0	0	0	0	0	1	1	0	1	0	1	1	1
A. arenicola	0	1	1	0	0	1	1	0	1	1	1	1	1
A. comptonii	0	1	1	0	0	1	1	0	1	1	1	1	1
A. dabenorisana	0	2	1	0	1	1	1	0	1	1	1	1	1
A. distans	0	1	1	0	0	1	1	0	1	1	1	1	1
A. erinacea	1	1	0	1	0	0	0	2	0	0	0	1	1
A. melanacantha	1	1	0	1	0	0	0	2	0	0	0	1	1
A. meyeri	0	2	1	0	1	1	1	0	1	1	1	1	1
A. mitriformis	0	1	1	0	0	1	1	0	1	1	1	1	1
A. pearsonii	0	0	1	0	0	1	1	0	1	1	1	1	1
A. peglerae	1	0	1	1	0	0	0	1	0	0	1	1	1
A. yavellana	0	1	0	0	0	1	1	0	0	0	1	1	1

The chemical and morphological characters discussed above are summarised in Table 2, and polarised using *Aloe* ser. *Macrifoliae* as outgroup. A cladistic analysis of these data (Fig. 4) shows the *A. melanacantha* clade to be sister to *A.* ser. *Mitriformes* s.l. (i.e. including *A. yavellana, A. angelica,* and *A. pearsonii*). The tropical *A. yavellana* is suggested to be ancestral or basal to *A.* ser. *Mitriformes* s.str. While the coherence of the clade as a whole is seemingly based on only two chemical characters (12-13 in Table 2), it is important to note that these actually represent a unique chemical profile, i.e., a combination of characters which reflects a distinctive biochemical pathway.

Conclusions

Aloe ser. *Mitriformes* and related species are characterised by a unique leaf exudate profile. A rigorous morphological and chemical analysis supports the notion that *A. pearsonii* should be included in the *"Mitriformes"* group. *A. yavellana* presents an interesting example of a chemical and biogeographical link between the aloes of South Africa and those of eastern Africa. The taxonomically problematic *A. angelica* is chemically identical with the species in the *"Mitriformes"* complex, and the branched panicle bearing capitate racemes is in agreement with the chemical pattern. The two closely related xerophytic species, *A. erinacea* and *A. melanacantha,* seem to be akin to *A. peglerae.*

The affinities of *A. melanacantha, A. erinacea, A. peglerae, A. yavellana,* and *A. angelica* have hitherto been a mystery. We propose that species containing aloeresin

E and F form a natural group or clade, as shown in Fig. 4. There are several morphological similarities (as discussed above; see Table 2) to support this rearrangement of species. The new infrageneric group comprises two subgroups (sister taxa), viz., *A.* ser. *Mitriformes* (expanded to include *A. yavellana, A. angelica,* and *A. pearsonii*) and a new, unnamed group *(A. peglerae, A. erinacea,* and *A. melanacantha).* We perceive this rearrangement of anomalous species as a small but noteworthy advance towards a natural classification system for the genus *Aloe.*

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