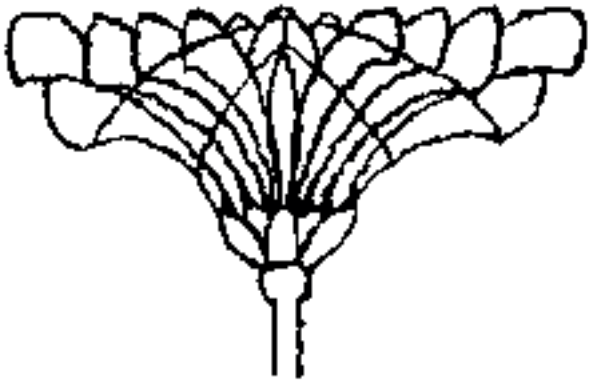

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Infrageneric classification of *Haworthia* (Aloaceae): perspectives from nectar sugar analysis

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Abstract. *– Species and genus concepts and infrafamilial delimitations in the Aloaceae (subfamily Alooideae of the Asphodelaceae) have often been controversial. Arguments are mostly based on evidence obtained from vegetative and floral morphology, but data from other fields have also been used for speculating on taxonomic affinities within the Aloaceae. For the present study, nectar sugar composition (glucose, fructose, sucrose) was determined for representatives of* Astroloba, Chortolirion, *the three subgenera of* Haworthia *and for interspecific haworthioid hybrids and miscellaneous taxa. Two nectar types were distinguished in* Haworthia *and the two related genera: a Haworthia type (subg.* Haworthia *only, usually less than 50% sucrose) and a Hexangulares type (subg.* Hexangulares, *subg.* Robustipedunculares, Chortolirion *and* Astroloba, *usually more than 60% sucrose). Results support Uitewaal's subdivision of* Haworthia *in two main groups, but reveal little substantiation for infra-subgeneric groupings.*

Key words*: Aloaceae,* Astroloba, Chortolirion, Haworthia, *nectar sugars, chemotaxonomy.*

Résumé. *–* **Classification infragénérique chez Haworthia (Aloaceae): perspectives à partir de l'analyse du sucre nectarifère.** *Les concepts spécifiques et génériques ainsi que les délimitations infrafamiliales chez les Aloaceae (sous-famille Alooideae des Asphodelaceae) ont souvent fait l'objet de controverses. L'argumentation se base principalement sur des données de morphologie végétative et florale mais des travaux dans d'autres domaines ont également conduit à spéculer sur des affinités taxonomiques au sein des Aloaceae. Dans la présente étude, la composition du sucre nectarifère (glucose, fructose, sucrose) a été déterminée chez des représentants de* Astroloba, Chortolirion, *des trois sous-genres de* Haworthia, *chez des hybrides interspécifiques haworthioïdes ainsi que chez divers taxons. Deux types de nectar ont été distingués chez* Haworthia *et chez les deux genres affins: un type Haworthia (subg.* Haworthia *seulement, généralement moins de 50% de sucrose) et un type Hexangulares (subg.* Hexangulares, *subg.* Robustipedunculares, Chortolirion *et* Astroloba, *généralement plus de 60% de sucrose). Les résultats corroborent la subdivision de Uitewaal du genre* Haworthia *en deux groupes principaux mais révèlent le peu de bien-fondé de groupements infra-subgénériques.* Traduit par le journal.

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Introduction

The Aloaceae, often regarded as subfamily Alooideae of the Asphodelaceae (Dahlgren & al. 1985; Smith & Van Wyk 1991, 1998), is a medium-sized family comprising five to seven genera and about 510 species (Smith & Van Wyk 1998) of succulent-leaved, petaloid monocotyledons. Most researchers recognise the family as a taxonomically difficult unit and species and genus concepts have often been controversial. The basic integrity of the three principal genera, *Aloe* L., *Gasteria* Duval and *Haworthia* Duval is usually accepted, but infrageneric classification in *Haworthia* and the legitimacy and intergeneric relationships of the remaining genera (*Astroloba* Uitewaal, *Chortolirion* A.Berger, *Lomatophyllum* Willd. and *Poellnitzia* Uitewaal) have frequently been debated (Uitewaal 1947; Rowley 1967, 1996; Parr 1971; Bayer 1972; Obermeyer 1973; Manning & Smith 2000). Arguments are mostly based on evidence obtained from vegetative and floral morphology, but data from other fields, e.g. *in vitro* callus growth (Hayashi 1987), cytogenetics (Rollins 1953; Brandham 1971; Riley & Majumbar 1979) and phytochemistry (Reynolds 1985; Viljoen & Van Wyk 1996), have also been used for speculating on taxonomic affinities within the Aloaceae.

The haworthioid genera of the Aloaceae

Three of the genera of the Aloaceae are included in the so-called haworthioid group. These are, chronologically and in descending order in terms of number of species, *Haworthia*, *Astroloba* and *Chortolirion*. On morphological grounds these genera are united by their small stature and dull-whitish, more or less or distinctly two-lipped or at least obsolescently zygomorphic flowers (Bayer & al. 1997). Geographically *Haworthia* is near-endemic to South Africa, with only one species, *H. venosa* (Lam.) Haw. entering Namibia in the west, and another, *H. limifolia* Marloth, extending into Mozambique in the east. *Astroloba* is restricted to the southern parts of South Africa while *Chortolirion* has a very wide distribution in the southern African grasslands, extending from Angola in the west, through Namibia, Botswana and Zimbabwe to the northern, central and eastern provinces of South Africa.

Data from nectar sugar analysis of Aloaceae

In a cladistic study of *Aloe* and related genera, Smith & Van Wyk (1991) used nectar sugars (glucose, fructose and sucrose) as one of an array of phylogenetically informative characters to investigate generic relationships in the Aloaceae. The data on which the character polarities were based were presented in a later paper (Van Wyk & al. 1993). During the latter study, high performance liquid chromatography (HPLC) analyses of nectar samples had shown distinct generic and suprageneric discontinuities in the family and three distinct nectar types could be distinguished. These comprised (a) an alooid type (*Aloe*, *Kniphofia*, *Lomatophyllum* and *Poellnitzia*) with less than 5% sucrose and more or less equal proportions of fructose and glucose; (b) a gasterioid type (*Gasteria* only) with sucrose dominant and about equal proportions of fructose and glucose; (c) a haworthioid type (*Astroloba*, *Chortolirion* and *Haworthia*) with sucrose dominant, but with much more glucose than fructose.

According to Smith & Van Wyk (1991) and Van Wyk & al. (1993), nectar sugar composition is remarkably consistent within each of genera of the Aloaceae. However, subsequent scrutiny of results obtained during the 1993 study showed that the nectar of a few haworthias did not conform to the haworthioid type: the dominant sugar in the nectar of *Haworthia arachnoidea* (L.) Duval, *H. comptoniana* G.G.Sm. and *H. herbacea* (Mill.) Stearn was not sucrose, but glucose; the sucrose content for these species was in fact unusually low at 36%, 42% and 35 %, respectively (tab.1 in Van Wyk & al. 1993). Since all three aberrant species belong to *H.* subg. *Haworthia*, it seemed possible that nectar sugar analyses might reveal infrageneric groups in this genus and help to elucidate intergeneric relationships in the family. To test this hypothesis, a rigorous comparison of nectar sugars was made for species

of all three subgenera of *Haworthia* as well as for representatives of *Chortolirion* and *Astroloba*. The results of our investigation are reported in the present communication.

Material and methods

Nectar was sampled from newly opened flowers on cultivated plants in private collections and various botanical gardens (see tab.1). Collecting voucher specimens was therefore not only unpractical, but also unnecessary (most of the co-authors of this paper are taxonomic experts on the various genera of the family). A total of 65 samples, representing 49 taxa as listed in tab. 1, were analysed. Nectar samples were applied as spots to Whatman no.1 filter paper with a micropipette, air-dried and stored at –18 °C. For analysis, the sugars were recovered from the filter paper by repeated rinsing with distilled water, followed by centrifugation (usually 3× with 25 to 50 ml, depending on the size of the spot). Analyses were done with a refractive index detector coupled to a isocratic HPLC system operating at 2.5 ml per minute, with a "Waters Sugarpack" column and acetonitrile-water (87:13) as eluent. The percentages of the sugars were calculated on a weight basis from peak area, using 2, 4, 6, and 8 mg per ml of fructose, glucose and sucrose as external standards.

Table 1. Nectar sugar composition in *Haworthia* (*sensu* Bayer 1982), *Astroloba* (*sensu* Groen 1987) and *Chortolirion* (*sensu* Smith 1991).

GFS, Personal collection G.F.Smith; JDV, Personal collection J.D.Venter;
KNBG, Kirstenbosch National Botanical Garden;
PNBG, Pretoria National Botanical Garden;
POTCH, Potchefstroom University for Christian Higher Education Botanical Garden;
WNBG, Karoo National Botanical Garden, Worcester.
fru, fructose; glu, glucose; su, sucrose.

No.	Genera and species	Locality	Nectar composition (%) fru	(%) glu	(%) su
	Haworthia* I. Subgenus *Haworthia				
1	*H. angustifolia* Haw.	WNBG	4	48	48
2	— fa. *baylissii* (C.L.Scott) M.B.Bayer	GFS	10	25	65
3	*H. arachnoidea* (L.) Duval sample 1	NBG	13	51	36
4	— sample 2	JDV	5	50	45
5	*H. blackburniae* W.F.Barker	WNBG	24	43	33
6	*H. bolusii* Baker	PNBG	6	39	55
7	*H. comptoniana* G.G.Sm.	POTCH	4	54	42
8	*H. cooperi* Baker	KNBG	5	39	56
9	*H. cymbiformis* (Haw.) Duval var. *cymbiformis*	GFS	14	55	31
10	*H. decipiens* Poelln. sample 1	WNBG	7	51	42
11	— sample 2	WNBG	9	61	30
12	*H. divergens* M.B.Bayer	WNBG	16	52	32
13	*H. emelyae* Poelln. sample 1	PNBG	8	54	38
14	— sample 2	GFS	12	59	29
15	*H. habdomadis* Poelln. var. *morrisiae* (Poelln.) M.B.Bayer	JDV	2	48	50
16	*H. herbacea* (Mill.) Stearn	POTCH	19	46	35
17	*H. maculata* (Poelln.) M.B.Bayer sample 1	WNBG	17	49	34
18	— sample 2	WNBG	16	58	26
19	*H. magnifica* Poelln. var. *maraisii* (Poelln.) M.B.Bayer	JDV	1	50	49
20	*H. marumiana* Uitewaal	WNBG	16	54	30
21	*H. maughanii* Poelln.	JDV	7	58	35
22	*H. nortierii* G.G.Sm. var. *nortierii*	WNBG	20	60	20

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23	*H. pubescens* M.B.Bayer	WNBG	11	46	43
24	*H. retusa* (L.) Duval	JDV	4	44	52
25	— var. *dekenahii* (G.G.Sm.) Bayer	JDV	10	39	51
26	*H. rycroftiana* M.B.Bayer	JDV	6	57	37
27	*H. semiviva* (Poelln.) M.B.Bayer	WNBG	16	52	32
28	*H. truncata* Schönland	JDV	7	47	46
29	*H. unicolor* Poelln.	WNBG	5	45	50
30	*H. xiphiophylla* Baker	JDV	25	48	27
31	*H. glauca* Baker	KNBG	1	19	80
II. Subgenus *Hexangulares*					
32	*H. koelmaniorum* Oberm. & D.S.Hardy — sample 1	PNBG	5	23	72
33	— sample 2	GFS	8	28	64
34	*H. limifolia* Marloth var. *limifolia* sample 1	KNBG	4	41	55
35	— sample 2	PNBG	3	24	73
36	— sample 3	GFS	11	29	60
37	— var. *gigantea* Bayer	WNBG	4	34	62
38	*H. longiana* Poelln. sample 1	KNBG	3	20	77
39	— sample 2	WNBG	7	19	74
40	*H. nigra* (Haw.) Baker	WNBG	–	25	75
41	*H. venosa* (Lam.) Haw. subsp. *granulata* (Marloth) M.B.Bayer sample 1	PNBG	5	25	70
42	— sample 2	PNBG	4	24	72
43	— sample 3	WNBG	8	30	62
44	subsp. *tessellata* (Salm-Dyck) Baker sample 1	KNBG	1	29	70
45	— sample 2	PNBG	2	24	74
46	*H. viscosa* (L.) Haw. sample 1	PNBG	2	32	66
47	— sample 2	GFS	4	33	63
48	*H. woolleyi* Poelln.	WNBG	1	22	77
III. Subgenus *Robustipedunculares*					
49	H. minima (Aiton) Haw.	PNBG	7	24	69
50	H. pumila (L.) Duval sample 1	WNBG	1	14	85
51	— sample 2	PNBG	3	17	80
52	— sample 3	PNBG	7	19	74
Interspecific hybrids and miscellaneous taxa					
53	*H. woolleyi* × *H. sordida*	WNBG	4	29	67
54	*H. viscosa* × *H. longiana*	WNBG	8	27	65
55	*H.* subg. *Haworthia* sp. nov.	WNBG	6	55	39
56	*H. tortuosa* Haw.	GFS	11	31	58
57	*H. mcmurtryi* C.L.Scott	PNBG	7	26	67
Astroloba					
58	*A. bullulata* (Jacq.) Uitewaal	GFS	20	46	34
59	*A. spiralis* (L.) Uitewaal subsp. *spiralis*	KNBG	2	13	85
60	— subsp. *foliolosa* (Haw.) Groen sample 1	POTCH	4	16	80
61	— — sample 2	PNBG	7	29	64
62	— — sample 3	KNBG	9	32	64
Chortolirion					
63	C. angolense (Baker) A.Berger sample 1	POTCH	8	21	71
64	— sample 2	POTCH	8	19	73
65	— sample 3	POTCH	7	20	73

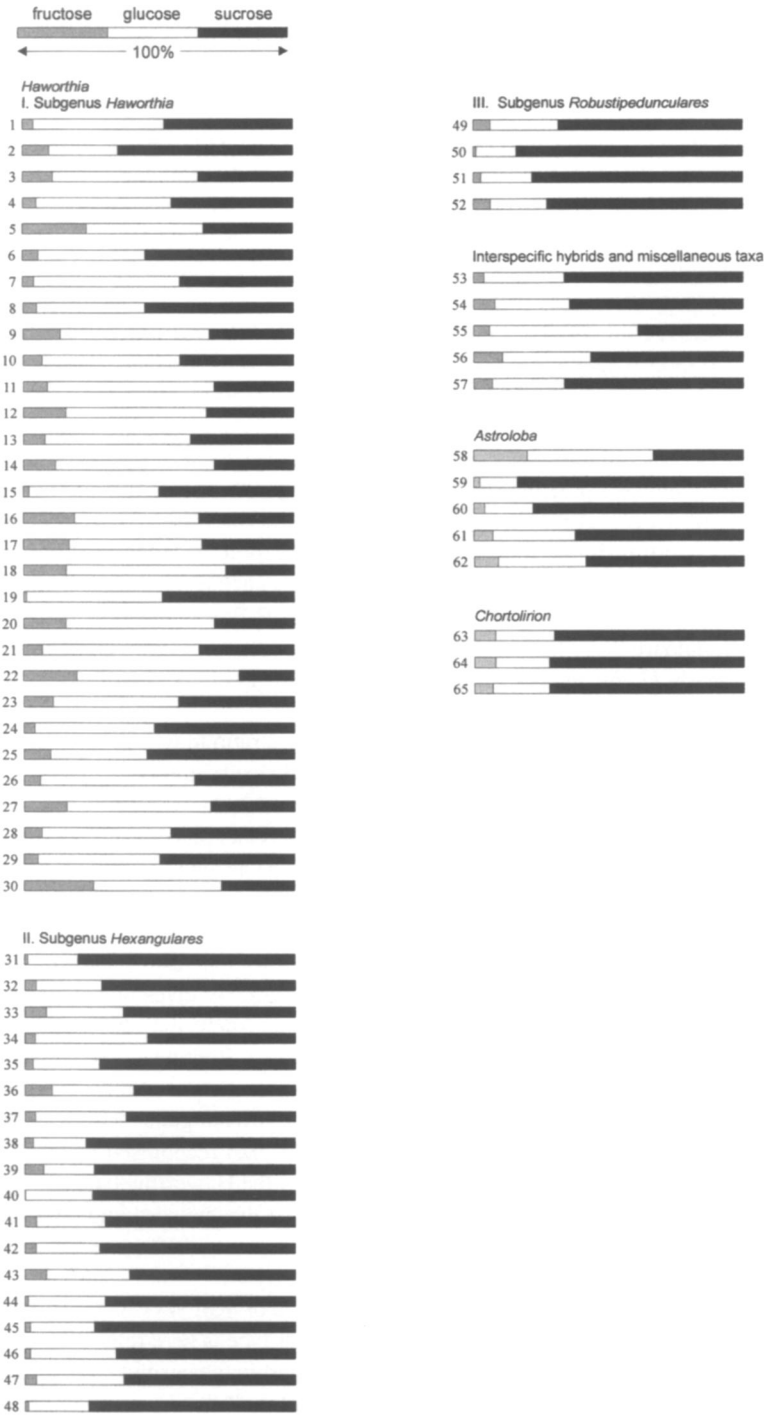

Figure 1.
Composition of nectar sugars in 65 samples taken from 49 taxa of Haworthia, Astroloba *and* Chortolirion.
Samples and species are numbered as in tab. 1.

Results and discussion

In the list of taxa studied (tab. 1), the composition of nectar sugars present in the samples are given. The results of the analysis are also depicted graphically (fig. 1) to ease comparison of species. The outcome of the study clearly shows that the composition of nectar sugars varies considerably among species of *Haworthia* and *Astroloba* and, especially, among those representing *H.* subg. *Haworthia*. Moreover, samples taken from plants belonging to the same species but from different localities, i.e. botanical gardens or private collections, vary in their ratio of sucrose to hexose, i.e. glucose plus fructose (compare Nos. 3 & 4; 10 & 11; 13 & 14; 34, 35 & 36). In samples of *Chortolirion* taken from plants of the same locality, the variation is negligible (compare Nos. 63, 64 & 65). Hence, results on *Haworthia* and *Astroloba* do not support the findings of Van Wyk & al. (1993) that "...the sugar composition of the nectar is remarkably invariable within each of the genera (of Aloaceae)".

The various nectar samples were selected so as to cover most of the sections and subsections or series within the subgenera of *Haworthia* as recognised by Pilbeam (1983) and by Breuer (1998), who both based their infrageneric subdivisions on leaf and rosette characters. Where our study provided data on the nectar of more than one representative of a subsection or series, it shows that some species, grouped together on account of morphological similarities such as *H. bolusi* Baker, *H. cooperi* Baker and *H. habdomadis* Poelln. var. *morrisiae* (Poelln.) M.B.Bayer, are remarkably uniform in sugar composition (compare Nos. 6, 8 & 15). Nevertheless, other species belonging to the same series (Limpidae), namely *H. decipiens* Poelln. and *H. semiviva* (Poelln.) M.B.Bayer (Nos.10 & 27), differ notably.

According to Van Wyk & al. (1993) the fructose/glucose ratio in intergeneric hybrids of Aloaceae is inherited from the pod parent. Our results on interspecific hybrids and *H. tortuosa*, a possible hybrid of *H. viscosa* (Pilbeam 1983), are inconclusive (compare Nos. 53, 54 & 56 with Nos. 46–48). The nectar composition of a new, undescribed species (No. 55) supports its placement under *H.* subg. *Haworthia*. Nectar of *H. mcmurthryi* (No. 57), regarded by Scott (1985) as allied to *H. koelmaniorum* (No. 32), is very similar to that of the latter species.

Despite the variation in nectar composition within the subgenera of *Haworthia* and in *Astroloba*, for example the deviation shown by *Astroloba bullulata* (No. 58), our results show a general trend. On the basis of the ratio of sucrose to hexose (fructose plus glucose) the 49 investigated taxa can be divided into two groups, namely those with sucrose-rich nectar (usually more than 60% sucrose) and those with nectar that is low in sucrose (usually less than 50% sucrose). Both nectar types have a relative higher proportion of glucose than fructose. The results of the present investigation suggest that sucrose-low nectar is generally characteristic of representatives of the largest subgenus within *Haworthia*, namely *H.* subg. *Haworthia*. This subgenus includes 93 of the 132 taxa recognised by Breuer (1998). Consequently, the characteristic nectar type is here referred to as the Haworthia type. On account of its lower proportion of sucrose, the Haworthia type is quite unlike the 'haworthioid' type of Van Wyk & al. (1993).

Sucrose-rich nectar, comparable to the 'haworthioid' type of Van Wyk & al. (1993) is produced by a second, smaller group of taxa within *Haworthia*. This nectar type is here referred to as the Hexangurales type, since it characterises the nectar of representatives within the second largest subgenus, namely *H.* subg. *Hexangulares*. The investigated species of *H.* subg. *Robustipedunculares*, *Chortolirion* and *Astroloba* also belong to this group. The correlation of nectar in *Astroloba* and members of *H.* subg. *Robustipedunculares* with the sucrose-rich Hexangulares type is surprising. Because of floral and other morphological features it was expected that the sugar composition of species belonging to these two units would be nearer to the sucrose-low *Aloe* type nectar of Van Wyk & al. (1993).

Conclusions

- Two nectar types occur in *Haworthia*, namely a sucrose-low Haworthia type (generally less than 50% sucrose and more glucose than fructose) and a Hexangulares type (generally more than 60% sucrose and more glucose than fructose).
- Haworthia type nectar characterises taxa within *H.* subg. *Haworthia*, while Hexangulares type nectar occurs in *H.* subg. *Hexangulares*, *H.* subg. *Robustipedunculares*, *Astroloba* and *Chortolirion*.
- Evidence from nectar sugar analyses knits *Haworthia* to *Astroloba* and *Chortolirion*, and also suggests that *Chortolirion* and *Astroloba* may not be closely linked to species within *H.* subg. *Haworthia*.
- Our findings support Uitewaal's (1947) subdivision of *Haworthia* in two main groups, but reveal little substantiation for infra-subgeneric groupings.

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