



# Genome size in *Anthurium* evaluated in the context of karyotypes and phenotypes

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## Abstract

### Background and aims

*Anthurium* is an important horticultural crop from the family Araceae, order Alismatales, a lineage considered to have diverged from other monocots prior to the cereals. Genome size and its distribution in *Anthurium* were investigated to gain a basic understanding of genome organization in this large genus and to forge a firm foundation for advancement of molecular approaches for the study of *Anthurium*. Currently, genome size estimates have been reported for only two *Anthurium* samples.

### Methodology

Bulk nuclear DNA content estimates were obtained by flow cell cytometry using leaf tissue collected from *Anthurium* species of different subgeneric groups and from commercial cultivars. The most current and well-supported topology of subgeneric, sectional relationships was applied to present genome size estimates in the context of reported chromosome counts, karyotypes, putative phylogenetic relationships, observed phenotypes and pedigree.

### Principal results

Genome size estimates based on bulk nuclear DNA content for 77 accessions representing 34 species and 9 cultivars were obtained, including initial estimates for 33 *Anthurium* species, and both the smallest (*Anthurium obtusum*; *Tetraspermium*) and largest (*Anthurium roseospadix*; *Calomystrium*) *Anthurium* genome sizes reported to date. Genome size did not distinguish any subgeneric section, but ranged 5-fold (4.42–20.83 pg/2 C) despite consistent  $2N = 30$  chromosome counts. Intraspecific genome size variation >20% is reported for *Anthurium ravenii*, *A. watermaliense* and *A. gracile*.

### Conclusions

Genome size estimates for *Anthurium* species spanning 13 recognized subgeneric sections indicate that genome size does not generally correlate with chromosome count or phylogenetic relationships. Mechanisms of genome expansion and contraction, including amplification and reduction of repetitive elements, polyploidy, chromosome reorganization/loss, may be involved in genome evolution in *Anthurium* as in other species. The new information on *Anthurium* genome sizes provides a platform for molecular studies supporting further research on genome evolution as well as cultivar development.

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## Introduction

*Anthurium* is the most speciose genus in the Araceae, a monocot family defined by its unique inflorescence composed of a spadix and spathe. The spadix holds hundreds of minute flowers compacted on a spike, which is subtended by a more or less showy sterile leaf-like organ, the spathe. *Anthurium* is comprised of ~900 published and 1500 estimated species endemic to the neotropical zones of northern Mexico and south through Central America to southern Brazil, and on the Caribbean Islands (Croat 1988, 1989; Mayo *et al.* 1997; Govaerts *et al.* 2011; Boyce and Croat 2012). *Anthurium andraeanum*, native to Colombia, was first introduced to the island of Oahu in 1889, where it flourished and became widely cultivated by amateur breeders and hobbyists who developed many attractive new varieties throughout the 1940s. Beginning in 1950, an intensive breeding programme at the University of Hawai'i at Manoa yielded many unique and improved cultivars using selective breeding, hybridization and *in vitro* propagation of *Anthurium* species. The novel colours and forms, as well as desirable horticultural attributes, generated in these cultivars contributed to the dominance of the anthurium industry by Hawaiian growers for much of the second half of the 20th century (Kamemoto and Kuehne 1996). We anticipate that future improvements to anthurium cultivars will utilize molecular resources developed to contribute to the basic understanding of this large genus while supporting applied science.

Genome size has implications for molecular biology work, genomics and overall successful implementation as a study organism. Genome size is also correlated with seed mass, cell size, stomatal size, stomatal density, length of cell cycle and a host of derivative phenotypes important for plant success (Beaulieu *et al.* 2007, 2008; Leitch *et al.* 2007; Hodgson *et al.* 2010). Genera with larger genome sizes have limited photosynthetic rates, tend to have limited distribution and tend to be less speciose (Knight *et al.* 2005). Genome size in angiosperms varies ~1000-fold (Leitch *et al.* 2005) with the largest genomes found among the monocots (Leitch *et al.* 2010; Zonneveld 2010). Variations in angiosperm DNA content (Bennett and Leitch 2005) have been interpreted in a robust phylogenetic context to reconstruct genome size evolution, revealing the ancestral genome size to be relatively small (1.4 pg/1 C) (Soltis *et al.* 2003). Significant increases in genome size have been attributed to polyploidy and to amplification of repetitive DNA content (SanMiguel *et al.* 1996; Vicent *et al.* 1999; Hawkins *et al.* 2006). Secondary downsizing in lineages embedded within clades having larger genome sizes counters the overall trend towards

genome size growth (Leitch *et al.* 1998; Bennetzen *et al.* 2005).

In the family Araceae, genome sizes tend to be moderate, including *Orontium aquaticum* (30 pg/2 C), a species derived from an early-diverging lineage in Araceae (Cabrera *et al.* 2008; Cusimano *et al.* 2011). Genome size estimates have been reported for only two *Anthurium* accessions: *A. schlechtendalii* (15.27 pg/2 C) and *A. grande* Hort. (27.00 pg/2 C) (26 July 2011; <http://data.kew.org/cvalues>). These are also of moderate size with the genome size estimate for *A. schlechtendalii* 2.8 times that of *Zea mays* (5.45 pg/2 C) (Bennett and Smith 1976), and the estimate for the accession *A. grande* Hort. nearly twice as large as that of *A. schlechtendalii* (Ghosh *et al.* 2001). However, in Lemnoideae, the sister group to the true Araceae, the group to which *Anthurium* belongs (Cabrera *et al.* 2008; Cusimano *et al.* 2011), the genera *Lemna* (1.20 pg/2 C) and *Spirodela* (0.60 pg/2 C, 0.74 pg/2 C) have quite small genome sizes (Geber 1989). The evolutionary relationship of these lineages suggests that the common ancestor of both Lemnoideae and the true Araceae may have had in place the genomic machinery to secondarily generate species with small genomes and that there may also be *Anthurium* species with small genome sizes.

Understanding the organization and composition of the *Anthurium* genome is a prerequisite to the development of molecular resources to support improvement of the *Anthurium* Hort. complex. We set out to document a wider range of *Anthurium* genome sizes and interpret them in the context of the most recent phylogenetic analysis of *Anthurium* species (Carlsen 2011), referencing cytological observations and known mechanisms of genome size evolution to identify trends in genome evolution in *Anthurium*. We sampled most deeply the natural, easily recognized sections *Calomystrium* and *Cardiolonchium* from which the *Anthurium* Hort. complex mainly derives in order to better explore the extent of evolutionary change and gain insight into the events influencing genome evolution in these clades.

## Materials and methods

Nuclear genome size estimations were obtained by flow cell cytometry following the protocol described by Arumuganathan and Earle (1991). Genome sizes were obtained for 81 accessions obtained from botanical gardens, anthurium industry growers and cultivar developers. Tissue samples of 50 mg fully expanded, non-senescent leaf tissue were collected and shipped to arrive for analysis within 24 h of collection. Flow cytometry involves chopping of fresh plant material together

with an internal standard (Galbraith *et al.* 1983). Ideal internal standards display minimal variability (Baranyi and Greilhuber 1996), match as closely as possible the configuration of DNA (e.g. chromosome structure) in the nucleus of the sample, and have a genome size larger than that of the sample, but not >4 times larger (Suda and Leitch 2010; Praça-Fontes *et al.* 2011). Monocots display a greater variability in chromosome organization and amount of DNA in the genome (Leitch *et al.* 2010), so we provided the monocots wheat (*Triticum aestivum* cv. 'Zak' 30.55 pg/2 C) and barley (*Hordeum vulgare* line NE86954 9.69 pg/2 C) as internal standards based on the existing 2-fold range of genome sizes for *Anthurium* found in the plant DNA C values database (26 July 2011; <http://data.kew.org/cvalues>). We also provided tobacco (*Nicotiana tabacum* cv. 'SR-1' 9.32 pg/2 C). Each nuclear preparation was sampled four times, under the direction of K. Arumuganathan, at the Flow Cell Core Lab, Benaroya Research Institute at Virginia Mason, 1202 Ninth Avenue, Seattle, WA 98101, USA. The mean bulk nuclear DNA content (2 C) of each sample (expressed as picograms) was based on 1000 scanned G0 + G1 nuclei from sample tissue, compared with nuclei of the internal standard.

## Results

### Genome sizing

The terms 'C value' and 'genome size' have specific meaning independent of the number of chromosomes or base pairs in the cell. The term 'C value' originally referred to a constant value observed across all different tissue types in animals, whereas the term 'genome size' is used to describe the bulk nuclear DNA content of cells, both the more easily obtained somatic (holoploid) cells and also gametic (monoploid) cells (Bennett and Leitch 2005; Greilhuber *et al.* 2005). The terms 2 C and 1 C have been proposed to distinguish between the DNA content of holoploid somatic cells and monoploid gametes, respectively, and we follow this convention (Greilhuber *et al.* 2005). Measurements were derived from somatic (i.e. leaf) tissue, therefore values for 1 C are obtained by dividing the measured 2 C value in half and are useful for estimating and comparing the DNA content of the monoploid genome. After excluding four accessions having uncertain provenance, we report the arithmetic mean of four instrument readings of nuclei,  $\pm$  standard deviation, for 77 accessions, increasing reported genome size estimates for *Anthurium* spp. by 33 species and 9 cultivars (Table 1).

Wheat and barley, both monocots, were chosen as internal standards to better reflect the DNA configuration

in our *Anthurium* samples. However, when barley was used, sample peaks frequently overlapped those of the internal standard, so most genome sizes are reported using wheat as the internal standard. When overlapping peaks prevented interpretation of results with wheat, or if the genome size was closer to that of the eudicot tobacco, results are reported using that species as an internal standard. In one case (*A. gymnopus*) the sample was processed using a previously evaluated *Anthurium* species as the internal standard (Table 1) because standards were not available at the time of sampling. Genome sizes for 26 accessions obtained using both tobacco and wheat as internal standards were generally within 10% of the mean, confirming that the use of either standard produces essentially the same results [see Additional Information].

The mean pg/2 C genome size of all accessions sampled for each species is presented (Fig. 1) along with previously published chromosome counts [see Additional Information], organized according to accepted sectional assignments based on traditional characters of morphology, habit, flower/inflorescence, secondary metabolites, karyotype and, most recently, molecular data (Croat 1983, 1991; Croat and Sheffer 1983; Carlsen and Croat 2007; Carlsen 2011). The most recent phylogenetic analysis of 102 samples broadly retains the composition and identity of natural sections, those found least controversial by traditional systematics, and proposes relative relationships among them, which can be extended to other species assigned to those sections. Carlsen sampled 84 species that we did not include, and we sampled 16 species that Carlsen did not include (Carlsen 2011). These are placed according to their existing sectional assignment (Carlsen 2011). The relationship of the monotypic section *Gymnopus* to other sections has not been determined (Fig. 1).

Published chromosome counts report  $2N = 30$  ( $N =$  monoploid chromosome number) for most *Anthurium* species (Fig. 1), with frequent reports of supernumerary chromosomes ('B', chromosomes, satellites or fragments) (Petersen 1989), distinguished mainly by size, dispensability and behaviour at meiosis (Jones and Rees 1982) [see Additional Information]. Of the species we sampled, recent cytogenetic analyses report supernumerary chromosomes exclusively in species assigned to sections *Calomystrium*, *Cardiolonchium*, *Porphyrochitonium* and *Pachyneurium* (Fig. 1) (Sharma and Bhattacharyya 1961; Sheffer and Kamemoto 1976; Sheffer and Croat 1983; Marutani *et al.* 1993; Cotias-de-Oliveira *et al.* 1999).

### Genome sizes in a phylogenetic framework

The organization of *Anthurium* species chromosome counts and genome sizes according to the accepted sub-generic grouping of species into sections suggests all

**Table 1** Genome sizes of accessions sampled, listed alphabetically by *Anthurium* species, followed by cultivars

Species name	Accession	Genome sizing standard	Genome size <sup>a</sup> (pg/2 C) ± S.D. (n = 4)	DNA content (Mbp/1 C) <sup>b</sup>
<i>A. amnicola</i> Dressler	ABG 19911372	W	10.53 ± 0.11	5147
	MBG 84952	W	10.81 ± 0.57	5287
	MSBG 1976-0053-002A	W	9.74 ± 0.21	4765
<i>A. andraeanum</i> Linden	ABG 19911368	W	9.59 ± 1.20	4688
<i>A. andraeanum</i> sp. aff. (presumed Hort.)	USBG s.n.	W	8.92 ± 0.05	4631
<i>A. antioquiense</i> Engler	MBG 81407a/b	W	10.35 ± 0.07	5059
	MSBG 1996-0276A	W	9.91 ± 0.06	4845
	NYBG 1383/78*A*C	W	9.23 ± 0.14	4512
<i>A. armeniense</i> Croat	MBG 63434e	W	11.38 ± 0.15	5563
	MSBG 1979-1055A	W	12.64 ± 0.35	6180
<i>A. bakeri</i> Hooker f.	MBG 78747c	W	9.89 ± 0.08	4835
	NYBG 897/63*A*B*C	W	8.71 ± 0.35	4260
	USBG 77-0090	W	9.24 ± 0.13	4519
<i>A. cerrocampaense</i> Croat	MBG 76663/b	W	11.73 ± 0.18	5734
	MSBG 1980-0429A	W	11.16 ± 0.09	5455
<i>A. clavigerum</i> Poepp.& Endl.	MSBG 1991-0174A	W	13.27 ± 0.17	6490
	ABG 20011433	T	15.26 ± 0.28	7462
<i>A. clidemioides</i> Standl.	JBVL 850002	W	9.47 ± 0.17	4632
	MBG 79567	W	8.86 ± 0.65	4333
<i>A. coriaceum</i> G. Don	NYBG 574/65*A	W	14.32 ± 0.17	7003
<i>A. esmeraldense</i> Sodiro	ABG 20072399	W	8.79 ± 0.17	4297
<i>A. flexile</i> ssp <i>muelleri</i> (J.F. Macbr.) Croat & Baker	MBG 100348	W	9.46 ± 0.19	4628
<i>A. formosum</i> Schott	MSBG 1991-0158A	W	8.77 ± 0.28	4286
<i>A. fragrantissimum</i> Croat	MBG 76860	W	6.21 ± 0.39	3036
<i>A. gracile</i> (Rudge) Lindl.	ABG 19980680	W	9.66 ± 0.20	4721
	MBG 95664	W	13.51 ± 0.22	6607
	MSBG 2001-0232A	W	13.98 ± 0.06	6835
<i>A. gymnopus</i> Griseb.	CJBN 2009.3.213	<i>A. coriaceum</i> NYBG 574/65*A	11.21 ± 0.14	5482
<i>A. hoffmanni</i> Schott	MBG 66203	W	9.56 ± 0.38	4674
	MSBG 1993-0176A	W	9.89 ± 0.09	4835
<i>A. kamemotoanum</i> Croat	UH s.n.	W	9.47 ± 0.07	4629
<i>A. lentii</i> Croat & Baker	MBG 35902	W	13.96 ± 0.06	6824
<i>A. leuconeurum</i> Lemaire	MSBG 1980-1683B	W	14.35 ± 0.07	7016
<i>A. lucens</i> Standl. ex Yuncker	MBG 78702	W	13.24 ± 0.50	6473
	MSBG 1980-1619A	W	12.71 ± 0.38	6216
	NYBG 103/81*A*B	W	13.16 ± 0.68	6435

Continued

Table 1 Continued

Species name	Accession	Genome sizing standard	Genome size <sup>a</sup> (pg/2 C) ± S.D. (n=4)	DNA content (Mbp/1 C) <sup>b</sup>
<i>A. microspadix</i> Schott	MBG 100186	W	12.25 ± 0.13	5992
<i>A. nymphaefolium</i> C. Koch & Bouché	MBG 55262	W	9.45 ± 0.27	4623
<i>A. obtusum</i> (Engl.) Grayum	ABG 19970478	T	5.80 ± 0.08	2835
	MBG 82905	T	4.42 ± 0.09	2160
<i>A. ochranthum</i> K. Koch	MBG 69861a	W	10.87 ± 0.12	5317
	MBG 75190a	W	10.64 ± 0.10	5201
<i>A. pittieri</i> Engl.	JBM 84-2010	T	5.61 ± 0.03	2745
<i>A. radicans</i> K. Koch & A. Haage	ABG 19911495	W	15.10 ± 0.32	7103
	MSBG 1975-0053-0003A	W	14.52 ± 0.11	7103
	USBG 98-2591	W	13.46 ± 0.33	6581
	MBG 74778	W	13.32 ± 0.29	6515
<i>A. ravenii</i> Croat & Baker	MSBG 1980-0425A	W	7.54 ± 0.15	3689
	MBG 74076	W	20.83 ± 0.36	10187
<i>A. scandens</i> (Aubl.) ssp. <i>pusillum</i> Engler	USBG 98-1900	W	5.12 ± 0.14	2506
<i>A. scandens</i> (Aubl.) ssp. <i>scandens</i> Scheffer	ABG 19911433	W	9.98 ± 0.57	4882
	ABG 19980667	W	9.67 ± 0.15	4726
	MBG 47671	W	9.28 ± 0.16	4537
<i>A. schlechtendalii</i> ssp. <i>schlechtendalii</i> Kunth	MBG 78640	W	11.54 ± 0.13	5643
	MSBG 1977-3108A	W	14.33 ± 0.15	7008
	NYBG 933/79*A	W	12.25 ± 0.18	5991
	NYBG 993/93*A-C	W	12.07 ± 0.10	5903
<i>A. solitarium</i> Schott	JBM 2083-2000	W	14.69 ± 0.23	7181
	MBG 53699	W	16.03 ± 0.21	7840
<i>A. warocqueanum</i> J. Moore	ABG 19930478	W	8.65 ± 0.14	4232
	MBG 101538	W	8.97 ± 0.09	4386
	MSBG 2006-0001A	W	8.95 ± 0.12	4374
<i>A. watermaliense</i> Hort ex. L.H. Bailey & Nash	MBG 78766	W	9.47 ± 0.32	4632
	MSBG 1977-2832A	T	7.57 ± 0.02	3701
<i>A. wendlingeri</i> Barroso	ABG 20072507	W	7.87 ± 0.20	3846
	MBG 95418	W	7.04 ± 0.15	3441
	MSBG 1977-1989A	T	6.59 ± 0.31	3221
	USBG 01-1412	W	8.31 ± 0.22	4066
cv. 'Marian Seefurth'	HAIA-MS1	W	9.22 ± 0.07	4509
cv. 'Midori'	HAIA-Md1	W	9.48 ± 0.25	4636
cv. 'Miss June Purple'	HAIA-MJP	W	14.05 ± 0.32	6870
cv. 'New Paho Red'	HAIA-NPR2	W	8.93 ± 0.08	4367
cv. 'Princess Aiko'	NG-10-02-Aiko	W	9.39 ± 0.27	4592
cv. 'Puanani'	NG-10-Pu	W	9.44 ± 0.10	4616

Continued

**Table 1** Continued

Species name	Accession	Genome sizing standard	Genome size <sup>a</sup> (pg/2 C) ± S.D. (n=4)	DNA content (Mbp/1 C) <sup>b</sup>
cv. 'Purple Passion'	NG-10-PP	W	10.82 ± 0.14	5291
cv. 'Regina'	NG-10-1-Reg	W	9.80 ± 0.22	4792
cv. 'Shibori'	NG-10-Shi	W	9.73 ± 0.20	4758

Mbp, million base pairs; W, wheat (*Triticum aestivum* cv. 'Zak,' 30.55 pg/2 C); T, tobacco (*Nicotiana tabacum* cv. 'SR-1' 9.32 pg/2 C); ABG, Atlanta Botanical Garden; CJB, Conservatoire et Jardins Botaniques de Nancy; JBM, Jardin Botanique de Montréal; JBVL, Jardin Botanique de la Ville de Lyon; MBG, Missouri Botanical Garden; MSBG, Marie Selby Botanical Gardens; NYBG, New York Botanical Garden; UH, University of Hawai'i College of Tropical Agriculture and Human Resources; USBG, United States Botanic Garden; NG, Novelty Greens; HAIA, Hawaiian Anthurium Industry Association.

<sup>a</sup>Genome size and standard deviation have been rounded to two decimal places.

<sup>b</sup>Mbp/1 C DNA for plant species is based on 1 pg=978 Mbp according to Doležel and Greilhuber (2010) and was calculated prior to rounding genome size (pg/2 C) to two decimal places.

clades derived from a progenitor lineage having  $2N = 30$  chromosomes, with occasional polyploidy and cytological variation in most sections. The genome sizes associated with  $2N = 30$  vary >4-fold (Table 1, Fig. 1). The genome sizes of the two species sampled in section *Polyphyllium* (9.17–9.46 pg/2 C) are within 10% of each other, a variance not greater than within-sample variance (K. Arumuganathan, Flow Cell Core Lab, Benaroya Research Institute, Seattle, WA, USA, pers. commun.). There is evidence for polyploidization in section *Polyphyllium*, with twice as many chromosomes reported for *A. flexile* ( $2N = 60$ ) as for *A. clidemioides* ( $2N = 30$ ) (Table 1, Fig. 1). The near equivalence of genome size despite the doubled number of chromosomes may reflect an early polyploidization in the *Polyphyllium* crown group which arose over 11 million years ago (mya) (Fig. 1). The genome sizes of the two species sampled in section *Urospadix* (14.32–14.36 pg/2 C) are also within 10% of each other, but are reported to share the same chromosome count (Table 1, Fig. 1) [see Additional Information].

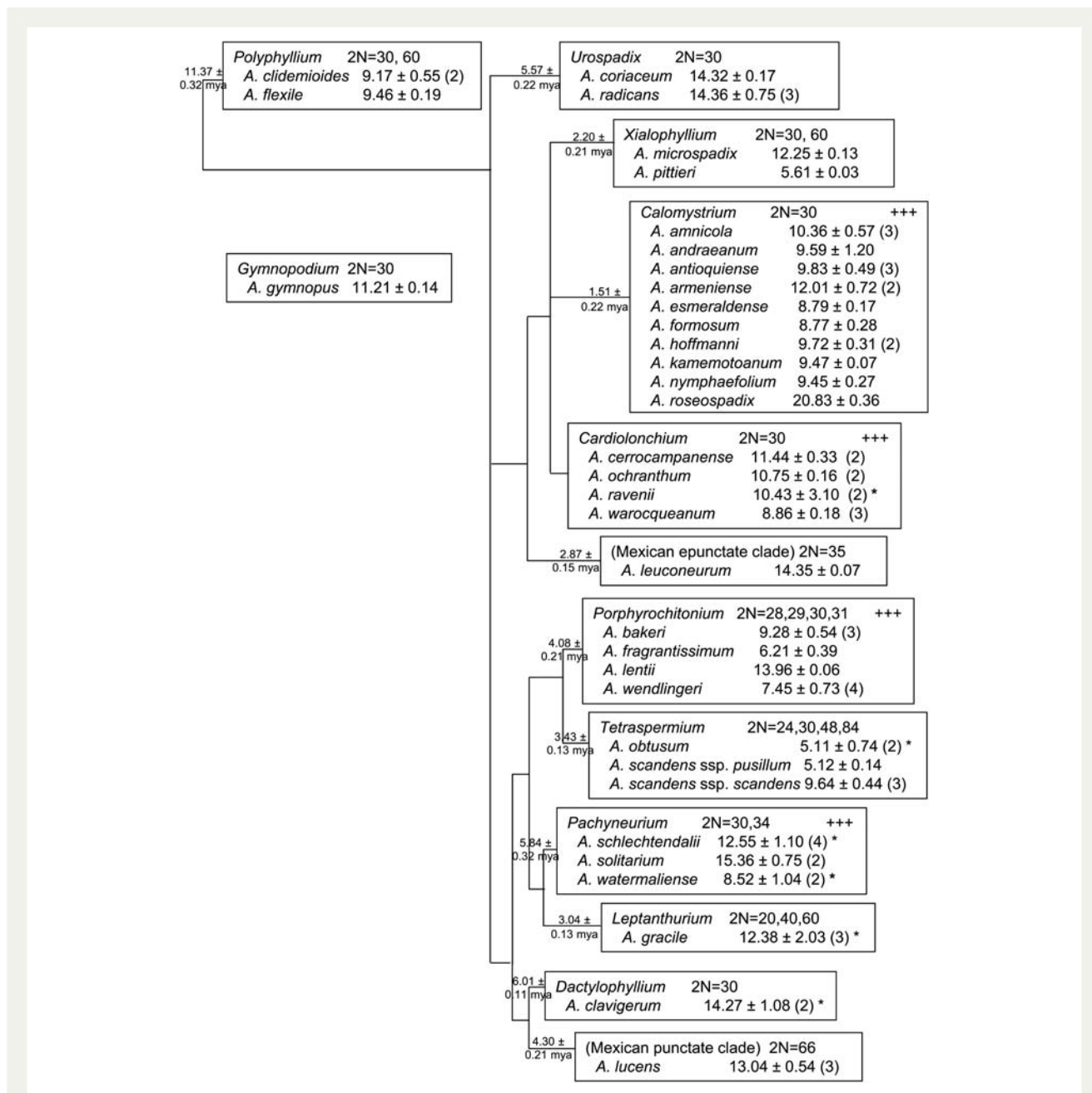
The phylogenetic relationships among the traditionally described sections *Xialophyllum*, *Calomystrium* and *Cardiolonchium* are unclear, although it is certain that section *Cardiolonchium* as traditionally defined is not monophyletic (Carlsen 2011). The genome sizes of two species sampled in section *Xialophyllum* are 5.61–12.25 pg/2 C, displaying an approximately proportional relationship between genome size and chromosome count between the two species, suggesting a polyploid event occurring no more than 2.20 mya, the estimated crown group divergence date (Fig. 1).

In section *Calomystrium*, the most recently established clade (1.5 mya), genome sizes of the 10 species sampled range from 8.77 to 20.83 pg/2 C, a 2.4-fold difference (Figs 1 and 2), despite a consistent number of chromosomes (Fig. 1). Among the *Calomystrium* species, the

genome sizes of *A. formosum* and *A. esmeraldense* are very similar (8.77–8.79 pg/2 C), as are the genome sizes of *A. nymphaefolium*, *A. kamemotoanum*, *A. andraeanum*, *A. hoffmanni* and *A. antioquiense* (9.45–9.83 pg/2 C) (Table 1, Fig. 1). The genome sizes of *A. amnicola* and *A. armeniense* are larger, but the genome size of *A. roseospadix* (20.83 pg/2 C), the largest *Anthurium* genome size to date, is two or more times that of most other species sampled in section *Calomystrium* (Table 1, Fig. 1). Although B chromosomes have been occasionally reported in section *Calomystrium*, there have been no reports of polyploidy as in other *Anthurium* sections having such large inter- and intraspecies differences in genome size (Fig. 1).

The mean genome size estimates for the four species represented in section *Cardiolonchium* range from 8.86 to 11.44 pg/2 C, with consistent  $2N = 30$  and reported occurrence of B chromosomes (Figs 1 and 2). The range of the mean genome sizes for these species is less than the range of genome size estimates reported for the two accessions sampled for the species *A. ravenii* (Table 1). The intraspecies variation reported for *A. ravenii* (7.54–13.32 pg/2 C) reflects ~43% difference in genome sizes between the two accessions sampled, each of which had reliable provenance information and resembled no species other than *A. ravenii*. The larger genome size reported for this species is 1.76 times that of the smaller one, suggesting some extent of autopolyploidy in *A. ravenii* and that we may have sampled an accession with a different cytotype. Other species sampled in this section display unremarkable intraspecies variance.

The genome size of *A. leuconeurum*, a member of a newly described (Carlsen 2011) and yet unnamed clade of epunctate Mexican species, is much larger than all those in its sister clade (consisting of *Cardiolonchium*,

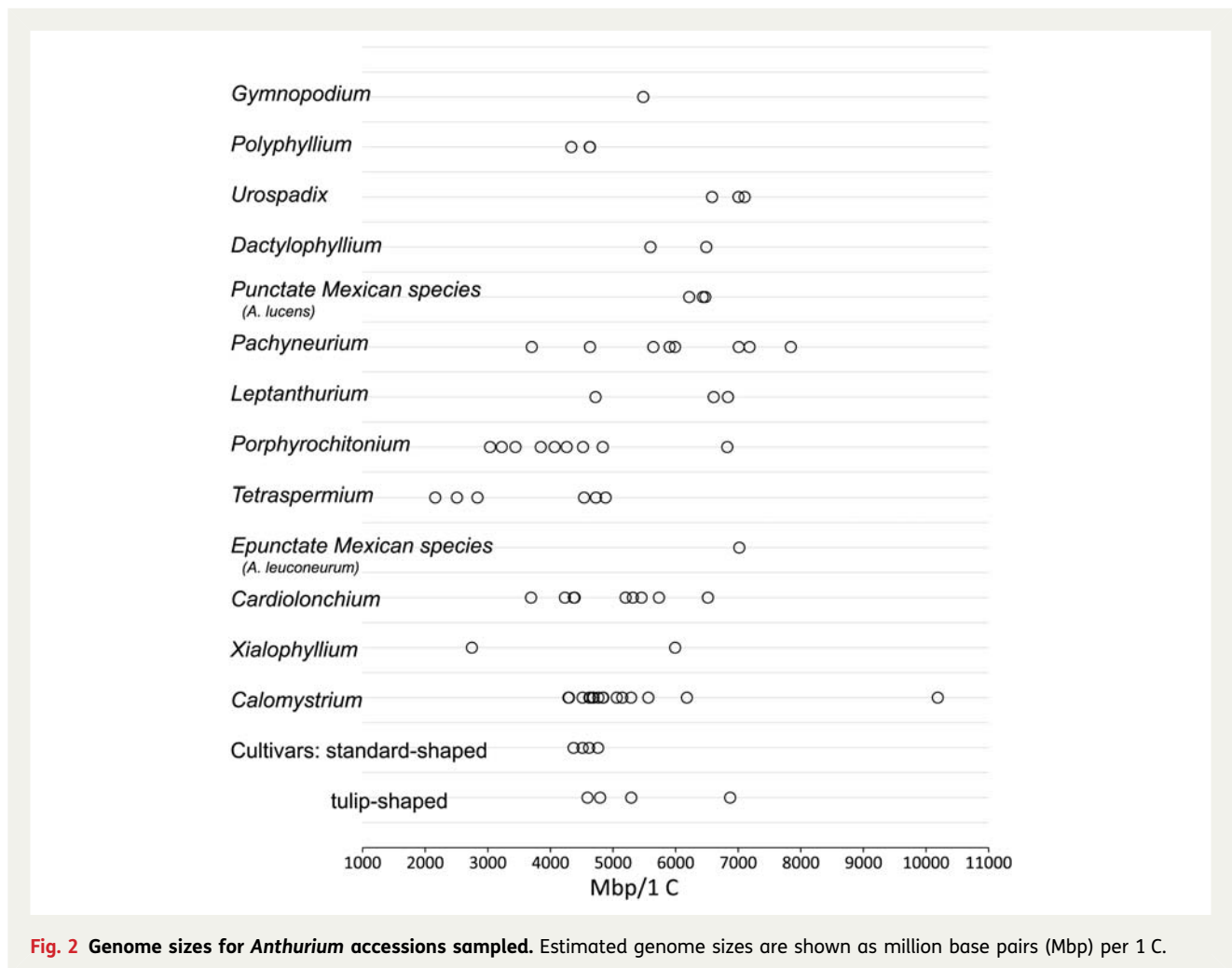


**Fig. 1** Genome size and chromosome counts of *Anthurium* species presented by subgeneric sections. Clades of punctate and epunctate Mexican species are proposed new sections (Carlsen 2011). Relative relationships of sections and estimated dates of crown group divergence are indicated at nodes, as per Carlsen (2011). Dates expressed in millions of years ago (mya). Chromosome counts for each section represent values reported for species in that section. ‘+++’ indicates supernumerary chromosomes have been observed in that section. Genome size is reported as the mean of all accessions sampled, in pg/2C ± S.D., followed by number of accessions sampled in parentheses. Superscript asterisk indicates between-sample (accession) variance >10% of the species mean.

*Calomystrium*, *Xialophyllum*) with the exception of *A. roseospadix* (Fig. 1). The 2N = 35 chromosome count report for *A. leuconeurum* has not been confirmed in over 50 years (Mookerjee 1955), although it is speculated

to have possibly included observation of 1–5 B chromosomes (Sheffer and Kamemoto 1976).

The four species sampled in section *Porphyrochitonium*, a section arising ~4.08 mya, display a more



**Fig. 2** Genome sizes for *Anthurium* accessions sampled. Estimated genome sizes are shown as million base pairs (Mbp) per 1 C.

than two-fold range in genome sizes (6.21–13.96 pg/2 C). Supernumerary chromosomes have been reported in *A. bakeri*, the only species sampled from section *Porphyrochitonium* varying from  $2N = 30$ . In section *Tetraspermium*, sister clade to *Porphyrochitonium*, *A. obtusum* and *A. scandens* ssp. *pusillum* have similar genome sizes, with one accession of *A. obtusum* having the smallest genome size (4.42 pg/2 C) reported to date in *Anthurium* (Fig. 1, Table 1). *Anthurium obtusum* has been reported as having  $2N = 30$ , and also as  $2N = 24$ , suggesting a ready loss of six chromosomes, or a 20% decrease. The genome sizes of the two accessions for this species differ by ~25% (Table 1, Fig. 1), suggesting that our accessions may have had the different numbers of chromosomes reported for this species. *Anthurium scandens* ssp. *pusillum* has been reported as having  $2N = 24$ . A polyploid event in *A. scandens* may be responsible for the  $2N = 48$

chromosomes found in *A. scandens* ssp. *scandens*. A different loss of six chromosomes appears evident in the  $2N = 84$  variant of *A. scandens* ssp. *scandens*, which could arise by the *A. scandens* ssp. *scandens*  $2N = 48$  cytotype losing six chromosomes to  $2N = 42$ , followed by a polyploidization event to yield the observed  $2N = 84$ .

The mean genome sizes of the three species in section *Pachyneurium* (estimated divergence 5.8 mya) range from 8.52 to 15.36 pg/2 C (Fig. 1). Relatively wide intraspecific variance is observed between accessions in *A. watermaliense*, which varied ~20% from the mean, approaching the intraspecific genome size changes that correlate with chromosome count changes in *A. scandens* of section *Tetraspermium*. However, supernumerary chromosome are the only cytotypic changes reported to occur in section *Pachyneurium*, suggesting that the genome size differences between different





**Fig. 3 Images of *A. andraeanum* and related cultivars.** (A) *A. andraeanum* Linden (used with permission of Michael Wenzel), (B) *A. andraeanum* Hort. cv. 'New Paho Red', (C) *A. andraeanum* Hort. cv. 'Marian Seefurth', (D) *A. andraeanum* Hort. cv. 'Midori', (E) *A. andraeanum* Hort. cv. 'Puanani', (F) *A. andraeanum* Hort. cv. 'Shibori', (G) *Anthurium* Hort. 'Princess Aiko', (H) *Anthurium* Hort. 'Regina' and (I) *Anthurium* Hort. 'Miss June Purple.'

accessions observed here might be related to the presence of extra-chromosomal DNA, or that somatic changes in the accessions sampled may have been extensive.

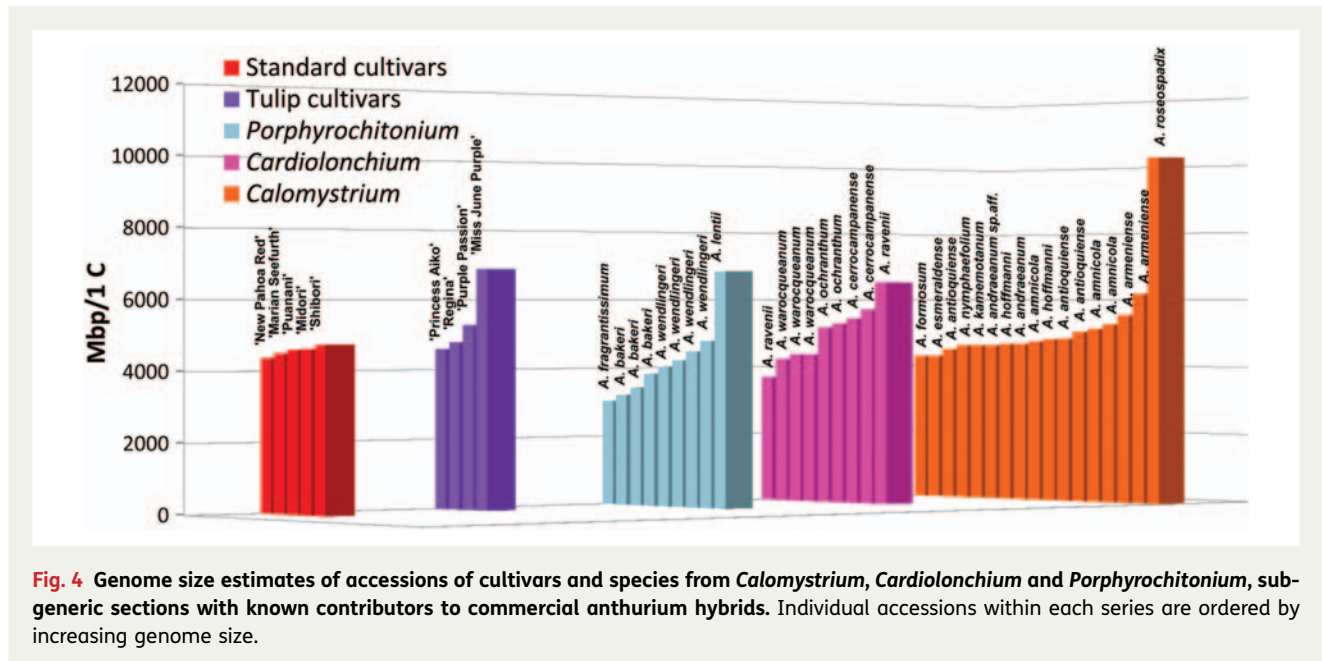
The species sampled of section *Leptanthurium*, section *Dactylophyllium*, and *A. lucens*, representing a newly described (Carlsen 2011) and yet unnamed clade of punctate Mexican species, had relatively larger genome sizes (Table 1, Figs 1 and 2). In section *Leptanthurium*, one of the three different accessions sampled of *A. gracile* has a genome size nearly 30% smaller than the other two, which are very similar, suggesting that we sampled two accessions having the same cytotype and a third accession having a different one.

### Genome size and phenotype

Species contributing to the pedigree of cultivars were expected to be reflected in the genome sizes of those

cultivars. *Anthurium andraeanum* hybridizes most easily with *A. amnicola*, *A. antioquiense*, *A. armeniense*, *A. formosum*, *A. hoffmanni*, *A. kamemotoanum*, *A. lindenianum*, *A. nymphaefolium* and *A. roseospadix*, all members of section *Calomystrium* (Kamemoto and Kuehnle 1996). Of these, *A. lindenianum* was not available for sampling. The cultivars 'Marian Seefurth', 'Midori', 'New Paho Red', 'Puanani' and 'Shibori' all share the 'standard' blistered, heart-shaped spathe of *A. andraeanum* (Fig. 3A–F) and lack any documented contribution by species other than *A. andraeanum*. Genome sizes for those five cultivars (Table 1, Fig. 2) are similar to those of most members of the *Calomystrium* series (Figs 2 and 4), but only the species *A. andraeanum* (Fig. 3A) has a heart-shaped spathe.

The cultivars with tulip-shaped spathes (Fig. 3G–I), 'Princess Aiko', 'Regina', 'Purple Passion' (photograph not available) and 'Miss June Purple', may have



been derived from tulip-shaped species in section *Calomystrium*, or from species in other sections (*Cardiolonchium*, *Porphyrochitonium*) known to contribute to hybrids derived from *A. andraeanum* (e.g. *A. antrophyoides*, *A. ochranthum*, *A. cerrocampaense* and *A. lentii*) (Kamemoto and Kuehnle 1996). Alternatively, cultivars with tulip-shaped spathes may be derived entirely from species reproductively incompatible with *A. andraeanum* (e.g. *Anthurium wendlingeri*, *A. bakeri*, *A. scherzerianum*, *A. lancifolium*, *A. caperatum* and *A. garagaranum*). The pedigrees of the cultivars 'Aiko' and 'Regina' are in fact known, as they were developed in the University of Hawai'i, Manoa, anthurium breeding programme. The genome size of the 'Princess Aiko' cultivar (Table 1, Fig. 3G) is similar to that of the cultivars with standard-shaped spathes (Figs 2 and 4) and other accessions sampled in *Calomystrium*, reflecting its derivation from the standard cultivar 'Tatsuta Pink Obake' and the tulip-shaped section *Calomystrium* species, *A. antioquiense* (Kuehnle et al. 2004). The cultivar 'Regina' (Table 1, Fig. 3H) is derived from earlier cultivars composed of contributions from the smaller-genome-sized *Calomystrium* species (i.e. *A. amnicola*, *A. formosum*, *A. andraeanum* and *A. kamemotoanum*) (Kamemoto and Kuehnle 1996; Kuehnle et al. 2004), and the genome size of 'Regina' is consistent with that pedigree (Figs 2 and 4). The cultivar 'Purple Passion' resembles (phenotypically) no species other than *A. amnicola*, and the genome size estimate is consistent with that (Table 1, Figs 2 and 4). The cultivar 'Miss June Purple' phenotypically resembles 'Regina' in many aspects (Fig. 3H and I), but the genome size is ~43 % larger (Table 1, Fig. 4), suggesting a species

with a larger genome size in its pedigree (perhaps *A. roseospadix* or *A. lentii*), or possibly an endogenous genome size change occurred in this cultivar.

## Discussion

Genome sequencing and comparative mapping have revealed the ancient polyploid nature of angiosperms and provided an insight into the effects of polyploidy on genome evolution in plants (The Arabidopsis Genome Initiative 2000; Vision et al. 2000; Blanc and Wolfe 2004; Adams and Wendel 2005; Cui et al. 2006; Fawcett et al. 2009), while studies in *Oryza* (Wang et al. 2005), *Arabidopsis* (Lagercrantz 1998; Yogeewaran et al. 2005) and others (Wendel 2000; Leitch and Bennett 2004; Leitch et al. 2008) reveal downsizing in genomes occurring after polyploidization events. We have used genome size to gain insight into some aspects of genome evolution in *Anthurium*, a genus for which the angiosperm genome size database previously contained data only for one species and one cultivar. In this study, apparent correlation between chromosome count and genome size is only clearly evident in species found in sections *Xialophyllum* and *Tetraspermium*. Although the correlations may be incidental, their importance as clues for the various ongoing processes involved in genome evolution in *Anthurium* should not be excluded.

Genome sizes and reported chromosome counts of two species are suggestive of polyploidy events in section *Xialophyllum*. In section *Tetraspermium*, with the smallest genome sizes reported to date, genome

sizes reflect reported intraspecific variations in chromosome counts in *A. scandens* (Sheffer and Kamemoto 1976; Sheffer and Croat 1983), including polyploidy, and suggest additional, subsequent genome changes occurred. The age of genome changes such as polyploidy influences the confidence with which they can be identified since subsequent mutations tend to obscure the original event (Doyle and Egan 2009). Although the ages of polyploidy events discussed here are not known, they are maximally the ages of the relatively young crown groups, *Xialophyllum* and *Tetraspermium*, to which they belong, which are estimated to have arisen 2.2 and 3.43 mya, respectively. In sections other than *Xialophyllum* and *Tetraspermium*, our data display incongruity between interspecific and intraspecific genome size and chromosome counts reported by others for *Anthurium* and other genera.

In sections *Calomystrium*, *Cardiolonchium*, *Porphyrochitonium* and *Pachyneurium*, we report interspecies genome size variation without any apparent relationship between genome size and base chromosome number, suggesting that the size difference may be unrelated to a polyploidization event. Therefore, interspecies genome size variation in these sections, particularly in the youngest crown group, *Calomystrium*, suggests a mechanism of genome size change capable of producing large differences in a short span of time. Transposable elements are capable of producing such changes. Up to 80% of the current *Z. mays* genome is composed of retroelements, most inserted in the last 1–3 million years (Rabinowicz and Bennetzen 2006). Transposable elements may be deleted after initial amplification (Shirasu et al. 2000), or may persist and play a part in local adaptation, as exemplified by the intraspecific expansion of BARE-1 retroelements in barley in response to elevation and aridity (Kalendar et al. 2000). In *Arabidopsis* and *Oryza*, genome size variations are associated with changes in repetitive DNA content occurring in the last 3 million years (Bennetzen et al. 2005). Considering *Calomystrium* is estimated to have arisen ~1.5 mya, genome changes due to rapid invasion and evolution of repetitive elements may play a role in genome size differences. However, the timeframe for comparable changes to occur in *Anthurium*, a long-lived tropical perennial, may be different than that of the annuals *Zea* spp., *Arabidopsis* spp. and *Oryza* spp.

Some genome size changes in these sections may be attributable to DNA changes associated with chromosomal reorganization (Kalendar et al. 2000). Chromosome reorganization in *Anthurium* was reported by Marutani et al. (1993), who detected differences in the karyotypes of *A. nymphaefolium* (*Calomystrium*) and *A. ochranthum* (*Cardiolonchium*), which she proposed to

have resulted from chromosomal rearrangement. She also observed very similar karyotypes among closely related species in section *Calomystrium*, noting that the *A. roseospadix* karyotype resembled those of *A. kamemotoanum* and *A. formosum* (Marutani et al. 1993). This is particularly interesting given that the genome size estimate for *A. roseospadix* is more than twice that of each of the other two, suggesting that this may be an example where similar karyotypes among related species may be composed of chromosomes of different structure or DNA mass. However, the cytotype of the *A. roseospadix* accession we sampled would have to be determined before further inferences can be made.

In *Polyphyllum*, the oldest crown group in *Anthurium*, we have a case of extreme differences between reported chromosome count and expected potential genome size, with reported ploidy difference between two species with measured genome sizes that were essentially the same. As ploidy differences within other *Anthurium* species do exist, it is possible that we may have sampled a previously unreported cytotype, as is suspected for *A. ravenii* in section *Cardiolonchium*. However, if verified, this apparent incongruity between chromosome count and measured genome size allows us to consider a loss or gain of bulk nuclear DNA and permits inferences based on evidence from mechanisms of genome evolution elucidated by studies in other species. For example, in rice, Wang et al. (2005) estimate that 35–60% of duplicated genes were lost shortly after genome size expansion as recently as 5 mya. In *Arabidopsis*, Lagercrantz (1998) estimated ~90 chromosomal rearrangements since *Arabidopsis* and *Brassica* diverged ~14–24 mya, and Yogeewaran et al. (2005) estimated ~10 chromosomal rearrangements occurred in the divergence of *Arabidopsis thaliana* and *Arabidopsis lyrata* ~5 mya. Carlsen (2011) estimated the crown group *Polyphyllum* arose ~11 mya, well within the time required to accomplish the scope of chromosomal changes as observed in the genus *Arabidopsis*.

The polyploid origin of *A. clidemioides* is unknown. However, polyploids arising by interspecies hybridization (allopolyploidy) are subject to mismatch repair during recombination of homeologous chromosomes which may generate large-scale deletions contributing to chromosome loss and reorganization (Leitch and Bennett 2004). The more broadly applicable mechanisms of ongoing unequal recombination and illegitimate recombination of homologous chromosomes also contribute to genome size reduction (Shirasu et al. 2000; Devos et al. 2002), in part by double-strand break repair, an essential but error-prone housekeeping function causing increases, decreases and chromosomal

reorganizations, which can lead to chromosome loss (Gorbunova and Levy 1999; Kirik *et al.* 2000). Although the suppositions are intriguing, the disparity between measured genome size and reported chromosome count of *A. flexile* compared with *A. clidemioides* warrants further investigation. It was not possible to evaluate cytotypes for the accessions included in this study. Most samples were contributed by botanical gardens, and thus we did not have the plants available locally for fresh root tip sampling and cytotype determination.

The genus *Anthurium* displays considerable flexibility of nuclear DNA quantity and organization, even within species. We report here different genome sizes for different accessions of *A. ravenii*, *A. watermaliense* and *A. gracile* varying >20% from the mean for the species. Conceptually, intraspecies variation can be viewed as incipient interspecies genome size variation (Greilhuber 1998). Once, genome size seemed to offer promise for delineating species (Ohri 1998; Ghosh *et al.* 2001), so reports of intraspecies genome size variations have been scrutinized to identify and eliminate systematic sources of variation, leading to standardization of methods, attention to detail in sample handling and careful selection of internal standards (Greilhuber 1998, 2005). Still, intraspecies variations persist: approximately 10% of *Curcuma* species sampled displayed intraspecies variation in genome size estimates (2007), while both genome sizes and ploidy levels varied widely in a survey of 244 *Dianthus broteri* individuals collected from 25 populations (Balao *et al.* 2009), similar to results reported here for *A. scandens*. While variant cytotypes may explain the largest differences observed, a lesser amount of intraspecific variation in bulk nuclear DNA content may be attributed to the presence of extra-chromosomal material, which can only be convincingly excluded from genome size estimates by determining the cytotype of each accession sampled (Teoh and Rees 1976). In particular, the origin and evolution of B chromosomes seems to be associated with amplification of tandem repeats on A chromosomes, and can be generated spontaneously following allopolyploidization (Jones and Houben 2003). It may be that activity of extra-chromosomal DNA in sections *Calomystrium*, *Cardiolonchium*, *Porphyrochitonium* and *Pachyneurium* has contributed to the range of genome sizes among accessions of the same species in those sections.

Furthermore, *Anthurium* cultural practices, including *in vitro* cultivation, clonal propagation and selection for sports, impose extreme selective pressures, capable of activating transposable elements causing intraspecies genome size variations without imposing a reproductive barrier (Peschke and Phillips 1991; Hirochika *et al.* 1996). Indeed, individual cultivars may be selected for

phenotypes associated with transposable element activity which has affected genome size, but has more noticeably affected phenotype. For example, variegated cultivars of maize (McClintock 1965–1966), *Antirrhinum* (snapdragon) (Coen and Carpenter 1986), *Convolvulus* (morning glory) (Hoshino *et al.* 1995), *Dahlia* (Ohno *et al.* 2011) and *Sorghum* carry transposable elements associated with variegation (Chopra *et al.* 1999), and it may be that the mottled ‘Shibori’ cultivar (Figs 3I and 4), with a slightly larger genome than that of the other standard cultivars, is accomplishing its variegation by similar means.

## Conclusions and forward look

Genome sizes in *Anthurium* display variation suggestive of repeated polyploidy, with evidence for possible re-diploidization in the oldest crown group *Polyphyllium*, and ongoing expansion in the youngest crown group, *Calomystrium*. *Anthurium* genome size distribution was not distinctly demarcated by ploidy level, as Leong-Škorničková *et al.* (2007) similarly reported in an analysis of nearly half the *Curcuma* (Zingiberales) species found on the Indian continent. Also, as in *Curcuma*, we found genome size to be useful, together with phenotypic similarities, for insight into the pedigree of cultivars (Leong-Škorničková *et al.* 2007). The new information on genome sizes in *Anthurium* will serve as a useful framework from which to launch molecular investigations including map- and sequence-based studies, which may provide further insight into the processes resulting in genome size variation observed in *Anthurium*, which may be similar to those described in other monocots.

## Additional information

The following additional information is available in the online version of this article –

File 1. Genome size estimates for 26 *Anthurium* accessions evaluated with two internal standards.

File 2. References for cytological observations summarized for *Anthurium* species sampled.

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## Contributions by the authors

B.J.B. and J.Y.S. designed the study. B.J.B. coordinated sampling and genome sizing, analysed data, organized figures and authored the manuscript. J.Y.S. supported

the research and contributed to the development and revision of the manuscript.

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## Conflict of interest statement

None declared.

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