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ORIGINAL ARTICLE

Genome sizes of *Eucomis* L'Hér. (*Hyacinthaceae*) and a description of the new species *Eucomis grimshawii* G.D.Duncan & Zonneveld

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Abstract Nuclear genome size, as measured by flow cytometry with propidium iodide, was used to investigate the relationships within the genus *Eucomis* L'Hér. (*Hyacinthaceae*). Most species of *Eucomis* have the same basic chromosome number, $x = 15$. However, the somatic DNA 2C-value (2C) is shown to range from 21 to 31 pg for the diploids. The largest genome contains roughly 10^{10} more base pairs than the smallest. Genome sizes are evaluated here in combination with available morphological and geographical data. Therefore, the taxonomy proposed here is not based on genome size alone. The genus *Eucomis*, as here determined, has 12 species. These can be divided into two groups: mainly dwarf diploid species and large-sized, tetraploid species. A small diploid plant, *Eucomis* (*autumnalis* subsp.) *amaryllidifolia*, is restored to species status, as a diploid subspecies seems incongruent with an allotetraploid *Eucomis autumnalis*. Moreover, as a diploid it is separated reproductively from the allotetraploid *E. autumnalis*. A new diploid species that has the lowest C value, *E. grimshawii*, is described here. On the basis of DNA content and other morphological characters, possible parents are suggested for all tetraploid species. Nuclear DNA content as measured by using flow cytometry may conveniently be used to produce systematic data. It is applicable even in dormant bulbs or sterile plants for the monitoring of the trade in bulbous species.

Keywords *Eucomis grimshawii* sp. nov · *Eucomis* species · DNA 2C-value · Taxonomy

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Introduction

Eucomis, a small genus of 12 species in the family Hyacinthaceae, is endemic to the southern African countries, South Africa, Botswana, Lesotho, and Swaziland, as well as Zimbabwe and Malawi in southern Tropical Africa (Duncan 2007). The first described species (now known as *E. regia*) was illustrated in the early eighteenth century in *Hortus Ethamensis* (Dillenius 1732). Five of the later species were described by Baker (1878, 1886, 1887, 1895), and the most recently described one was *E. schijffii* (Reyneke 1976). *Eucomis* is characterized by the presence of a coma or tuft of leaf-like bracts that develop above the inflorescence. The most recent classification of *Eucomis* (Reyneke 1972) recognized ten species based on morphological characters, namely *E. autumnalis* (Mill.) Chitt., *E. bicolor* Baker., *E. comosa* (Houtt.) Wehrh., *E. humilis* Baker, *E. montana* Compton, *E. pallidiflora* Baker, *E. regia* (L.) L'Hér., *E. schijffii* Reyneke, *E. vandermerwei* Verd. and *E. zambesiaca* Baker. In the World Checklist for *Eucomis* (Govaerts 2006), 33 names were listed, and Reyneke's 10 species and 3 subspecies were accepted there. The present study follows Reyneke's ten recognised species and in addition restores *E. amaryllidifolia* Baker to species level and describes the new species *E. grimshawii* G.D.Duncan & Zonn. The species are here divided into two groups: seven mainly small-sized diploids ($2n = 2x = 30$)

and five large-sized tetraploids ($2n = 4x = 60$) (Reyneke and Liebenberg 1980).

Eucomis regia (L.) L'Hér. was the first *Eucomis* to be cultivated in Britain close to 3 centuries ago, but the species that have featured most prominently in hybridization and selections have been the fully hardy *E. comosa* (Houtt.) Wehrh. (syn: *E. punctata* L'Hér.) and *E. autumnalis* (Mill.) Chitt. (syn: *E. undulata* Aiton), as well as the less hardy, tall-growing *E. pallidiflora* Baker.

All the species are summer-flowering with the single exception of *E. regia*, which hails from the winter rainfall zone (Duncan 2007).

In South Africa the bulbs of several species, particularly *E. autumnalis*, are used in traditional medicine to treat a number of diseases. Recent investigations have validated this use by finding that extracts of *Eucomis* contain a high COX-1 inhibitory activity (Taylor and van Staden 2001). Within the bulb trade, the dwarf species have potential as flowering pot plants and the larger members as cut flowers. The only drawback to the appeal of certain species is the foul-smelling flowers (especially *E. bicolor* Baker, *E. humilis* Baker, *E. schijffii* Reyneke and *E. vandermerwei* Verd.).

The only comprehensive taxonomic treatment of the genus remains that of W.F. Reyneke, in the form of an unpublished M.Sc. thesis produced in South Africa in 1972. J. Compton wrote an excellent account of the genus (1990), and the present article could be regarded as an extension and DNA-based backing to these treatises.

To elucidate the relationships between *Eucomis* species, the classical taxonomic traits based on morphological characters and geographical distribution are here supplemented with data on nuclear DNA content. These were not investigated earlier in the systematic study of *Eucomis*. Taxonomy of *Eucomis* is rather difficult as the flowers are very similar. The main useful characters are fragrance, plant size and leaf color. More than 80 different accessions representing all accepted species were measured in an attempt to understand the relationships within *Eucomis* better.

Nuclear DNA content can conveniently be measured by flow cytometry using propidium iodide, a stoichiometric DNA stain that intercalates in the double helix. Where many species in a genus have the same chromosome number, differences in DNA 2C value have proven to be very effective in delimiting infrageneric divisions in a number of taxa (Ohri 1998). Greilhuber (1998, 2005) has clearly shown that intraspecific variation of genome size is much less than assumed.

The evolution of genome size [Cx-value (Greilhuber 1979)] has received increased attention during recent years. Primitive angiosperms are now supposed to have had small genomes; increases up to a factor of 1,000 have occurred independently in various modern taxa (Leitch et al. 1998). Flow cytometry was successfully used to measure the 2C-value for the genera *Hosta* Tratt., *Helleborus* L., *Clivia* Lindl., *Nerine* Herb., *Agapanthus* L'Hér., *Galanthus* L., *Narcissus* L., *Gasteria* Duval., *Tulipa* L., etc., by Zonneveld (2003, 2008, 2009), Zonneveld and Van Iren (2001), Zonneveld and Duncan (2003, 2006), Zonneveld and Van Jaarsveld (2005) and Zonneveld et al. (2003). In this paper it is shown that genome size alone is not sufficient to discriminate all species of *Eucomis*. Also one subspecies is returned to species status, and a new species is described.

Materials and methods

Plant material

Plant material was obtained mainly from the collections of Kirstenbosch Botanical Garden, J. des Brisay (UK), C. McMaster (RSA) and J. Grimshaw (UK). Where possible, material of known wild origin was used, and care was taken to ensure correct identification of all material. Vouchers of material from the Kirstenbosch and McMaster collections (including all known species) are lodged in the Compton Herbarium at Kirstenbosch, Cape Town, RSA.

Flow cytometric measurement of DNA 2C value

For the isolation of nuclei, about 5 cm of root was chopped together with a piece of *Agave americana* L. 'Aureomarginata' as an internal standard (see below). The chopping was done with a new razor blade in a Petri dish in 0.25 ml nuclei-isolation buffer to which 0.25 mg RNase/ml was added (Zonneveld and Van Iren 2001). After adding 1.75 ml propidium iodide solution (50 mg PI/l in isolation buffer), the suspension with nuclei was filtered through a 30-µm nylon filter. The fluorescence of the nuclei was measured ½ h and 1 h after addition of propidium iodide, using a Partec CA-II flow cytometer. The optical path contained a HBO mercury lamp, filters KG1, BG12, dichroic mirror TK500, filter OG570 and a Leitz 50 × 1 water immersion objective. Data were analyzed by means of DPAC software (Partec GmbH). The 2C DNA content of the sample was calculated as the sample peak mean, divided by the *Agave* peak mean, and multiplied with the amount of DNA of the *Agave* standard. At least three different samples, each with at least 5,000 nuclei, were measured twice for each clone. Most histograms revealed a coefficient of variation of less than 5%. The standard deviation was calculated for the DNA content of each species, using all relevant measurements.

Internal standard and absolute DNA content values

When measuring nuclear DNA content by means of flow cytometry, it is necessary to chop tissue from the plant of

interest together with an internal standard: this standard must be as close as possible to the plants of interest. In this way, variation in signal intensities due to staining kinetics, to light absorption and quenching by sample components, as well as to instrument and other variables, is reduced to a minimum. *Agave americana* was chosen as internal standard for *Eucomis*. *Agave americana* is available year round, does not mind several weeks without water and, being a large plant, a single specimen can serve a lifetime, thereby further reducing variation in readings. It also has a low background in propidium iodide measurements and shows a single G_0 peak, almost lacking G_2 arrest.

Fresh male human leucocytes (2C = 7.0 pg; 1 pg = 10^{-12} g = 0.978 × 10^9 base pairs; Dolezel et al. 2003) were chosen as primary standard (Tiersch et al. 1989). This yields 2C = 15.9 pg for nuclei of *Agave americana* L. Based on a published male human genome size of 6.294 × 10^9 base pairs, the nucleus was calculated as containing 6.436 pg (Dolezel et al. 2003). However, this is based on a human sequence where the size of the very large repeat sequences could not accurately be determined. So in the end, the genome size could be closer to 7 pg than now envisioned.

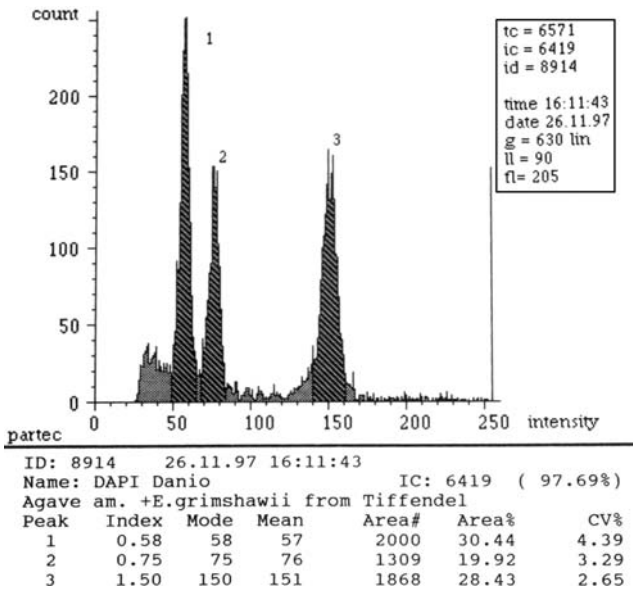

Fig. 1 Flow cytometry histogram of fluorescence intensity of more than 6,000 nuclei isolated and stained simultaneously. Peak *1* the nuclei of the standard: *Agave americana* (15.9 pg). *2* The diploid nuclei of the roots of *Eucomis grimshawii* (21.2 pg). *3* The 59% nuclei with a tetraploid amount of nuclear DNA of the roots of *Eucomis grimshawii* (42.1 pg)

Results

General

Eucomis leaves, like most members of the *Hyacinthaceae*, produce a lot of mucus when cut, clogging the flow cytometer. Therefore, nuclei of *Eucomis* were extracted from the thicker roots. Remarkable was the high content of root nuclei with a doubled DNA content. Usually between 25–50% nuclei are found in roots in most genera investigated (Zonneveld, unpublished) that have doubled their DNA content by endopolyploidy. However, in both diploid and tetraploid *Eucomis* between 50 and up to 75% of the nuclei are found to have doubled their nuclear DNA content (Fig. 1). Five of the diploid species hardly differ in DNA 2C value, and the same is true for the tetraploid species. The main morphological distinctions between the species are fragrance, the presence or absence of a purple color to the leaf base or flower, the cylindrical or clavate shape of the scape and overall plant size.

The diploid species

E. schijffii Reyneke

A dwarf native of the Drakensberg of KwaZulu-Natal and Lesotho, and the mountains of the southern Eastern Cape, it is the *Eucomis* species with the highest altitudinal limit, occurring at up to 3,200 m. The rosette has intensely bluish-grey leaves with maroonish undersides. The purplish leaf margins are minutely toothed. The plant grows to 15 cm high, the coma bracts are large, sometimes overhanging the inflorescence, and its purplish-maroon flowers emit a strong, fetid scent. It has clavate, purple scapes, and its DNA 2C value is 22.8 pg.

E. vandermerwei Verd.

This dwarf species reaching up to 24 cm high in flower is restricted to rocky outcrops in the grassland at high altitude in Mpumalanga in northeastern South Africa (Verdoorn 1944). Its brownish-maroon flowers are extremely long lasting, it has cylindrical scapes, and the plant has cryptically marked foliage. It has a fetid smell, and its DNA 2C value is 23.5 pg.

E. zambesiaca Baker

Occurring in the northern parts of South Africa's Limpopo Province and in the highlands of Malawi and Zimbabwe, this species has creamy-white, sweet-scented racemes that mature to bright green and cylindrical scapes. Reaching 30 cm high, it flowers from mid to late summer. The leaves are always uniformly pale green, and it is a variable species as regards inflorescence width; in some forms these can be as narrow as 15 mm. Its DNA 2C-value is 23.3 pg.

Eucomis grimshawii G.D.Duncan & Zonn., sp. nov.

Haec species habitu E. schijffii similis sed foliis leviter viridibus, margine cartilagineo, floribus albovirentibus, parvioribus, breviviventibus, suaveolentibusque, filamentis triangularibus, antheribus parvis, polline cremeo, fructibus et ovariis valde inflatis differt.

Type: South Africa, Eastern Cape, 3027 (Lady Grey): Hill below Tiffendel Ski Resort north of Rhodes, on shaded moist, grassy, south and southwest facing slopes, under overhanging rocks near a stream (-DD) Dec 2008, *J.C. McMaster s.n.* (NBG, holo.!) (see Fig. 2).

Dwarf geophyte, deciduous, summer-growing, 80–100 mm high. *Bulb* ovoid, 35–40 × 25 × 30 mm solitary or offset-forming, scales cream, apices obtuse; tunic 1- or 2-layered, membranous, pale brown; cataphyll oblong, 30–40 × 10–12 mm, translucent white, subterranean, adhering to leaf bases, apex obtuse. *Leaves* 4 or 5, broadly lanceolate, 90–120 × 40–60 mm, spreading to suberect, pale green, longitudinal veins prominent on upper surface, depressed, 5–7 mm apart; midrib prominent on lower surface, yellowish green; margins cartilaginous, flat to weakly undulate, hyaline. *Inflorescence* few to many flowered, 30–50 mm long, erect, dense; scape clavate, 50–60 × 10–15 mm, erect, pale green, lower half heavily flushed with deep maroonish-magenta, upper half sporadically spotted or blotched with maroonish-magenta; rachis pale green; fertile bracts lanceolate, slightly canaliculate, lengthening towards inflorescence apex, 5–15 × 2–5 mm. *Perianth* campanulate, weakly sweet-scented, spreading, nectar sweet; lower flowers sessile, pedicels of upper flowers up to 1 mm long; tepals oblong, 3–5 × 3–4 mm, greenish white, curved inwards, soft, short-lived; sterile coma bracts 9–16, broadly lanceolate to ovate, 15–35 × 10–25 mm, pale green, suberect to spreading or weakly deflexed, often obscuring upper flowers; margins cartilaginous, hyaline. *Stamens* 6, included; filaments narrowly triangular 3–4 × 0.2–1 mm, white, curved inwards; anthers oblong, 1.5 × 0.8 mm, pollen cream. *Ovary* trilocular, bright green; locules more or less ovoid, 4–5 × 4–5 mm, strongly inflated; style weakly decurved, pale green, 3–4 mm long, stigma penicillate. *Capsule* flat-topped, membranous, dehiscent, pale brown, ripening rapidly; locules strongly inflated, 7–10 × 10–15 mm. *Seed* ovoid, 3–3.5 × 2.5 mm, dull blackish brown, 2 or 3 per locule, surface sculpturing reticulate.

Fig. 2 *Eucomis grimshawii* G.D.Duncan & Zonn. sp. nov. as photographed 29 December 2006 at Tiffendell, Eastern Cape, South Africa by C. McMaster

Note

It is the smallest species in the genus, and with 21.1 pg it also has the lowest amount of nuclear DNA of them all and might therefore be the most plesiomorphic one. However, we have no further arguments to substantiate this claim. Four accessions of *E. grimshawii* were measured. They differed consistently about 7% in nuclear DNA content, this despite the fact that they were morphologically indistinguishable. It cannot be excluded that aneuploidy was involved.

Distribution and habitat

Eucomis grimshawii was discovered in 2002 by Dr. J. Grimshaw at Tiffendel Ski Resort, north of Rhodes Hamlet, Southern Drakensberg, just south of Lesotho between 2,720 and 2,740 m altitude. The plant was subsequently found by J.C. McMaster at the same locality and close by at Naude's Nek Pass to the northeast. They grow in shaded, seasonally wet, south-facing grassy slopes below overhanging rocks, in rich, heavy black soil. They are also encountered in boggy conditions, growing in shade in association with *Kniphofia caulescens* and *K. northiae* (Grimshaw, pers. comm.).

The species is most similar to *E. schijffii.* The latter differs in its intensely glaucous leaves with strongly cartilaginous, minutely crisped purple margins. It is further a taller plant (10–15 cm high) with a purple scape. The tepals are dark purple (instead of greenish white), long-lasting (8–9 days instead of 2–3) and firm (instead of soft), and the flowers emit a strong fetid scent (instead of sweet). Its filaments are purple (instead of white) and broadly triangular with 2-mm-wide bases and have larger anthers (2.5 × 1.5 mm) with bright yellow pollen (instead of cream). Its ovary is much less inflated, has prominent apical grooves, and the capsule with a purple pericarp is also much less inflated, and takes 2 months to ripen (instead of 3–4 weeks). The two species are unusual within the genus in having dull blackish brown seeds with reticulate sculpturing (instead of glossy and smooth).

Eucomis schijffii grows in moist terrain on exposed basalt cliffs and in open rocky, grassy fields, and has a wider distribution along the Lesotho/KwaZulu-Natal border, in northern and southern Lesotho and in the mountains of the southern part of the Eastern Cape (Reyneke 1976). It has a longer flowering period extending from late November to late February.

Eucomis amaryllidifolia Baker

In the type description (Baker 1878) *E. amaryllidifolia* has a leaf length of 30–37 cm and a slightly shorter inflorescence. It is a plant from the Eastern Cape and only differs from *E. autumnalis* in its much narrower leaves of 3 cm. However, it must be kept in mind that this was a plant cultivated in a greenhouse in England. This might explain the seemingly larger size in the type description. One of the authors (G.D.) has never seen a long and narrow leaved plant of this species in nature or in culture. It was later reassessed as a subspecies of *E. autumnalis* as *E. autumnalis* subsp. *amaryllidifolia* (Baker) Reyneke, restricted to the Eastern Cape and Free State in South Africa (Reyneke 1980). In the description of Reyneke (1980), it is a small plant with a leaf length of 13–30 cm, a clavate scape and an inflorescence of only 3–7 cm. The tetraploid chromosome count that was found for a "subsp. *amaryllidifolia*" from Fauresmith, Free State, is most likely a different subspecies (Reyneke and Liebenberg 1980), probably subsp. *clavata*. In describing the subspecies of *E. autumnalis*, four taxa are actually mentioned by Reyneke (1980). Going from south to north in RSA, they are (1) a smaller subsp. *autumnalis* from the south (Eastern Cape), (2) subsp. *clavata* (widespread in north and northeast of South Africa), (3) subsp. *amaryllidifolia* (Eastern Cape) and (4) a larger subsp. *autumnalis* from the north (KwaZulu-Natal and Limpopo provinces in South Africa, and Zimbabwe, but not Malawi).

Eucomis autumnalis subsp. *autumnalis* and subsp. *clavata* have a tetraploid amount of nuclear DNA (Tables 1, 2). All plants measured here as subsp. *amaryllidifolia* were small, diploid plants with on average 23.4 pg and came from the south, i.e., Eastern Cape. Reyneke and Liebenberg (1980) concluded also that all tetraploids were allotetraploid, based on the absence of identical chromosome pairs for the large chromosomes. However, if a diploid is considered as incongruent as a subspecies of an allotetraploid, then *Eucomis amaryllidifolia* is a good species. Moreover, as a diploid it is separated reproductively from the allotetraploid *E. autumnalis.* Therefore, it seems best to consider the large tetraploid form as subsp. *autumnalis* with or without a narrow leaf and restore the small diploid form as *E. amaryllidifolia*.

Eucomis bicolor Baker

The well-known and very hardy *E. bicolor* frequents various high-altitude habitats from the northern part of the Eastern Cape to KwaZulu-Natal, Lesotho and southern Mpumalanga. The name *E. reichenbachii* that has crept into some nursery lists is a mistake and is in fact *E. bicolor*. *E. bicolor* is the only diploid that has a large size. It has a fetid smell, cylindrical scapes and often a purple base to the leaves. Its amount of nuclear DNA of 25.7 pg deviates from that of the other diploids.

Eucomis regia (L.) L'Hér.

Eucomis regia is native to the winter rainfall zone of the Northern and Western Cape. This rather variable plant is confined to heavy clay soil in open aspects or between large rocks or low bushy cover. The bulbs are usually solitary, and the uniformly green leaves lie flat on the ground or spread over rocks. The inflorescence reaches up to 20 cm high. In some specimens the leafy bracts of the coma are disproportionately large, almost completely obscuring the green, unpleasant-smelling flowers. It has clavate scapes and is the only *Eucomis* that flowers from late winter to late spring. It has a rather deviating amount of nuclear DNA with 31.3 pg. It is unclear if there is any relationship between the differences in flowering time and amount of DNA for this species.

The tetraploid species

Eucomis autumnalis (Mill.) Chitt.

By far the most widespread *Eucomis* is the greenish-cream flowering *E. autumnalis*. *Eucomis autumnalis* leaves have undulate edges (Chittenden 1951) and according to Reyneke (1980) it comprises three subspecies: subsp. *autumnalis,* subsp. *clavata* and subsp. *amaryllidifolia*. *Eucomis amaryllidifolia* Baker from the Eastern Cape is here considered as a separate, diploid species. However, the diploid is apart from its overall size very similar to the tetraploid, 'southern' forms of subsp. *autumnalis*, the flowers differing only in their shorter tepals (6–8 mm long). Reyneke and Liebenberg (1980) consider all tetraploids as likely allotetraploids based on the fact that none has identical chromosome pairs. If that is accepted, an allotetraploid *E. autumnalis* cannot be derived solely from a doubling of the diploid *E. amaryllidifolia*.

The subsp. *autumnalis* has a cylindrical scape and semi-erect leaves, occurring in open grassland in the Eastern Cape, KwaZulu-Natal and Limpopo provinces of South Africa, and in Zimbabwe. It includes naturally occurring

Table 1 *Eucomis* accessions with their 2C amount of DNA per nucleus, average, standard deviation (SD), locality and origin

Species	DNA in pg	Average	SD	Locality	Origin
Diploid species					
Eucomis grimshawii Duncan & Zonn. sp. nov.	22.5	21.1	1.0	Tiffendell E.Cape, A	J. Grimshaw s.n.
Eucomis grimshawii Duncan & Zonn. sp. nov.	21.2			Tiffendell E.Cape, A	J. Grimshaw s.n.
Eucomis grimshawii Duncan & Zonn. sp. nov.	20.5			Tiffendell E.Cape, B.	C. McMaster s.n.
Eucomis grimshawii Duncan & Zonn. sp. nov.	20.3			Tiffendell E.Cape, B.	C. McMaster s.n.
Eucomis schijffii Reyneke	22.7	22.8	0.4	Drakensberg, KZN	van Jaarsveld 6551
Eucomis schijffii Reyneke	22.8			Sani Pass KZ Natal	J. Grimshaw s.n.
Eucomis schijffii Reyneke	22.9			Sani Pass KZ Natal	J. Grimshaw s.n.
Eucomis zambesiaca Baker	22.7	23.3	0.6	Hort.	G. Duncan
Eucomis zambesiaca Baker	23.4			Hort.	Longwood Grds.
Eucomis zambesiaca Baker	23.8			Hort.	J. des Brisay
Eucomis vandermerwei Verd.	23.2	23.5	0.6	Hort.	J. Agoston
Eucomis vandermerwei Verd.	23.3			Dullstroom, Mpumal.	H. de Lange s.n.
Eucomis vandermerwei Verd.	23.9			Hort.	J. des Brisay
Eucomis vandermerwei Verd.	23.5			Middelburg, Mpumal.	McDonald
Eucomis bicolor Baker	26.1	25.7	0.6	Harrismith, Free State	B. Szabo 197
Eucomis bicolor Baker	25.1			Cathedral Peak KZN	L. van der Walt s.n.
Eucomis bicolor Baker	25.1			Drakensberg, KZN	NBG 221/76
Eucomis bicolor Baker	26.3			Hort.	J. des Brisay
Eucomis bicolor Baker	25.4			Hort.	J. des Brisay
Eucomis bicolor 'Reichenbachii'	25.8			Hort.	J. des Brisay
Eucomis bicolor 'Reichenbachii'	25.4			Hort.	J. des Brisay
Eucomis bicolor 'Alba'	26.0			Hort.	J. des Brisay
Eucomis bicolor 'White'	26.2			Hort.	J. des Brisay
Eucomis bicolor (as sp. Jack Elliot)	26.0			Hort.	J. des Brisay
E. regia (L.) L'Hér.	30.4	31.3	1.0	Nieuwoudtville, N.Cap	R. Saunders s.n.
E. regia (L.) L'Hér.	32.0			Napier, W.Cape	G. Duncan s.n.
E. regia (L.) L'Hér.	32.1			Caledon, W. Cape	G. Duncan 466
E. regia (L.) L'Hér.	30.6			Dassiesfontein, W.C.	J. Grimshaw s.n.
E. regia (L.) L'Hér.	31.4			Hort.	J. des Brisay
Eucomis amaryllidifolia Baker	22.9	23.4	0.3	Kinross, E.Cape	C. McMaster s.n.
Eucomis amaryllidifolia Baker	22.6			Bombazi, E.Cape	C. McMaster s.n.
Eucomis amaryllidifolia Baker	23.5			Quagga Heights, E.C	C. McMaster s.n.
Eucomis amaryllidifolia Baker	23.5			Stutterheim, E. Cape	C. McMaster s.n.
Eucomis amaryllidifolia Baker	23.2			Bedford, E. Cape	C. McMaster s.n.
Eucomis amaryllidifolia Baker	23.2			Bedford, E. Cape	C. McMaster s.n.
Eucomis amaryllidifolia Baker	23.3			Hort.	J. des Brisay
Eucomis amaryllidifolia 'White Dwarf'	24.0			Hort.	J. des Brisay
Eucomis amaryllidifolia Baker	24.2			Hort.	J. des Brisay
Tetraploid species					
Eucomis autumnalis (Mill.) Chitt.	47.2	47.6	1.0	Triple Streams, E.C.	J. des Brisay
Eucomis autumnalis (Mill.) Chitt.	48.1			Sherwood, KZN	J. des Brisay
Eucomis autumnalis (Mill.) Chitt.	49.7			Baakens Valley, E.C	J. des Brisay
Eucomis autumnalis (Mill.) Chitt.	47.1			Wakkerstroom, Mpu.	J. des Brisay
Eucomis autumnalis (Mill.) Chitt.	48.4			Hort.	J. des Brisay
Eucomis autumn. (Mill.) Chitt. ssp. *autumnalis*	46.5			Triple Streams, E.C.	C. McMaster s.n.
Eucomis autumn. (Mill.) Chitt. ssp. *autumnalis*	47.5			Kirstenbosch BG	NBG 671/83
Eucomis autumn. (Mill.) Chitt. ssp. *autumnalis*	47.8			Aliwal North, E.Cape	C. McMaster s.n.

Table 1 continued

Species	DNA in pg	Average	SD	Locality	Origin
Eucomis autumn. (Mill.) Chitt. ssp. *autumnalis*	47.7			Nieu Bethesda, E.C.	C. McMaster s.n.
Eucomis autumn. (Mill.) Chitt. ssp. *autumnalis*	47.6			Egglestone, E. Cape	C. McMaster s.n.
Eucomis autumn. (Mill.) Chitt. ssp. *autumnalis*	45.5			Katberg, E. Cape	C. McMaster s.n.
Eucomis autumn. (Mill.) Chitt. ssp. *autumnalis*	46.0			Drakensberg; 'Purple'	G. Duncan s.n.
Eucomis autumnalis ssp. *clavata* (Baker) Reyneke	47.2	46.9		Oxbow, Lesotho	G. Matthews 870
Eucomis autumnalis ssp. *clavata* (Baker) Reyneke	46.2			Port Edward, KZN	D. Govender 42
Eucomis autumnalis ssp. *clavata* (Baker) Reyneke	47.3			Qwa Qwa, Free State	D. Govender 40
Eucomis pallidiflora Baker ssp. *pallidiflora*	48.1	47.7	1.2	Kirstenbosch BG	NBG 63/09
Eucomis pallidiflora Baker ssp. *pallidiflora*	46.3			Wakkerstroom, Mpu.	C. McMaster s.n.
Eucomis pallidiflora Baker ssp. *pallidiflora*	48.3			Hort.	J. des Brisay
Eucomis pallidiflora Baker ssp. *pallidiflora*	48.3			Hort.	R. MacKenzie
E. pallidiflora ssp. *pole-evansii* (N.E.Br.) Reyneke	45.9	46.4		Hort.	J. Grimshaw
E. pallidiflora ssp. *pole-evansii* (N.E.Br.) Reyneke	46.2			Hort.	J. Agoston
E. pallidiflora ssp. *pole-evansii* (N.E.Br.) Reyneke	47.2			Hort.	J. des Brisay
Eucomis comosa (Houtt.) Wehrh. var. *comosa*	48.0	48.6	1.0	Kirstenbosch BG	NBG 818/89
Eucomis comosa (Houtt.) Wehrh. var. *comosa*	48.7			Port Edward, KZN	D.Govender 56a
Eucomis comosa (Houtt.) Wehrh. var. *comosa*	49.1			Mnt Kubusi, E. Cape	C. McMaster s.n.
Eucomis comosa (Houtt.) Wehrh var. *striata*	61.0	61.0	1.9	Hort.	J. des Brisay
Eucomis humilis Baker	48.9	47.8	0.7	Drakensberg, KZN	D. Human s.n.
Eucomis humilis Baker	48.0			Drakensberg, KZN	D. Human s.n.
Eucomis humilis Baker	46.6			Qwa Qwa, Free State	D. Govender 39
Eucomis humilis Baker	47.1			Drakensberg, KZN	NBG 291/57
Eucomis humilis Baker	48.2			Drakensberg, KZN	NBG 222/99
Eucomis humilis Baker	48.1			Drakensberg, KZN	NBG 837/82
Eucomis humilis Baker	48.5			Hort.	J. des Brisay
Eucomis humilis Baker	47.3			Sani Pass, KZN	van Jaarsveld 6596
Eucomis humilis Baker	47.7			Hort.	J. des Brisay
Eucomis humilis Baker	47.8			Cathcart, E. Cape	C. McMaster s.n.
Eucomis montana Compton	47.6	48.7	0.9	Vryheid, KZ Natal	D. Govender s.n.
Eucomis montana Compton	48.4			Hort.	J. des Brisay
Eucomis montana Compton	48.3			Hort.	L. Nieuwoudt
Eucomis montana Compton	49.8			Tafelberg KZ Natal	J. des Brisay
Eucomis montana Compton	49.6			Swaziland	H. Hay
Eucomis autumnalis 'Sparkling Burgundy'	46.1			Hort.	Plant Delight Nurs.
Eucomis comosa 'Reuben'	46.6			Kirstenbosch BG	E. Welsh
Eucomis comosa 'Coco'	46.4			Kirstenbosch BG	E. Welsh
Eucomis comosa 'Concolor'	47.3			Hort.	J. des Brisay
Eucomis comosa var. *striata* 'Tugela Jade'	48.2			Kirstenbosch BG	E. Welsh
Eucomis comosa var. *striata* 'Can Can'	46.3			Kirstenbosch BG	E. Welsh

forms with striking burgundy-pink blooms whose leaves are purplish-burgundy when grown in full sun (Duncan 2007). Its DNA 2C value is 47.6 pg.

The subsp. *clavata* (Baker) Reyneke has, as its name suggests, clavate scapes, although not always (Reyeneke 1980). The description calls for a large leaved plant with a comparable rather short inflorescence of 7–13 cm. Further, it has hard, double-layered pericarps contrary to the thin pericarp of ssp. *autumnalis*. It is widespread in the north and northeast of South Africa, and its DNA 2C value is 46.9 pg.

Eucomis comosa (Houtt.) Wehrh.

The sweet-scented *E. comosa* (syn: *E. punctata*), is found from the Eastern Cape to Limpopo in northern South

Table 2 *Eucomis* accessions with their avarage 2C amount of DNA per nucleus, number of different clones measured and 12 morphological characters

Species	DNA in pg	Clones no.	Flower smell	Plant size	Scape + infloresc.	Leaf length	Leaf width	Pedicel length	Tepal color	Filament color	Ovary color	Leaf base color	Upper leaf color	Scape shape
Diploid species														
Eucomis grimshawii Duncan&Zonn.	21.1	4	Sweet	Small	8–10 cm	9–12 cm	4–6 cm	0–1 mm	Green-white	White	Green	Green	Green	Clavate
Eucomis zambesiaca Baker	23.3	3	Sweet	Small	20–30 cm	30 cm	5 cm	2–5 mm	Cream	Green/white	Cream	Green	Green	Cylindrical
Eucomis amaryllidifolia	23.4	9	Sweet	Small	9–20 cm	13–30 cm	1.5–4 cm	2–5 mm	Cream	Green	Green	Green	Green	Clavate
Eucomis vandermerwei Verd.	23.5	4	Fetid	Small	15–24 cm	40 cm	4–5 cm	2–4 mm	Maroon	White	Maroon	Purple	Maroon/purple	Cylindrical
Eucomis schijffii Reyneke	22.8	3	Fetid	Small	10–15 cm	6–10 cm	4–7 cm	0–1 mm	Purple	Purple	Green/purple	Purple	Glaucous	Clavate
Eucomis bicolor Baker	25.7	10	Fetid	Large	50–100 cm	50 cm	10 cm	20 mm	Cream/purple	Purple	Green	Purple	Green	Cylindrical
E. regia (L.) L'Hér.	31.3	5	Fetid	Small	10–20 cm	8–15 cm	1.5 × 4 cm	0 mm	Green	Green/white	Green	Green/purple	Green	Clavate
Tetraploid species														
Eucomis autumnalis (Mill.) Chitt.	47.4	13	Sweet	Large	12–55 cm	15–55 cm	6–13 cm	3–9 mm	Cream/purple	Green/purple	Cream/purple	Usually green	Green	Cylindrical
E.aut. ssp. clavata (Baker) Reyneke	46.9	3	Sweet	Large	12–26 cm	15–45 cm	6–13 cm	3–8 mm	Cream	Green	Green	Green	Green	Clavate/cyl
Eucomis pallidiflora Baker	47.2	4	Sweet	Large	50–90 cm	65 cm	10–12 cm	15–20 mm	Cream	Green	Green	Green	Green	Cylindrical
E.pal. ssp. pole-evansii (N.E.Br.) Reyneke	46.4	3	Sweet	Large	100–200 cm	65 cm	15 cm	25–50 mm	Cream	Green	Green	Green	Green	Cylindrical
Eucomis comosa (Houtt.) Wehrh.	48.6	9	Sweet	Large	40–60 cm	30–80 cm	3–10 cm	15 mm	Cream	White	Purple	Purple	Green	Cylindrical
Eucomis humilis Baker	47.8	10	Fetid	Small/large	14–40 cm	35 cm	6–7 cm	2–4 mm	Cream/green	Purple/maroon	Cream	Purple	Green/glaucous	Clavate/cyl
Eucomis montana Compton	48.7	5	Fetid	Large	30–50 cm	50 cm	18 cm	5 mm	Green	Purple	Purple	Purple	Green/glaucous	Clavate

Africa. The typical variety occurs in a range of dry to moist habitats, while the var. *striata* (Donn) Wild. is confined to swampy conditions, having distinctive stripes instead of spots on the outer leaf bases. It has cylindrical scapes, and the DNA 2Cvalue of *E. comosa* var. *comosa* is 48.6 pg. Surprisingly, one of the three accessions received as var. *striata* turned out to be a pentaploid with 61.0 pg.

Eucomis pallidiflora Baker

Giant within the group, the plain green *E. pallidiflora* subsp. *pallidiflora* is native to wetland marshes in the Eastern Cape, Lesotho and KwaZulu-Natal. It has sweet-smelling and greenish-cream flowers, and cylindrical scapes. Its DNA 2C value is 47.7 pg. *Eucomis pallidiflora* subsp. *pole-evansii*, previously *E. pole-evansii* (Brown 1918), occurs further north in Mpumalanga and Swaziland and is even more robust than subsp. *pallidiflora*, its racemes reaching up to 2 m high or more. Its DNA 2C-value is 46.4 pg, but this difference falls within the standard deviation (Table 1). Both subspecies are further characterized by their long pedicels of 15–50 mm.

Eucomis humilis Baker

Eucomis humilis has greenish-cream, foul-smelling flowers on a cylindrical or clavate scape. We found *E. humilis*, despite its often small size, to be tetraploid with a DNA 2C value of 47.8 pg.

A perusal of W.F. Reyneke's thesis on *Eucomis* and examination of herbarium material annotated by him shows that *E. humilis* is an extremely variable species, which has given rise to some confusion among gardeners. *Eucomis humilis* grows to 40 cm high and occurs in small groups at high altitude in moist grassland below rocky overhangs in northeastern Lesotho and western KwaZulu-Natal. It comprises several distinct forms, including a short form with pale green flowers arranged tightly together on a short, compact inflorescence that is shorter than the length of the leaves, and a very different-looking, robust form with larger, cream-coloured flowers arranged more loosely on a much longer inflorescence, a portion of which overtops the leaves. The flowers of the green form have pinkish-maroon filaments, but in the cream form they are broader and deep purple. The leaves of both forms are broadly lance-shaped with crisped or undulate margins, and are usually heavily spotted with maroon or purple on the undersides, as well as on the scape. The leaves of the short form are bright green, while those of the robust form are much larger and dark green with a purple tinge, with deep purple margins. According to Reyneke (1972), these two forms occur in association in the wild, and at certain localities a number of intermediate forms occur between the two extremes, making it difficult to assign taxonomic rank to any particular form. In addition to these, a dwarf form exists, having rather short green leaves with purple spotting on the undersides, and short creamy-green inflorescences that appear above the leaves.

It has become clear that a robust, cream-flowering form of *E. humilis* has for years been incorrectly listed and illustrated by nurseries in the Northern Hemisphere as *E. montana* Compton. According to the literature and herbarium material, the true *E. montana* has a more northerly distribution, with green flowers, purple ovaries and broader leaves (see the description below).

E. montana Compton

A native of Swaziland, the northeastern Free State and eastern Mpumalanga in South Africa, *E. montana* is a robust plant up to 50 cm high with very large, semi-erect, ovate leaves that are spotted on the underside towards the base, and usually have flat margins (Compton 1967). It has clavate scapes and green flowers with purple/dark-brown filaments, and the rather short inflorescence is produced well below the tips of the leaves. It has a DNA 2C value of 48.7 pg. *Eucomis montana* grows in large groups at high altitude on rocky mountain slopes in partial shade of boulders, flowering in midsummer. We are aware of only one authentic illustration, a watercolor painting (Fabian 1982), and the species is very rare in cultivation. It is very similar to large forms of *E. humilis*, including its fetid flowers. The only difference seems to be the green tepals and the purple ovary.

Discussion

Nuclear DNA content was measured in 85 accessions of *Eucomis*. The ploidy results are generally in agreement with the results of Reyneke (see below for the exceptions). It cannot be excluded that, as has been stated by Reyneke and Liebenberg (1980), the fact that *Eucomis* could have 30/32 or 60/64 chromosomes might indicate that aneuploidy may play a role. Four dwarf species with 22.6, 23.3, 23.4 and 23.5 pg had very close values. The same is true for all the tetraploid plants varying only from 46.4–48.6 pg, well within the range of variation. Therefore, it was often not possible based on the DNA 2C value alone to subscribe an unknown accession to a certain species. Exceptions are *E. bicolor* with 25.7 pg and *E. regia* with 31.3 pg and the new species *E. grimshawii* with 21.1 pg.

Moreover, very few morphological characters are useful in *Eucomis*. Those that have been investigated are compared in Table 2. Genome size as investigated here (Tables 1, 2) complements the work based mainly on morphological characters of (Reyneke 1972).

The evolutionary origin of the tetraploid species

Ploidy seems to play a bigger role than envisioned in the speciation of *Eucomis*. Earlier cytological investigation of *Eucomis* has shown that about half the species are diploid (Reyneke and Liebenberg 1980), and this is confirmed here (Table 1). Only in *E. comosa* was a pentaploid found.

Polyploid tulips are concentrated in the middle and upper mountains, whereas the diploids are mainly found in the deserts and lower mountains (Botschantzeva 1962). The opposite seems true for *Eucomis* with all the diploid species (except certain forms of *E. regia*) occurring high up in the mountains. In most cases polyploidy is not an argument (any longer) to assign a taxon specific status (Woods and Bamford 1937). The origin of the tetraploid species of *Eucomis* is unknown so far. Reyneke and Liebenberg (1980) state that all tetraploids are allotetraploids. These allotetraploids must be derived from two different diploid parents. Moreover, it seems likely that plants with sweet smells have pollinators different from those with fetid smells. Besides, sweet-smelling *E. autumnalis, E. pallidiflora* and *E. comosa* have morphologically most in common with sweet-smelling *E. amaryllidifolia*. So if these sweet tetraploids have two different sweet diploid parents, they are likely to be: *E. amaryllidifolia* and *E. zambesiaca*. The very large coma bracts and lower amount of nuclear DNA of the sweet-scented *E. grimshawii* do not seem to fit in here. Different forms of each diploid species and local adaptations might have resulted in the end in "different" tetraploid species. The two fetid tetraploids *E. humilis* and *E. montana* are morphologically most similar to *E. bicolor*. Therefore, their parents could be the fetid *E. bicolor* and *E. schijffii*. The heavily maroon spotted upper leaf surfaces of *E. vandermerwei* seem to exclude it as a parent. Just doubling *E. bicolor* (→51.4 pg) would give too high a value, unless genome downsizing has taken place, as often happens in tetraploids. The above hypotheses are not in contradiction with the amounts of nuclear DNA as measured here.

Conclusions

Flow cytometry can be considered as a quick and useful method to produce a systematic data source. Moreover, it can be used to investigate imported bulbs, precluding the need to grow them to maturity for identification purposes. The difference between the highest and lowest DNA contents for the diploids is about 10 pg. This 50% increase in DNA content without changing the number of chromosomes must be the result of a vast number of genomic changes. Depending on the size of the total genome, 1 pg amounts to several thousand genes. Therefore, flow cytometry is not a one-character-based taxonomy as the largest genome contains roughly 10^{10} more base pairs than the smallest and has chromosomes that are on average 50% longer. The data presented here for DNA content agree in most respects with the most recent classification of *Eucomis*. Flow cytometry as a taxonomic and diagnostic tool is applicable even in the case of dormant bulbs or sterile plants, and therefore has applications for conservation monitoring.

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References

Baker JG (1878) New garden plants: *Eucomis amaryllidifolia* and *Eucomis bicolor*. Gard Chron 10:492
Baker JG (1886) *Eucomis zambesiaca*. Gard Chron 25:9
Baker JG (1887) New or noteworthy plants: *Eucomis pallidiflora*. Gard Chron (2):154
Baker JG (1895) *Eucomis humilis*. Bull Misc Inform Kew 152
Botschantzeva ZP (1962) Tulips: taxonomy, morphology, cytology, phytogeography, and physiology. English translated edition by HQ Varekamp (1982) p. 1–230. Balkema, Rotterdam
Brown NE (1918) New or noteworthy plants: *Eucomis pole-evansii*. Gard Chron III 63:185
Chittenden FJ (1951) *Eucomis autumnalis*. Roy Hort Soc Dict Gard 2:787
Compton RH (1967) Plantae Novae Africanae: *Eucomis montana*. J S Afr Bot 33:293–294
Compton J (1990) *Eucomis* L'Héritier. The Plantsman 12–3:129–139
Dillenius JJ (1732) *Hortus Ethamensis*. Sumptibus Auctoris, London
Dolezel J, Bartos J, Voglmayer H, Greilhuber J (2003) Nuclear DNA content and genome size of trout and human. Cytometry 51a:127–128
Duncan GD (2007) Lesser known *Eucomis*. The Plantsman 6:98–103
Fabian A (1982) Transvaal Wild Flowers: 26 (plate 8b). Macmillan Publication, USA
Govaerts R (2006) World checklist series RBG Kew, UK: genus *Eucomis*. http://apps.kew.org/wcsp/qsearch.do77
Greilhuber J (1979) Evolutionary changes of DNA and Heterochromatin amounts in the *Scilla bifolia* Group (*Liliaceae*). Plant Syst Evol Suppl 2:263–280
Greilhuber J (1998) Intraspecific variation in genome size: a critical reassessment. Ann Bot 82:27–35
Greilhuber J (2005) Intraspecific variation in genome size in angiosperms, identifying its existence. Ann Bot 95:91–98
Leitch I, Chase MW, Bennett MD (1998) Phylogenetic analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants. Ann Bot 82(Suppl. A):85–94
Ohri D (1998) Genome size variation and plant systematics. Ann Bot 82(Suppl. A):750–812
Reyneke WF (1972) 'n Monografiese studie van die genus *Eucomis* L'Hérit. in Suid Afrika. Department of Botany, University of Pretoria. unpublished Msc thesis

Reyneke WF (1976) A new species of *Eucomis* L'Hérit. (Liliaceae) from South Africa. J S Afr Bot 42–4:361–364
Reyneke WF (1980) Three subspecies of *Eucomis autumnalis*. Bothalia 13:140–142
Reyneke WF, Liebenberg H (1980) Karyotype analysis of the genus *Eucomis* (Liliaceae). J S Afr Bot 46–4:355–360
Taylor JLS, van Staden J (2001) COX-1 inhibitory activity in extracts from *Eucomis* L'Herit' species. J Ethnopharm 75:257–265
Tiersch TR, Chandler RW, Wachtel SSM, Ellias S (1989) Reference standards for flow cytometry and application in comparative studies of nuclear DNA content. Cytometry 10:706–710
Verdoorn IC (1944) *Eucomis vandermerwei*. Fl Pl S Africa 24:t. 955
Woods MW, Bamford R (1937) Chromosome morphology and number in *Tulipa*. Am J Bot 24:174–184
Zonneveld BJM (2001) Nuclear DNA contents of all species of *Helleborus* discriminate between species and sectional divisions. Plant Syst Evol 229:125–130
Zonneveld BJM (2003) The systematic value of nuclear DNA content in *Clivia*. Herbertia 57:41–47
Zonneveld BJM (2008) The systematic value of nuclear DNA content for all species of *Narcissus* L. (Amaryllidaceae). Plant Syst Evol 275:109–132
Zonneveld BJM (2009) The systematic value of nuclear genome size for all species of *Tulipa* L. *(Liliaceae)*. Plant Syst Evol (in press)
Zonneveld BJM, Duncan GD (2003) Taxonomic implications of genome size and pollen color and vitality for species of *Agapanthus* L'Héritier (*Agapanthaceae*). Plant Syst Evol 241:115–123
Zonneveld BJM, Duncan GD (2006) Genome size for the species of *Nerine* Herb. (Amaryllidaceae) and its evident correlation with growth cycle, leaf width and other morphological characters. Plant Syst Evol 257:251–260
Zonneveld BJM, Van Iren F (2001) Genome size and pollen viability as taxonomic criteria: application to the genus *Hosta*. Plant Biol 3:176–185
Zonneveld BJM, Van Jaarsveld EJ (2005) Taxonomic implications of genome size for all species of the genus *Gasteria* Duval (Aloaceae). Plant Syst Evol 251:217–227
Zonneveld BJM, Grimshaw JM, Davis AP (2003) The systematic value of nuclear DNA content in *Galanthus*. Plant Syst Evol 241:89–102