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The chemotaxonomic value of 10-hydroxyaloin B and its derivatives in *Aloe* series *Asperifoliae* Berger

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Summary. A chemotaxonomic and cladistic study of *Aloe* series *Asperifoliae* and related species is presented. The leaf exudate of most species included in, or pertaining to series *Asperifoliae* have littoraloin, deacetyllittoraloin and 10-hydroxyaloin B. *Aloe viridiflora* is misplaced in series *Asperifoliae*, as the chemical composition of the exudate and morphological characters are remarkably different from all other members of the group, but similar to the more distantly related *A. hereroensis* (both have homonataloin as major anthrone). *Aloe littoralis* is a logical outgroup for the series *Asperifoliae* because it is chemically identical to most members of the group. A cladistic analysis, based on chemical and morphological data, is presented, together with an interpretation of distribution patterns. Our results give a new perspective on natural relationships and are consistent with the hypothesis that the *Asperifoliae* is an extremely xerophytic southern clade of tropical origin, with *A. littoralis* as the basal species.

INTRODUCTION

Reynolds (1950) included five species in his delineation of *Aloe* series *Asperifoliae* Berger; *A. asperifolia* Berger, *A. pachygaster* Dinter, *A. falcata* Baker, *A. claviflora* Burch. and *A. viridiflora* Reynolds. Since his benchmark publication, four new species of this group have been described, namely *A. argenticauda* Merxm. & Giess, previously confused with *A. pachygaster* (Giess 1974), *A. corallina* I. Verd. (Verdoorn 1979), *A. namibensis* Giess (Giess 1970) and *A. dewinteri* Giess (Giess 1973). The species are all endemic to the western parts of southern Africa and occur in Namibia, the north western Cape and the Karoo (Reynolds, 1950 and Jankowitz, 1972 and 1975). Reynolds (1950) emphasised the obvious morphological similarity between *A. asperifolia, A. pachygaster, A. falcata* and *A. claviflora. Aloe viridiflora* was included with comments suggesting that it should be viewed as a marginal species in the series *Asperifoliae.* A survey of the leaf exudate of 280 species in the genus *Aloe* revealed that 10-hydroxyaloin B is found only in *Aloe* series *Asperifoliae* and in *A. littoralis* and *A. esculenta.* We have recently isolated and characterized 10-hydroxyaloin B (Fig. 1A) and its two nilic acid esters, littoraloin (Fig. 1B) and deacetyllittoraloin (Fig. 1C) from *A. littoralis* (Dagne *et al.* in press). The purpose of this paper is to illustrate the chemotaxonomic value of 10-hydroxyaloin B in the genus *Aloe.* We have followed a cladistic approach to provide, for the first time, a rigorous hypothesis of relationships in *Aloe* series *Asperifoliae* and related species.

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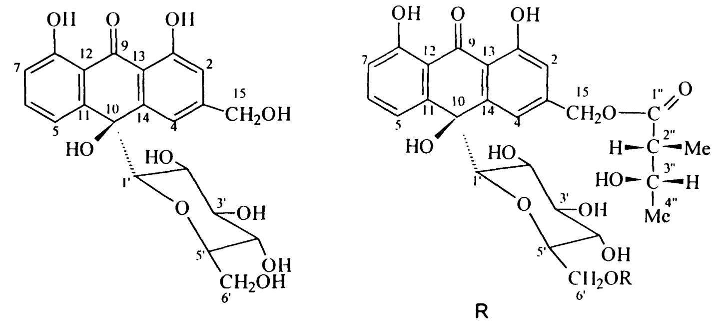
MATERIAL & METHODS

Leaf exudate chemistry

Leaf exudate was collected *in situ* or in the following botanical gardens (Table 1): Johannesburg Botanical Garden (JBG), Kirstenbosch (NBG) and the National Botanical Institute, Pretoria (NBI). The exudate was investigated on both TLC and HPLC. Samples were collected on filter paper and air-dried within a few minutes. The exudate was eluted from the filter paper with methanol and pre-cleaned through C_{18} cartridges. The 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 μm particle size, 250 mm × 4.6 mm internal diameter; flow rate 1 ml min^{-1}; 20 μl sample loop). The solvent system comprised a 30% to 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 ± 70 nm; B set at 365 ± 40 nm). TLC was carried out on silica gel 60 (Merck) plates (0.25 mm layer thickness) using ethylacetate-methanol-water (100 : 16,5 : 13,5) as mobile phase. Compounds were identified by direct comparison (Rf values, visibility/colour under UV 254 and 366 nm, retention times, UV/VIS spectra) with reference samples. The anthrones were isolated from *A. littoralis* and *A. speciosa* (see Fig. 1): 10-hydroxyaloin B (**A**) and two nilic acid esters, littoraloin (**B**) and deacetyllittoraloin (**C**) were isolated from *A. littoralis* (Rf values respectively 0.45, 0.62 and 0.52) and homonataloin A and homonataloin B were isolated from *A. speciosa* (Herbst, unpublished, Rf value 0.57) where they are known to occur as major compounds. Chromone reference samples were kindly supplied by G. Speranza.



1

R
2 Ac
3 H

FIG 1. Chemical structures of the major leaf exudate compounds in *A. littoralis*. **A** = 10-hydroxyaloin B; **B** = littoraloin; **C** = deacetyllittoraloin.

Data employed in cladistic analysis

For the purpose of this study, 13 morphological and chemical characters were used (see Table 2). *A. littoralis* was chosen as outgroup for the polarization of character states, on the basis of the shared presence of 10-hydroxyaloin as a convincing synapomorphy since it occurs in the ingroup and outgroup. *A. esculenta* L.C. Leach is here considered to be a geographical form of the polymorphic and geographically widespread *A. littoralis* and was not included in the cladistic analysis as a separate taxon. The data matrix given in Table 2 was analysed using the "HENNIG 86" software package (Farris 1988).

RESULTS & DISCUSSION

Table 1 gives a summary of chemical characters of the species pertaining to *Aloe* series *Asperifoliae,* together with *A. littoralis, A. esculenta* and *A. hereroensis* Engler. All except two of them have 10-hydroxyaloin B in the leaf exudate, often in combination with littoraloin and deacetyllittoraloin. Small amounts of aloin and an unknown derivative of aloin occur less frequently. The HPLC profiles of selected samples are shown in Fig. 2, to illustrate the two major chemical groups. The combination of major chemical compounds in *A. claviflora* (2A) and *A. littoralis* (2B) are identical,

TABLE 1. Distribution of major chemical compounds in the leaf exudate of *Aloe* series *Asperifoliae* and some other species. 1 = aloesin (Rt 6.5); 2 = unidentified chromone derivative (Rt 7.6); 3 = 10-hydroxyaloin B (Rt 15.1); 4 = unidentified compound (Rt 22.4, UV data identical to 3, 5 and 6); 5 = deacetyllittoraloin(Rt 26.1); 6 = littoraloin (Rt 31.9); 7 = aloin B (Rt 25.6) and aloin A (Rt 26.7); 8 = unidentified derivative of aloin (Rt 33.7); 9 = homonataloin B (Rt 24.6) and homonataloin B (Rt 26.7); + = present, tr = trace .

Species	Voucher	1	2	3	4	5	6	7	8	9
A. argenticauda	ex hort JBG		+	+	+	+	+	tr		
A. asperifolia	*Van Jaarsveld 2850*							+	+	
A. asperifolia	ex hort JBG			+				+	+	
A. asperifolia	NBI 432/72			+				+	+	
A. claviflora	Strydenburg		tr	+	+	+	+	+	tr	
A. claviflora	Graaff-Reinet		+	+	+	+	+	tr		
A. claviflora	Beaufort West		+	+	+	+	+	tr		
A. claviflora	Namibia		+	+	+	+	+	tr		
A. corallina	NBI 20079	+						+	+	
A. dewinteri	ex hort NBG		+	+				+	+	
A. dewinteri	Warmbad		+	+				+	+	
A. falcata	Van Rhynsdorp			+	+	+	+	tr		
A. namibiensis	NBI 28193		+	+				+	+	
A. pachygaster	ex hort JBG		+	+	+		+	tr		
A. pachygaster	NBG 1120/70	+	+	+		+	+	tr		
A. viridiflora	NBI 28700	+								+
A. viridiflora	ex hort JBG	+								+
A. hereroensis	NBI 28966	+	+							+
A. hereroensis	ex hort JBG	+	+							+
A. littoralis	Windhoek	tr		+	tr	+	+			
A. littoralis	Vivo	+	+	+	+	+	+			
A. littoralis	Etosha	+	+	+	+	+	+			
A. esculenta	NBI 27823	tr		+	tr	+	+	tr		

and closely similar patterns are found in other species (Table 1). Both species produce an unknown chromone, together with compounds **A** to **C** (Fig. 1). Namibia's rare endemic aloe, *A. viridiflora*, was included in the series *Asperifoliae* by Reynolds (1950) but the morphology and the leaf exudate chemistry now unambiguously show it to be misplaced in the section. *Aloe viridiflora* is closely allied to *A. hereroensis*, with which it shares several characters. Both species produce homonataloin as major anthrone constituent of the leaf exudate (Fig. 2C & 2D). This chemical similarity is amplified on the morphological level as both species produce a branched panicle with capitate racemes. There is also agreement in the leaf surface characters and flowering time. Various authors have mentioned the morphological similarity between the two species (Bruyns 1988, Jankowitz 1975 and Van Jaarsveld 1989), and the chemical evidence presented here establishes beyond doubt that *A. viridiflora* should be excluded from the series *Asperifoliae*. The remaining species, together with *A. littoralis* and *A. esculenta*, are likely to be monophyletic because they share a remarkable chemical similarity.

TABLE 2. Characters and polarization of morphological and chemical character states in *Aloe* section *Asperifoliae*.

Species	Characters and character states												
	1	2	3	4	5	6	7	8	9	10	11	12	13
A. argenticauda	1	0	1	1	1	1	0	2	1	1	0	0	0
A. asperifolia	1	0	1	1	1	1	1	1	0	0	1	1	1
A. claviflora	1	0	1	1	1	1	1	2	0	0	0	0	0
A. corallina	1	1	0	0	1	1	0	1	0	0	2	1	1
A. dewinteri	1	1	0	0	1	1	0	1	1	0	1	1	1
A. falcata	1	0	1	1	1	1	0	0	0	0	0	0	0
A. namibensis	1	0	0	1	1	1	0	1	1	0	1	1	1
A. pachygaster	1	0	1	1	1	1	1	2	1	1	0	0	0
A. littoralis	0	0	0	0	0	0	0	0	0	0	0	0	0

1	Caulescence	caulescent = 0; acaulescent or short procumbent stem = 1
2	Stem	erect or procumbent = 0, hanging = 1
3	Habit	solitary = 0; clustered = 1
4	Leaf orientation	decurved = 0; incurved = 1
5	Leaf maculation	spotted = 0; immaculate = 1
6	Leaf texture	smooth = 0; asperous = 1
7	Inflorescence orientation	erect = 0; oblique = 1
8	Inflorescence structure	much-branched panicle = 0; invariably 2- or 3-branched = 1; usually single racemes = 2
9	Bract length	bracts much shorter than the flowers = 0, bracts as long as the flowers = 1
10	Perianth shape	cylindrical = 0; ventricose = 1
11	10-Hydroxyaloin B	present in high quantities = 0; low quantities = 1; absent = 2
12	Littoraloin and deacetyllittoraloin	present = 0; absent = 1
13	Derivative of aloin (compound 8 in Table 1)	present = 0; absent = 1

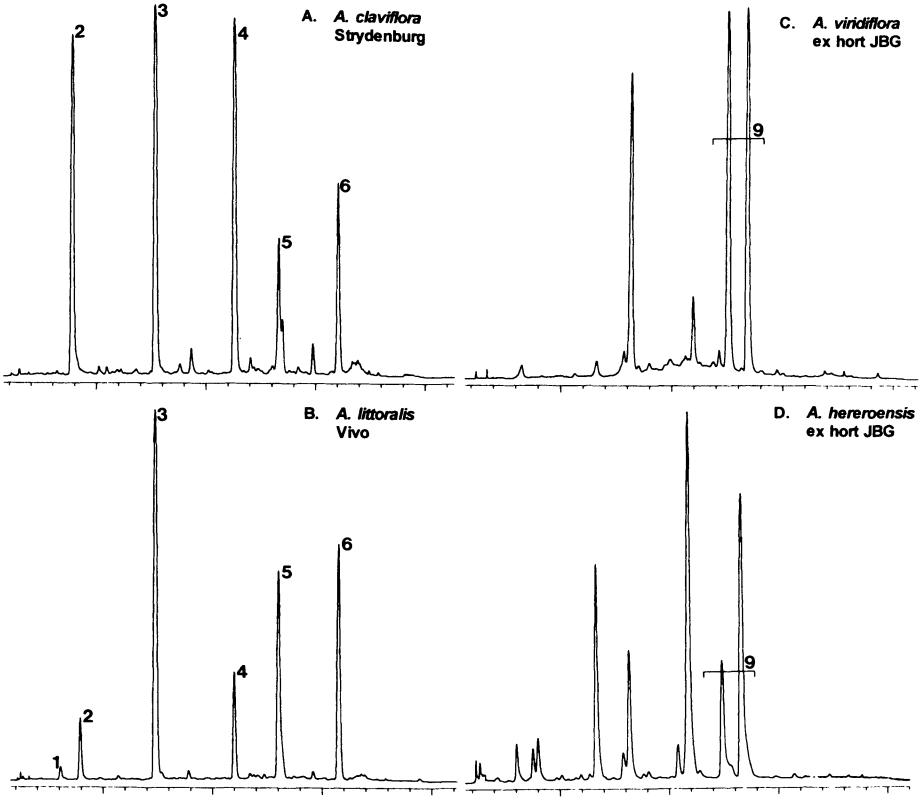


FIG. 2. A comparison of characteristic chemical compositions of leaf exudates from *Aloe* series *Asperifoliae* and some other species. Examples of HPLC profiles from *A. claviflora* (A), *A. littoralis* (B), *A. viridiflora* (C) and *A. hereroensis* (D) are shown. The major compounds are numbered as in Table 1.

In order to find out how the interesting chemical pattern should be interpreted, we studied various morphological characters and polarized them according to the outgroup method. Our interpretation of the evolution of character states is summarised in Table 2. A brief discussion of salient morphological features is given below.

Habit

All the species in series *Asperifoliae* are acaulescent or have a short procumbent stem. *Aloe argenticauda, A. asperifolia, A. pachygaster, A. claviflora* and *A. falcata* always form dense clusters, while the three species from northern Namibia, (*A. namibensis, A. dewinteri* and *A. corallina*) are all solitary. *Aloe littoralis* is generally caulescent and solitary, but a form with short stems and a clustered habit has been described as a separate species, *A. esculenta* (Leach 1971). In contrast to all the other species, *A. dewinteri* and *A. corallina* are cliff-hanging species and have pendulous stems.

Leaf Characters

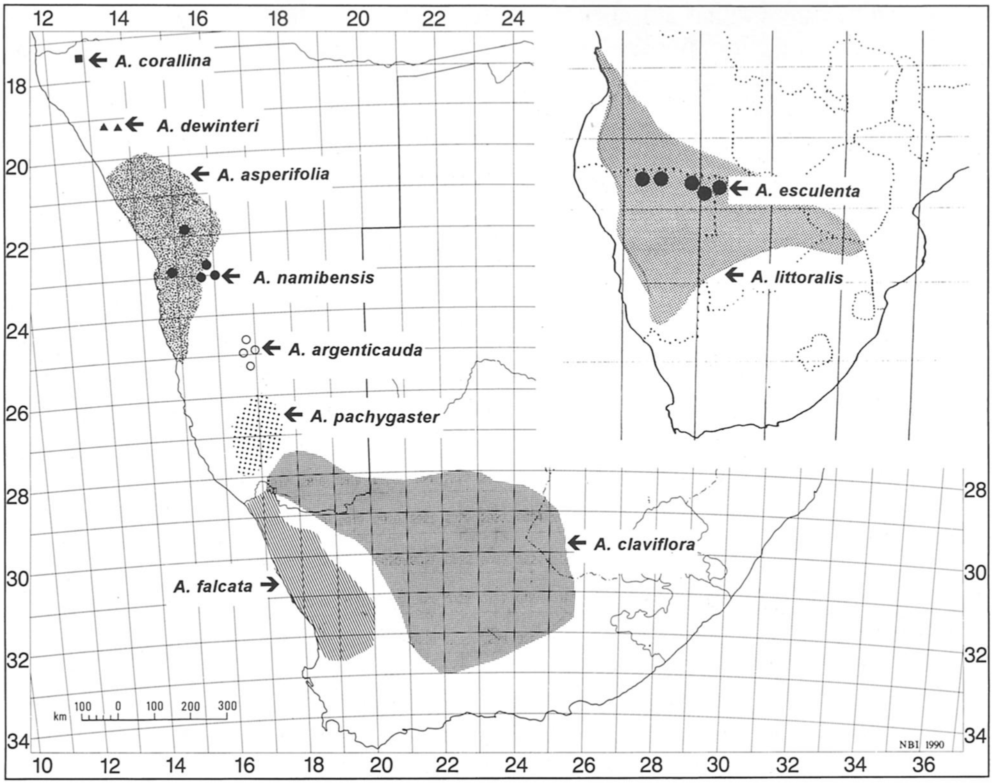
As the name indicates, all the species pertaining to *Aloe* series *Asperifoliae* have a more or less granular leaf texture and the leaves have a distinct glaucous bloom. In *A. littoralis*, the leaves may be somewhat glaucous but the surface is not asperous. *Aloe argenticauda, A. pachygaster, A. asperifolia, A. claviflora, A. falcata* and *A. namibensis* are characterized by incurved leaves, while *A. littoralis, A. corallina* and *A. dewinteri* produce decurved leaves. Juvenile plants of *A. littoralis* also have incurved leaves and although generally spreading and recurved in the mature plants, some individuals occasionally have the leaves incurved (Reynolds, 1966).

Inflorescence and Flower Characters

Inflorescence characters vary considerably in the series *Asperifoliae.* A simple (unbranched) inflorescence structure is characteristic of *A. argenticauda* and *A. pachygaster.* In the southern part of its distribution area, *A. claviflora* usually also produces simple racemes, while a branched panicle is more common in the northern parts of its distribution (Reynolds 1938). *Aloe falcata* and *A. littoralis* both produce a much branched panicle, laterally spreading in the former and more compressed in the latter. *Aloe asperifolia, A. namibensis, A. corallina,* and *A. dewinteri* have a 2- to 3-branched inflorescence (rarely simple in the latter). In *A. corallina* though, the inflorescence is branched in the upper half, while in *A. dewinteri* and *A. namibens* it is branched near the base (Verdoorn 1977a). The homology of flower shape deserves further study. A clavate or subclavate perianth is characteristic of *A. claviflora, A. corallina, A. esculenta* and the unrelated *A. viridiflora,* species that otherwise do not share any obvious synapomorphies. The ventricose shape of the perianth in *A. argenticauda* and *A. pachygaster* however, is here interpreted as a shared derived character. Flower colour varies considerably. All the species have flowers with various shades of pink or red, while *A. viridiflora* (green) and *A. hereroensis* (orange or greenish) are notable exceptions. The flower colour is seemingly correlated with the equally exceptional capitate inflorescence structure of these two species.

Leaf exudate chemistry

If *A. viridiflora* is excluded from the series and if *A. littoralis* is included, then 10-hydroxyaloin B becomes a chemical marker for the *Asperifoliae. Aloe littoralis* is currently placed in group 18 of Reynolds (1966) but the leaf exudate chemistry clearly shows a relationship with the *Asperifoliae.* The chemical profiles of *A. argenticauda, A. pachygaster, A. claviflora, A. falcata* and *A. littoralis* are practically identical. All these species contain high levels of 10-hydroxyaloin B, together with littoraloin and deacetyllittoraloin (Table 1). *Aloe dewinteri* and *A. namibensis* have lower levels of 10-hydroxyaloin B, and produce a unique derivative of aloin (compound 8 in Table 1) which is also present in *A. corallina,* the only species lacking 10-hydroxyaloin B. It is interesting to note from Table 1 that the unknown derivative of aloin also occurs in *A. asperifolia,* and that this species also lacks littoraloin and deacetyllittoraloin, characters which emphasise its relationship with *A. namibensis* and *A. dewinteri* (Verdoorn 1977b and 1977c).



Map 1. Approximate geographical distributions of the species pertaining to *Aloe* series *Asperifoliae*.

Distribution

The geographical distributions depicted in Map 1 were compiled from literature (Reynolds 1950, Jankowitz 1972 and 1975) and from specimens housed in the National Herbarium, Pretoria (PRE). The species occur mainly in the Namib Desert, and in the central and north western Cape. This area is prone to warm dry conditions and periodic droughts, receiving an average rainfall of only 125 mm per annum. Jankowitz (1977) pointed out the correlation between the summer rainfall area of Namibia and the distribution area of *Aloe littoralis* and *A. claviflora,* while *A. pachygaster* is always associated with dolomite and limestone. *Aloe littoralis* has a wide distribution range extending from Angola in the north, southwards to southern Namibia and eastward to the northern Transvaal. *Aloe corallina, A. dewinteri* and *A. namibensis* are geographically localized and the sympatric distribution of the latter species with *A. asperifolia* supports their chemical and morphological similarity. We propose that the present-day distributions reflect allopatric evolutionary changes in response to the extremely arid conditions of the western part of southern Africa.

Cladistic analysis

Fig. 3 shows the single, fully resolved cladogram of hypothetical relationships in the *Asperifoliae*, based on the morphological and chemical characters listed in Tables 1 and 2. *Aloe littoralis* is shown to be basal to the group, with *A. falcata* basal to the *Asperifoliae sensu stricto. Aloe littoralis* and *A. falcata* are similar in inflorescence structure and flower morphology, and the leaf exudate chemistry of the two species are identical. *Aloe pachygaster* and *A. argenticauda* form a well-defined clade based on inflorescence structure and flower morphology. This is not unexpected, as A. *argenticauda* has been confused with *A. pachygaster* (Reynolds 1950) and later described by Giess (1974) as a distinct species. Both species produce an unbranched inflorescence, with ventricose flowers and long bracts. *Aloe claviflora* and *A. asperifolia* are also very similar, but the leaf exudate chemistry revealed notable differences. As expected, *A. namibensis, A. corallina* and *A. dewinteri* form a well supported clade. The latter two species are considered to be more closely related as they are both cliff-hanging species with pendulous stems and both have the leaves decurved in the upper half. Based on all the data presented, *A. asperifolia* could be viewed as somewhat intermediate between

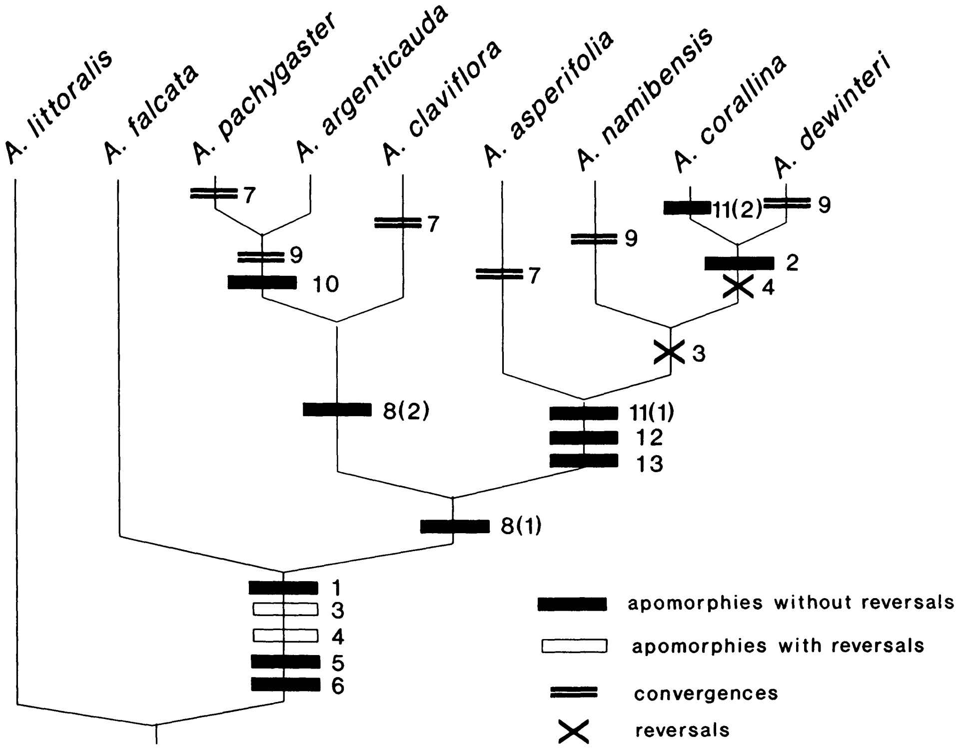


FIG. 3. Cladogram of phylogenetic relationships in *Aloe* series *Asperifoliae*, based on the data in Table 2. A single, fully resolved cladogram with 21 steps and a consistency index of 71 was obtained, using the "ie" command of HENNIG 86.

the *Asperifoliae sensu stricto* (as described by Reynolds 1950) and the three species more recently described from the north. It is particularly similar, both chemically and morphologically, to the sympatric *A. namibensis* (see Map 1). The cladogram supports the notion that the *Asperifoliae* developed from a geographically widespread basal species (*A. littoralis*), with subsequent adaptive radiation from south to north. Note the remarkable correlation between the sequence of branching in the cladogram, and the geographical replacement of species from south to north.

Conclusions

A rigorous comparison of morphological and chemical characters has resulted in a new and improved understanding of natural affinities within the series *Asperifoliae* and related species. The leaf exudate chemistry is relatively conservative and shows that the circumscription of the series should be modified to exclude *A. viridiflora* but to include *A. littoralis.*

A. viridiflora was clearly misplaced and should be grouped with *A. hereroensis.* These two species are morphologically and chemically very similar (both produce homonataloin) and lack the defining synapomorphies of the *Asperifoliae.*

The inclusion of *A. littoralis* would result in a chemically uniform group and would be consistent with our hypothesis that the *Asperifoliae* represents a southern, drought-adapted clade of tropical origin, with *A. littoralis* as the basal species. Adaptive radiation and allopatric speciation seems to have occurred in a northerly direction along the dry western parts of southern Africa.

Acknowledgements

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