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# Evolutionary trends in Iridaceae: new cytogenetic findings from the New World

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With the present work, we aim to provide a better understanding of chromosome evolutionary trends among southern Brazilian species of Iridoideae. Chromosome numbers and genome sizes were determined for 21 and 22 species belonging to eight genera of Tigridieae and two genera of Trimezieae, respectively. The chromosome numbers of nine species belonging to five genera are reported here for the first time. Analyses of meiotic behaviour, tetrad normality and pollen viability in 14 species revealed regular meiosis and high meiotic indexes and pollen viability (> 90%). The chromosome data obtained here and compiled from the literature were plotted onto a phylogenetic framework to identify major events of chromosome rearrangements across the phylogenetic tree of Iridoideae. Following this approach, we propose that the ancestral base chromosome number for Iridoideae is  $x = 8$  and that polyploidy and dysploidy events have occurred throughout evolution. Despite the variation in chromosome numbers observed in Tigridieae and Trimezieae, for these two tribes our data provide support for an ancestral base number of  $x = 7$ , largely conserved in Tigridieae, but a polyploidy event may have occurred prior to the diversification of Trimezieae, giving rise to a base number of  $x_2 = 14$  (detected by maximum-parsimony using haploid number and maximum likelihood). In Tigridieae, polyploid cytotypes were commonly observed ( $2x$ ,  $4x$ ,  $6x$  and  $8x$ ), whereas in Trimezieae, dysploidy seems to have been the most important event. This feature is reflected in the genome size, which varied greatly among species of Iridoideae, 4.2-fold in Tigridieae and 1.5-fold in Trimezieae. Although no clear difference was observed among the genome sizes of Tigridieae and Trimezieae, an important distinction was observed between these two tribes and Sisyrinchieae, with the latter possessing the smallest genome sizes in Iridoideae.

**ADDITIONAL KEYWORDS:** chromosome evolution – chromosome number – DNA content – dysploidy – polyploidy.

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## INTRODUCTION

Iridaceae (Asparagales; APG III, 2009) are a monocot family composed of 65–75 genera and > 2030 species divided into seven monophyletic subfamilies (Goldblatt *et al.*, 2008). Among these subfamilies, Iridoideae is the only one with representatives in the Neotropics, the second most important centre of diversity for Iridaceae, with 900 known species in 30 genera (Goldblatt & Manning, 2008). In South America, three of the five tribes of Iridoideae (Sisyrinchieae, Tigridieae and Trimezieae) are represented (Goldblatt & Manning, 2008; Goldblatt *et al.*, 2008). The 165 native species of Iridoideae in Brazil, including those endemic to the region, belong to 18 genera, 12 occurring in southern Brazil (Eggers *et al.*, 2014): *Calydorea* Herb., *Catila* Ravenna, *Cypella* Herb., *Eleutherine* Herb., *Gelasine* Herb., *Herbertia* Sweet,

*Kelissa* Ravenna, *Onira* Ravenna, *Phalocallis* Herb. (Tigridieae); *Neomarica* Sprague and *Trimezia* Salisb. ex Herb. (Trimezieae); and *Sisyrinchium* L. (Sisyrinchieae) (Fig. 1).

Iridoideae, as in other subfamilies of Iridaceae, have diverse karyotypes, with polyploidy and dysploidy playing an important role in karyotype diversification (Goldblatt & Takei, 1997). Iridaceae are cytologically well known, with chromosome numbers determined for > 1330 species (almost 65% of the species in the family), although most records are from the Northern Hemisphere and Africa (Goldblatt, 1982; Kenton & Heywood, 1984; Rudall, Kenton & Lawrence, 1986; Goldblatt & Takei, 1997). Chromosome data from South American species are scarce (Goldblatt, 1982; Kenton, Rudall & Johnson, 1986), particularly for natural populations (Alves, Lima & Felix, 2011; Souza-Chies *et al.*, 2012; Tacuatiá *et al.*,



**Figure 1.** Iridaceae flowers. A, *Calydorea campestris*; B, *C. crocoides*; C, *Cypella fucata*; D, *C. hauthalii* subsp. *opalina*; E, *Gelasine coerulea*; F, *Herbertia pulchella*; G, *H. quareimana*; H, *H. zebrina*; I, *Kelissa brasiliensis*; J, *Onira unguiculata*; K, *Phalocallis coelestis*; L, *Trimezia spathata*. All photographs were taken by A. P. Moraes, except D, H and K taken by L. Eggers. Bar in K and L represents 1 cm.



2012), and this has hampered any comprehensive analysis of chromosome evolution in subfamily Iridoideae. Furthermore, although the analysis of chromosome data in a phylogenetic context is essential for understanding chromosome evolution and its importance in species diversification, such studies have not been carried out in Iridaceae to date.

Despite the numerous species that have been analysed in this family, the base chromosome number is still uncertain, probably due to frequent chromosomal rearrangements throughout the family. Goldblatt (1990) suggested  $x = 10$ , with polyploidy associated with dysploidy giving rise to derived numbers such as  $x_2 = 14, 12, 11, 9, 8, 7$  and  $5$  (Goldblatt & Takei, 1997). In addition to mitotic chromosome number, meiotic stability, tetrad normality (meiotic index) and pollen grain viability from natural populations are important characteristics that provide insight into the reproductive success of natural populations. Through an analysis of meiosis, chromosome number and chromosome behaviour are observed, and the morphology and viability of pollen grains can also be evaluated. Such analyses have been largely neglected in species of Iridaceae.

The nuclear DNA content, or genome size (GS or 2C), plays a role during species evolution (Bennett & Leitch, 2005) and has become a valuable trait in biosystematics (Šmarda, Bureš & Horová, 2007; Bennett & Leitch, 2011; Pellicer *et al.*, 2013, 2014). However, despite its importance, genome sizes have been estimated for only *c.* 1.8% of all angiosperm species (Bennett & Leitch, 2012). Specifically in Iridaceae, DNA content has been reported for *c.* 150 out of 2030 species (7.4%), with 2C values ranging from 0.96 to 62.76 pg (Siljak-Yakovlev *et al.*, 2010; Bennett & Leitch, 2012; Bou Dagher-Kharrat *et al.*, 2013; Pustahija *et al.*, 2013). In Iridoideae, no data are available for 42 species of Trimezieae (Goldblatt *et al.*, 2008), and the GS values of just 12 of 1725 species (Goldblatt *et al.*, 2008) of Tigridieae (0.69%) have been reported: two *Cypella* spp. and three species of *Hesperoxiphion* Baker (2C values ranging from 2.48 to 8.76 pg; Kenton *et al.*, 1990), *Kelissa brasiliensis* (Baker) Ravenna (2C = 17.50 pg), *Gelasine elongata* (Graham) Ravenna (= *G. azurea* Herb.) (2C = 3.50 pg), *Cipura paludosa* Aubl. (2C = 16.9 pg) and four species of *Tigridia* Juss. (from 7.75 to 16.20 pg) (Zonneveld, Leitch & Bennett, 2005; Bennett & Leitch, 2012). Concerning Sisyrinchieae, most reports are for *Sisyrinchium* L., with 33 estimates of 2C ranging between 1.0 and 8.4 pg (Bennett & Leitch, 2012).

Given the paucity of karyotype information about species of Iridoideae from southern Brazil, the present work was undertaken to increase our knowledge concerning (1) chromosome number and ploidy, (2) genome size and (3) meiotic behaviour, meiotic index and pollen

viability of species of Iridoideae. Our data, combined with information taken from the literature, have been analysed in a phylogenetic framework (Chauveau *et al.*, 2012) to understand chromosome evolution better and reconstruct the ancestral base chromosome number in Iridoideae, with special attention paid to Tigridieae and Trimezieae.

## MATERIAL AND METHODS

Most samples were collected in southern Brazil between 2006 and 2009 (Table 1). Part of the studied material is held in the Botanical Garden of Porto Alegre, Fundação Zoobotânica do Rio Grande do Sul, Brazil. Flower buds, seeds and bulbs were collected for chromosome studies and fresh leaves for flow cytometry analyses. Vouchers were deposited in the herbarium ICN (acronym according to Thiers, 2014). Scientific names follow Chauveau *et al.* (2012).

### MITOTIC ANALYSIS

Mitotic analyses were performed using root meristems and ovary walls. Materials were pre-treated with 8-hydroxyquinoline (2 mM) for 24 h at 10 °C, fixed in absolute ethanol/glacial acetic acid (3:1, v/v) for 24 h at room temperature and stored at -20 °C. For squash chromosome preparation, roots were hydrolysed in 5 M HCl for 20 min at room temperature and macerated in a drop of 45% acetic acid. Slides were frozen in liquid nitrogen and air dried. The best slides were stained in 2% Giemsa and mounted in Entellan (Merck). Alternatively, some slides were prepared following the standard Feulgen method. All observations were conducted using a Zeiss Axioplan Universal photomicroscope.

### MEIOTIC ANALYSIS

Collected flower buds were fixed in ethanol/glacial acetic acid (3:1, v/v) for 24 h at room temperature and kept thereafter at -20 °C. Anthers were dissected, and pollen mother cells were mounted on a slide in a drop of 60% acetic acid. Slides were frozen in liquid nitrogen and air dried. The best slides were stained with 2% Giemsa and mounted in Entellan. Meiotic behaviour was evaluated by analysing the first 500 pollen mother cells observed. All available phases of meiosis I and II were analysed. Abnormalities, such as non-orientated bivalents and multivalents in metaphase I, bridges and laggards in anaphase and telophases I and II, were evaluated. Meiotic indexes were calculated using the formula: MI = (number of normal tetrads observed/total number of tetrads observed) × 100. Microcytes and micronuclei, bridges,

**Table 1.** Species investigated in Tigridieae and Trimezieae tribes of Iridaceae, voucher specimen and geographical origin

Taxa	Collector number	Origin [State, City]	Latitude	Longitude
<b>Tigridieae (Clade A)</b>				
<i>Calydorea</i> Herb.				
<i>C. alba</i> Roitman & J.A.Castillo	ESC 519	RS, Uruguaiiana	-29.909	-57.150
<i>C. approximata</i> R.C.Foster	ESC 542	RS, Santo Antônio das Missões	-28.493	-55.401
<i>C. basaltica</i> Ravenna	ESC 390	PR, Palmas	-26.585	-51.867
<i>C. campestris</i> Baker	ESC 324	PR, Tijucas do Sul	-25.836	-49.135
	ESC 330	PR, Balsa Nova	-25.451	-49.737
	ESC 343	PR, Jaguariaíva	-24.354	-49.806
	ESC 357	PR, Balsa Nova	-25.465	-49.748
<i>C. crocoides</i> Ravenna	ESC 218	RS, São José dos Ausentes	-28.802	-49.950
	IRI 118	RS, São José dos Ausentes	-28.748*	-50.068*
<i>Catila amabilis</i> Ravenna	Eggers 697	RS, Uruguaiiana	-29.728*	-56.742*
<i>Cypella</i> Herb.				
<i>C. fucata</i> Ravenna	ESC 295	RS, Viamão	-30.364	-51.022
	ESC 442	RS, Pinheiro Machado	-31.518	-53.519
	ESC 444	RS, Piratini	-31.439	-53.215
<i>C. hauthalii</i> subsp. <i>opalina</i> Ravenna	ESC 541	RS, Santo Antônio das Missões	-28.416	-55.020
<i>C. herbertii</i> Herb.	ESC 453	RS, Porto Alegre	-30.068	-51.120
	ESC 490	RS, São Gabriel	-30.326	-54.372
	ESC 547	RS, São Borja	-28.685	-55.937
<i>C. laxa</i> Ravenna	ESC 559	RS, São Pedro do Sul	-29.634	-54.268
<i>Herbertia</i> Sweet				
<i>H. crosae</i> Roitman & J.A.Castillo	ESC 528	RS, Uruguaiiana	-30.043	-57.250
<i>H. darwinii</i> Roitman & J.A.Castillo	ESC 502	RS, Santana do Livramento	-30.874	-55.481
	ESC 548	RS, São Borja	-28.894	-55.654
<i>H. lahue</i> (Molina) Goldblatt	ESC 162	RS, Taquara	-29.689	-50.816
	ESC 466	RS, Caçapava do Sul	-30.832	-53.503
	ESC 488	RS, São Gabriel	-30.326	-54.372
	ESC 494	RS, Santana do Livramento	-30.804	-55.263
	ESC 495	RS, Santana do Livramento	-30.803	-55.261
	ESC 504	RS, Santana do Livramento	-30.888	-55.475
	ESC 521	RS, Barra do Quaraí	-30.188	-57.469
	ESC 535	RS, Entre-Ijuís	-28.455	-54.398
<i>H. pulchella</i> Sweet	ESC 279	RS, São Lourenço do Sul	-31.372	-52.098
	ESC 446	RS, Piratini	-31.282	-53.050
	IRI 31	RS, Porto Alegre	-30.025	-51.213
	TLSALVES168	RS, Caçapava do Sul	-30.695	-53.392
<i>H. quareimana</i> Ravenna	ESC 513	RS, Quaraí	-30.195	-56.488
	ESC 520	RS, Uruguaiiana	-29.909	-57.150
	IRI 36	RS, Quaraí	-30.275*	-56.119*
<i>H. zebrina</i> Deble	ESC 179	RS, Encruzilhada do Sul	-30.519	-52.695
	ESC 421	RS, Encruzilhada do Sul	-30.517	-52.696
	IRI 92	RS, Dom Feliciano	-30.700	-52.103
<i>Kelissa brasiliensis</i> (Baker) Ravenna	ESC 467	RS, Caçapava do Sul	-30.832	-53.503
	IRI 38	RS, Caçapava do Sul	-30.589*	-53.478*
<i>Onira unguiculata</i> (Baker) Ravenna	ESC 273	RS, Rio Grande	-31.985	-52.293
	ESC 500	RS, Santana do Livramento	-30.874	-55.481
<b>Tigridieae (Clade B)</b>				
<i>Gelasine</i> Herb.				
<i>G. coerulea</i> (Vell.) Ravenna	ESC 335	PR, Jaguariaíva	-24.203	-49.647
<i>Phalocallis</i> Herb.				
<i>P. coelestis</i> (Lehm.) Ravenna	IRI 78	RS, São Lourenço do Sul	-31.216	-52.150*

Table 1. Continued

Taxa	Collector number	Origin [State, City]	Latitude	Longitude
<b>Trimezieae</b>				
<i>Neomarica</i> Sprague				
<i>N. caerulea</i> (Ker Gawl.) Sprague	IRI 02†			
<i>N. candida</i> (Hassl.) Sprague	IRI 04†			
	IRI 43	RS, Dom Pedro de Alcântara	–29.363*	–49.853*
<i>Neomarica</i> sp.	ESC 338	PR, Sengés	–24.061	–49.521
<i>Trimezia</i> Salisb. ex Herb.				
<i>T. spathata</i> Baker	ESC 368	PR, Guarapuava	–25.367	–51.334
	IRI 09	RS, Derrubadas	–27.264*	–53.865*
	IRI 12	RS, Julio de Castilhos	–29.230*	–53.677*
	IRI 61	RS, Lajeado	–29.451*	–52.010*
	TS (no number)‡	RS, Caçapava do Sul	–30.589*	–53.478*

ESC, Eggers, L. & Souza-Chies, T.T.; IRI, Porto Alegre Botanical Garden collection; RS, Rio Grande do Sul; PR, Paraná; SC, Santa Catarina.

\*Municipality geographical coordinates obtained from GoogleEarth.

†Without data of origin and in cultivation at Botanical Garden.

‡No number available, live specimen in cultivation, awaiting voucher.

and unequally sized cells were considered abnormal. Chromosome numbers were determined in diakinesis (prophase I).

#### POLLEN STAINABILITY

Pollen stainability and pollen morphology were used to assess pollen viability. Flowers at anthesis were collected, fixed and stored as described above. Slides were prepared following Alexander's method (Alexander, 1980), in which empty, non-viable pollen grains stain green, and full, viable pollen grains stain purple. Samples of 500 pollen grains per flower were analysed from at least five individuals per species.

#### GENOME SIZE ESTIMATION

Total DNA content was assessed by flow cytometry according to Marie & Brown (1993) and Doležal *et al.* (1998). *Petunia hybrida* Vilm. 'PxPc6' (2C = 2.85 pg; Marie & Brown, 1993) and *Solanum lycopersicum* 'Stupicke' (1.96 pg; Doležal *et al.*, 1998) were used as internal standards. Details about which standard species was used for each species of Iridaceae analysed are available upon request. Leaves of the studied species and the internal standards were chopped using a razor blade in a Petri dish with 600 µL Galbraith *et al.* (1983) nuclear-isolation buffer, supplemented with 10 mM sodium metabisulphite and 1% polyvinylpyrrolidone 10 000. The suspension was passed through a 50-µm mesh nylon filter (Cell-Trics, Partec) and nuclei were stained with 50–70 µg mL<sup>-1</sup> propidium iodide with RNase (2.5 U mL<sup>-1</sup>) at 4 °C. The DNA content of 5000–10 000 stained

nuclei was determined for each sample using an Elite ESP flow cytometer (Beckman-Coulter) with an argon 488-nm laser, a Partec CyFlow 532-nm laser cytometer or a FACSCalibur 488-nm laser (Becton, Dickinson). The total 2C DNA value was calculated using the linear relationship between the fluorescent signals from the stained nuclei of the unknown specimen and the internal standard. Generally, three or five samples were assessed for each taxon and the standard deviation (SD) was calculated. However, for seven species [*Calydorea campestris* Baker, *C. crocoides* Ravenna, *Catila amabilis* Ravenna, *Herbertia zebrina* Deble, *Phalocallis coelestis* (Lehm.) Ravenna, *Neomarica caerulea* (Ker Gawl.) Sprague and *Neomarica candida* (Hassl.) Sprague] only one individual was available, and no SD could be calculated. The term 'monoploid genome size' (1Cx) was used to represent the DNA content in a basic chromosome set (*x*) of a somatic cell (Greilhuber, 2005), whereas 2C refers to the whole GS of a somatic cell.

#### CHROMOSOME EVOLUTION

The updated chromosome numbers described for Iridoideae were compiled from the literature and the IPCN (Index to Plant Chromosome Numbers – Missouri Botanical Garden; <http://www.tropicos.org/Project/IPCN>) (Table 2). For each taxon, the haploid (*n*) and base chromosome numbers (*x*) were recorded. Following standard conventions, throughout this paper, we refer to 'x' as the ancestral base chromosome number of a lineage, *x*<sub>2,3</sub> ... as the derived secondary, tertiary (... ) base chromosome number of a

**Table 2.** Chromosome numbers, ploidy levels, 2C DNA content (pg and Mbp) and monoploid genome sizes (1Cx value) of the analysed species and other Iridoideae

Taxa	2n (ploidy level)	2C (pg)	1Cx (pg)	2C (Mbp)	Reference(s)
<b>Tigridaeae (15 / 1725)</b>					
<b>Clade A</b>					
<i>Ainea conzatti</i> (R.C.Foster) Ravenna: (21 / 2n = 14, 28, 42 / x = 7 / 2.29–4.17)	28 (4x)				AOC12
<i>Calydorea</i> Herb: (21 / 2n = 14, 28, 42 / x = 7 / 2.29–4.17)					
<i>C. alba</i> Roitman & A. Castillo	<b>14 (2x)</b>	<b>8.34</b>	<b>4.17</b>	<b>8160</b>	PW
<i>C. approximata</i> R.C.Foster	<b>14 (2x)</b>	<b>4.58</b>	<b>2.29</b>	<b>4480</b>	PW
<i>C. azurea</i> Klatt	28 (4x)	–	–	–	GT97
<i>C. basaltica</i> Ravenna	<b>14 (2x)</b>	<b>7.72</b>	<b>3.86</b>	<b>7550</b>	PW
<i>C. campestris</i> Baker	<b>14 (2x)</b>	<b>6.96</b>	<b>3.48</b>	<b>6810</b>	PW
<i>C. crocoides</i> Ravenna	<b>14 (2x)</b>	<b>6.90</b>	<b>3.45</b>	<b>6750</b>	PW
<i>C. nuda</i> Baker	14 (2x)	–	–	–	G82
<i>C. pallens</i> Griseb.	28 (4x)	–	–	–	GT97
<i>C. undulata</i> Ravenna	14 (2x)	–	–	–	TRB08
<i>C. xiphioides</i> (Poepp.) Espinosa	42 (6x)	–	–	–	GT97
<i>Catila amabilis</i> Ravenna: (1/ 2n = 14 / x = 7 / 2.04)	14 (2x)	<b>4.08</b>	<b>2.04</b>	<b>3990</b>	GT97, R83, PW
<i>Cipura</i> Aubl. (8 / 2n = 14, 28 / x = 7 / 4.22)					
<i>C. paludosa</i> Aubl.	14 (2x)	16.9	4.22	16528	A07, A11, BG90, G82, GT97, GWZ84, S70
	28 (4x)	–	–	–	G82, GWZ84
<i>C. xanthomelas</i> Mart. ex Klatt	28 (4x)				A11
<i>Cypella</i> Herb.: (21 / 2n = 10, 14 / x = 5, 7 / 1.95–2.41)					
<i>C. aquatilis</i> Ravenna	14 (2x)	4.78	2.39	4674	K90, R81
<i>C. fucata</i> Ravenna	14 (2x)	–	–	–	GT97, PW
<i>C. aff. fucata</i> Ravenna	<b>14 (2x)</b>	–	–	–	
<i>C. hauthalii</i> subsp. <i>opalina</i> Ravenna	14 (2x)	<b>4.18</b>	<b>2.09</b>	<b>4090</b>	GT97, KH84, PW
<i>C. herberti</i> Herb.	14 (2x)	<b>3.90</b>	<b>1.95</b>	<b>3810</b>	G82, GT97, PW
<i>C. laxa</i> Ravenna	–	<b>4.83</b>	<b>2.41</b>	<b>4720</b>	PW
<i>C. linearis</i> (Kunth) Baker	14 (2x)	–	–	–	G82
<i>C. plumbea</i> Lindl.	10 (2x)	–	–	–	G82
<i>Herbertia</i> Sweet: (9 / 2n = 14, 28, 42, 56 / x = 7 / 1.93–2.34)					
<i>H. crosae</i> Roitman & J.A.Castillo	14 (2x)	<b>4.13</b>	<b>2.07</b>	<b>4040</b>	RC04, PW
<i>H. darwinii</i> Roitman & J.A.Castillo	14 (2x)	<b>4.60</b>	<b>2.30</b>	<b>4500</b>	M09, RC08, PW
<i>H. lahue</i> (Molina) Goldblatt	14 (2x)	–	–	–	W59
	28 (4x)	–	–	–	GT97, KH84, W59
	42 (6x)	<b>11.61</b>	<b>1.93</b>	<b>11360</b>	GT97, KH84, PW
	56 (8x)	<b>15.43</b>	<b>1.93</b>	<b>15090</b>	GT97, M09, PW
<i>H. pulchella</i> Sweet	14 (2x)	–	–	–	GT97, KH84, W59
	<b>28 (4x)</b>	<b>8.60</b>	<b>2.15</b>	<b>8410</b>	
	42 (6x)	–	–	–	GT97, KH84
<i>H. quareimana</i> Ravenna	28 (4x)	<b>8.70</b>	<b>2.17</b>	<b>8510</b>	GT97, M09, PW
<i>H. zebrina</i> Deble	<b>14 (2x)</b>	<b>4.69</b>	<b>2.34</b>	<b>4590</b>	PW
<i>Kelissa</i> Ravenna (1 / 2n = 14, 56 / x = 7 / 2.16–2.36)					
<i>K. brasiliensis</i> (Baker) Ravenna	<b>14 (2x)</b>	<b>4.73</b>	<b>2.36</b>	<b>4625</b>	PW
	56 (8x)	17.36	2.16	16978	BL12
<i>Nemastylis</i> Nutt. (7 / 2n = 14, 56 / x = 7)					
<i>N. floridana</i> Small	56 (8x)	–	–	–	G75
<i>N. geminiflora</i> Nutt.	56 (8x)	–	–	–	G82



Table 2. Continued

Taxa	2n (ploidy level)	2C (pg)	1Cx (pg)	2C (Mbp)	Reference(s)
<i>N. tenuis</i> subsp. <i>pringlei</i> (S.Watson) Goldblatt	14 (2x)	–	–	–	G75
<i>N. tenuis</i> var. <i>nana</i> (S.Watson) R.C.Foster	14 (2x)	–	–	–	G82
<i>Onira unguiculata</i> (Baker) Ravenna: (1 / 2n = 14 / x = 7 / 2.28)	<b>14 (2x)</b>	<b>4.57</b>	<b>2.28</b>	<b>4470</b>	PW
<b>Clade B</b>					
<i>Aliphia</i> Herb.: (5 / 2n = 28 / x = 14)					
<i>A. drummondii</i> (Graham) R.C.Foster	14 (2x)+1B	–	–	–	A11
	28 (2x)	–	–	–	A11, GT97
	42 (4x)	–	–	–	A11
	56 (6x)	–	–	–	A11
<i>A. silvestris</i> (Loes.) Goldblatt	28 (2x)	–	–	–	G82
<i>A. veracruzana</i> Goldblatt & T.M.Howard	28 (2x)	–	–	–	GH82
<i>Cobana</i> Ravenna: (1 / 2n = 28 / x = 14)					
<i>C. guatemalensis</i> (Standl.) Ravenna	28 (2x)	–	–	–	GT97
<i>Eleutherine</i> Herb.: (4 / 2n = 12 / x = 6)					
<i>E. bulbosa</i> (Mill.) Urb.	12 (2x)	–	–	–	BG90, G82, G88, G91, GS91
<i>E. latifolia</i> (Standl. & L.O.Williams) Ravenna	12 (2x)	–	–	–	GS91
<i>Ennealophus</i> N.E.Br.: (5 / 2n = 14 / x = 7)					
<i>E. euryandrus</i> (Griseb.) Ravenna	14 (2x)	–	–	–	GT97
<i>E. foliosus</i> (Kunth) Ravenna	14 (2x)	–	–	–	GT97
<i>Gelasine</i> Herb.: (7 / 2n = 12, 14 / x = 6, 7 / 1.75–2.52)					
<i>G. coerulea</i> (Vell.) Ravenna	<b>14 (2x)</b>	<b>5.05</b>	<b>2.52</b>	<b>4938</b>	PW
<i>G. elongata</i> (Graham) Ravenna (cited as <i>G. azurea</i> Herb.)	12 (2x)	–	–	–	K87
	12 (2x)	3.50	1.75	3423	BL12
	14 (2x)	–	–	–	G82
<i>G. uruguayensis</i> Ravenna	14 (2x)	–	–	–	R84
<i>Hesperoxiphion</i> Baker (5 / 2n = 14 / x = 7 / 1.33–4.38)					
<i>H. herrerae</i> (Diels ex R.C.Foster) Ravenna	14 (2x)	2.65	1.33	2591	K90
<i>H. huilense</i> Ravenna	14 (2x)	8.75	4.38	8557	K90
<i>H. peruvianum</i> (Baker) Baker	14 (2x)	2.70	1.35	2640	K90
<i>Phalocallis</i> Herb.: (1 / 2n = 10 / x = 5 / 3.6)					
<i>P. coelestis</i> (Lehm.) Ravenna	10 (2x)	<b>7.21</b>	<b>3.60</b>	<b>7051</b>	GT97, R83, PW
<i>Sessilanthera</i> Molseed & Cruden: (3 / 2n = 28 / x <sub>2</sub> = 14)					
<i>S. citrina</i> Cruden	28 (2x)	–	–	–	G82
<i>S. latifolia</i> (Weath.) Molseed & Cruden	28 (2x)	–	–	–	G82
<i>Tigridia</i> Juss. (35; 2n = 28 [26?]; x = 14 / 3.88–4.75)					
<i>T. alpestris</i> Molseed	28 (2x)	7.75	3.88	7579	BL12
<i>T. durangense</i> Molseed ex Cruden	28 (2x)	16.20	8.10	15843	BL12
<i>T. ehrenbergii</i> (Schltld.) Molseed		9.50	4.75	9291	BL12
<i>T. galanthoides</i> Molseed	28 (2x)	–	–	–	G82
<i>T. hallbergii</i> Molseed	28 (2x)	–	–	–	G82
<i>T. flammaea</i> (Lindl.) Ravenna	28 (2x)	–	–	–	G82
<i>T. meleagris</i> (Lindl.) G. Nicholson	28 (2x)	–	–	–	G82
<i>T. mexicana</i> Molseed	28 (2x)	–	–	–	G82
<i>T. multiflora</i> (Baker) Ravenna	28 (2x)	–	–	–	G82
<i>T. orthantha</i> (Lem.) Ravenna	28 (2x)	–	–	–	G82

Table 2. Continued

Taxa	2n (ploidy level)	2C (pg)	1Cx (pg)	2C (Mbp)	Reference(s)
<i>T. pavonia</i> (L.f.) DC.	26 (2x)* 28 (2x)	8.70 –	4.35 –	8508 –	BL12, K02 S70
<i>T. vanhouttei</i> Roez. ex Van Houtte	28 (2x)	–	–	–	G82
<b>Trimezieae (3 / 42)</b>					
<i>Neomarica</i> Sprague: (21 / 2n = 16, 18, 22, 28, 32 / x = 8, 9, 14 / 3.80–3.96)					
<i>N. altivallis</i> (Ravenna) A.Gil	18 (2x)	–	–	–	GIL
<i>N. brachypus</i> (Baker) Sprague	18 (2x)	–	–	–	GIL
<i>N. caerulea</i> (Ker Gawl.) Sprague	18 (2x) 32 (4x)	7.92 –	– –	7750 –	A11 GIL, G82, GT97, KH84, ZBS85
<i>N. candida</i> (Hassl.) Sprague	18 (2x)	7.60	3.80	7430	A07, A11, PW
<i>N. castaneomaculata</i> A.Gil & M.C.E.Amaral	18 (2x)	–	–	–	GIL
<i>N. decora</i> (Ravenna) A.Gil	18 (2x)	–	–	–	GIL
<i>N. decumbes</i> (Ravenna) A.Gil	18 (2x)	–	–	–	GIL
<i>N. eburnea</i> A.Gil & M.C.E.Amaral	18 (2x)	–	–	–	GIL
<i>N. floscella</i> A.Gil & M.C.E.Amaral	18 (2x)	–	–	–	GIL
<i>N. glauca</i> (Seub. ex Klatt) Sprague	18 (2x)	–	–	–	A11, GIL, G82, GT97, KH84
<i>N. gracilis</i> (Herb.) Sprague	16 (2x)* 18 (2x)	– –	– –	– –	TRB08 GIL, GH92
<i>N. humilis</i> (Klatt) Capell.	18 (2x)	–	–	–	A11, GIL
<i>N. involuta</i> A.Gil & M.C.E.Amaral	18 (2x)	–	–	–	GIL
<i>N. imbricata</i> (Hand. Mazz.) Sprague	16 (2x)*	–	–	–	GIL
<i>N. itatiaica</i> (Ravenna) A.Gil	18 (2x)	–	–	–	GIL
<i>N. longifolia</i> (Link et Otto) Sprague	18 (2x)	–	–	–	GIL
<i>N. mauroi</i> A.Gil & M.C.E.Amaral	18 (2x)	–	–	–	GIL
<i>N. northiana</i> (Schneev.) Sprague	18 (2x)	–	–	–	A07, GIL, GT97, TRB08
<i>N. portosecurensis</i> (Ravenna) Chukr	18 (2x)	–	–	–	GIL
<i>N. cf. paradoxa</i> (Ravenna) Chukr	28 (2x)*	–	–	–	A11
<i>N. rupestres</i> (Ravenna) Chukr	28 (2x)	–	–	–	CG08
<i>N. sabinei</i> (Lindley) Churk	18 (2x)	–	–	–	GIL
<i>N. sancti-vicentei</i> A.Gil & M.C.E.Amaral	18 (2x)	–	–	–	GIL
<i>N. sergipensis</i> A.Gil & M.C.E.Amaral	18 (2x)	–	–	–	GIL
<i>N. silvestres</i> (Vellozo) Churk	18 (2x)	–	–	–	GIL
<i>N. unca</i> (Ravenna) A.Gil	18 (2x)	–	–	–	GIL
<i>Neomarica</i> sp. 1	28 (2x)*	–	–	–	PW
<i>Neomarica</i> sp. 2	18 (2x)	–	–	–	A11
<i>Neomarica</i> sp. 3	<b>28 (2x)*</b>	–	–	–	PW
<i>Trimezia</i> Salisb. ex Herb.: (41 / 2n = 26, 52; 28; 40, 60, 80, 82 / x <sub>2</sub> = 10, 13, 14 / 4.35)†					
<i>T. brevicaulis</i> Ravenna	28 (2x)	–	–	–	GIL
<i>T. connata</i> Ravenna	82 (6x) 52 (4x)	– –	– –	– –	A11 GT97, TRB08
<i>T. fistulosa</i> Foster	28 (2x)	–	–	–	CG08
<i>T. fosteriana</i> Steyererm.	26 (2x) 52 (5x)	– –	– –	– –	TRB08 A07, A11, GT97
<i>T. juncifolia</i> (Klatt) Benth. & Hook.	28 (2x)	–	–	–	CG08
<i>T. martinicensis</i> (Jacq.) Herb.	40 (4x) 54 (5x) 80 (8x)	– – –	– – –	– – –	GT97, CG08 A07, A11, CG08, M09 GT97, CG08

Table 2. Continued

Taxa	2n (ploidy level)	2C (pg)	1Cx (pg)	2C (Mbp)	Reference(s)
<i>T. spathata</i> Baker	<b>28 (2x)</b> 60 (6x)	<b>8.70</b>	<b>4.35</b>	<b>8510</b>	PW CG08
As <i>Trimezia martii</i> and <i>T. sincorana</i>	60 (6x)	–	–	–	KH84
<i>T. steyermarkii</i> R.C.Foster	52 (5x)	–	–	–	GT97, TRB08
<i>T. truncata</i> Ravenna	26 (2x) 28 (2x)	–	–	–	CG08 CG08
<b>Sisyrinchieae</b> ‡ (6 / c. 177)					
<i>Sisyrinchium</i> L. (194 / 2n = 18, 36, 34, 48, 96 / x <sub>2</sub> = 8; 9; 17 / 0.35–2.10)					
<i>S. angustifolium</i> Miller	96 (12x)				MM76
<i>S. californicum</i> (Ker Gawl.) Dryand.	32 (4x)	1.40	0.35	1369	KRJ86
<i>S. chilense</i> Hook.	16 (2x)	1.46	0.73	1427	G82, KH84, KRJ86
<i>S. convolutum</i> Nooca	36 (4x) 72 (9x)	1.90 4.20	0.48 0.53	1858 4107	G82, KH84, KRJ86 KRJ86
<i>S. longipes</i> (E.P.Bicknell) Kearney & Peebles	34 (4x)	1.40	–	1369	KRJ86
<i>S. micranthum</i> Cav.	32 (4x) 48 (6x)	– 2.90	– 0.48	– 2836	G82 KRJ86
<i>S. minus</i> Engelman & A.Gray	10 (?)				OL62
<i>S. pachyrhizum</i> Baker	96 (12x)	7.00	0.58	6846	KRJ86
<i>S. platense</i> I.M.Johnst.	48 (6x)	3.40	0.57	3325	KRJ86
<i>S. striatum</i> Sm.	18 (2x)	2.50	1.25	2445	KRJ86
<i>S. tenuifolium</i> Humb. & Bonpl. ex Willd.	36 (4x)	2.40	0.60	2347	KRJ86
<i>S. tinctorium</i> Kunth	36 (4x)	1.00	0.25	978	KRJ86
<i>S. vaginatum</i> Spreng. (cited as <i>Sisyrinchium alatum</i> Hook.)	36 (4x)	8.40	2.10	8215	KRJ86
<i>Olsynium</i> Raf.: (14 / 2n = 18 / x = 8, 9 / 3.55)					
<i>O. biflorum</i> Goldblatt (cited as <i>Phaiophleps biflora</i> (Thunb.) R.C.Foster)	18 (2x)	7.10	3.55	6943	G82, KRJ86
<i>O. douglasii</i> (A.Dietr.) E.P.Bicknell	64 (8x)	–	–	–	CH02
<i>O. douglasii</i> (A.Dietr.) E.P.Bicknell (cited as <i>Sisyrinchium grandiflorum</i> Dougl. ex Lindl.)	18 (2x)	–	–	–	KH84
<i>O. douglasii</i> (A.Dietr.) E.P.Bicknell (cited as <i>Sisyrinchium douglasii</i> A. Ditr.)	96 (12x)	–	–	–	KRJ86, RKL86
<i>O. filifolium</i> (Gaudich.) Goldblatt (cited as <i>Sisyrinchium filifolium</i> Gaudich.)	18 (2x)	–	–	–	KH84, KRJ86
<i>O. junceum</i> (E.Mey. ex C.Presl) Goldblatt (cited as <i>Sisyrinchium junceum</i> E.Mey.)	20 (2x)	–	–	–	KH84, R86, RKL86
<i>O. scirpoideum</i> subsp. <i>scirpeum</i> (Phil.) R.A.Rodr. & Martic. [cited as <i>Sisyrinchium scirpoideum</i> subsp. <i>scirpeum</i> (Phil.) Rodr.Rios.]					
<i>O. scirpoideum</i> subsp. <i>scirpoideum</i> (Poepp.) Goldblatt (cited as <i>Sisyrinchium scirpoideum</i> subsp. <i>scirpoideum</i> Poepp.)	18 (2x)				R86
<i>Libertia</i> Spreng.: (14 / 2n = 18, 38, 76, 228 / x = 9 / x <sub>2</sub> = 19 / 3.55)					
<i>L. biflorum</i> (Thunb.) Goldblatt [cited as <i>Phaiophleps biflora</i> (Thunb.) R.C.Foster]	18 (2x)	7.10	3.55	6943	G82, KRJ86
<i>L. cranwelliae</i> Blanchon, B.G.Murray & Braggins					

**Table 2.** *Continued*

Taxa	2n (ploidy level)	2C (pg)	1Cx (pg)	2C (Mbp)	Reference(s)
<i>L. chilensis</i> (Molina) Gunckel [cited as <i>L. formosa</i> Graham]	76 (4x)				B00
<i>L. ixiooides</i> (G.Forst.) Spreng.	228 (12x)				B00
<i>L. edgariae</i> B lanchon, B.G.Murray & Braggins					
<i>L. grandiflora</i> (R.Br.) Sweet					
<i>L. micrantha</i> A.Cunn.					
<i>L. mooreae</i> Blanchon, B.G.Murray & Braggins					
<i>L. peregrinans</i> Ckn. & Allan					
<i>L. paniculata</i> (R.Br.) Spreng.					
<i>L. pulchella</i> Spreng.					
<i>L. sessiliflora</i> Skottsb. [cited as <i>L. coerulea</i> Kunth & Bouché]	38 (2x)				B00
<i>Orthrosanthus</i> Sweet: (9 / 2n = 40, 50, 54 and 84/ x = 10)†					
<i>O. acorifolius</i> (Kunth) Ravenna	54 (5x)	–	–	–	GT97
<i>O. chimboracensis</i> (Kunth) Baker	54 (5x)	–	–	–	GT97
<i>O. var. exsertus</i> R.C.Foster	50 (5x)	–	–	–	KH84
<i>O. polystachyus</i> Benth.	40 (4x)	–	–	–	KH84
<i>Solenomelus</i> Miers:					
<i>S. segethi</i> Kuntze [cited as <i>Solenomelus</i> <i>sisyrinchium</i> (Griseb. in Lechler) Pax]	48 (2x)				RKL86
<b>Outgroup</b>					
<i>Dietes</i> Salisbury ex Klatt (Irideae): (6 / 2n = 60 / x = 10)					
<i>D. robinsoniana</i> (F.Meull.) Klatt	60 (2x)	–	–	–	GT97
<i>Diplarrena</i> Labill. (Diplarreneae): (2 / 2n = 32 / x = 16)					
<i>D. moraea</i> Labill.	32 (2x)	–	–	–	KH84
<i>D. latifolia</i> Benth.	32 (2x)	–	–	–	KH84

Unpublished counts are in bold type. Parentheses after tribes indicate (total number of genera/total number of species), while parentheses after genera indicate (total number of species/chromosome number recorded for the genera/base chromosome number suggested/1Cx range).

\*1 pg = 978 Mbp (Doležel *et al.*, 2003).

†Due to the dynamic chromosome number variation with recurrent dysploidy, we could not be sure about the ploidy level for the majority of species. When just one species is uncertain in the genus, the ploidy level was indicated by “\*”.

‡The list of species from Sisyrinchieae is not an exhaustive collection, especially for *Sisyrinchium*. Species were chosen according to phylogeny (used in Chauveau *et al.*, 2012) and those that have both chromosome number and genome size published.

No *Iris* species are listed here as the genus presents many diploid chromosome numbers, and both polyploidy and dysploidy are frequent, hampering analysis of chromosome number evolution.

Underlined number in *Neomarica* and *Trimezia* indicates the most common data observed.

References: A07, Alves & Felix (2007); A11, Alves *et al.* (2011); AOC02, Aaron & Ortiz-Catedral (2002); BG90, Beltrão & Guerra (1990); B00, Blanchon *et al.* (2000); BL12, Bennett & Leitch (2012); CH02, Cholewa & Henderson (2002); CG08, Chukr & Giulietti (2008); G75, Goldblatt (1975); G82, Goldblatt (1982); GWZ84, Goldblatt (1984); GS91, Goldblatt & Snow (1991); GH92, Goldblatt & Howard (1992); GT97, Goldblatt & Takei (1997); G88, Guerra (1988); G91, Guerra (1991); GIL, A. Gil (unpubl. data); K02, Kalaiselvi (2002); KH84, Kenton & Heywood (1984); KRJ86, Kenton *et al.* (1986); K87, Kenton & Rudall (1987); K90, Kenton *et al.* (1990); M09, Moreno *et al.* (2009); MM76, Murin & Majovsky (1976); OL62, Oliver & Lewis (1962); R81, Ravenna (1981); R83, Ravenna (1983); R84, Ravenna (1984); R86, Rodriguez (1986); RKL86, Rudall *et al.* (1986); RC04, Roitman & Castillo (2004); RC08, Roitman & Castillo (2008); S70, Sharma (1970); TRB08, De Tullio *et al.* (2008); W59, Winge (1959); ZBS85, Zaman, Begum & Saha (1985); PW, present study.

lineage,  $2n$  as the chromosome number in somatic tissues and  $n$  as the haploid chromosome number observed in gametes (Stebbins, 1966; Guerra, 2008; Peruzzi, 2013). Based on the recent phylogenetic hypothesis of Chauveau *et al.* (2012), the ancestral haploid and base chromosome numbers were reconstructed as categorical data with Mesquite v.2.73 (Maddison & Maddison, 2011) under unordered maximum-parsimony (MP), applying ancestral character reconstruction analysis. For species without published chromosome data, but with consistent chromosome numbers across the genus, those chromosome numbers were assumed for the species without data (for codification details, see supporting information, Appendix S1).

In addition, the reconstruction of ancestral chromosome numbers was analysed with CHROM-EVOL v.2 (<http://www.zoology.ubc.ca/prog/chromEvol.html>) (Mayrose, Barker & Otto, 2010; Glick & Mayrose, 2014), which uses a maximum likelihood estimation (MLE) approach. This likelihood-based method tests a number of different models to evaluate chromosome number changes along the phylogenetic branches and infers which model best explains the chromosome number variation in extant species. We ran all four constant rate models because the best model has usually been found to be one of these four models (Escudero *et al.*, 2014). For each species, the haploid chromosome number was defined and plotted into a counts file (Appendix S2). These models were fitted to our data, each with 10 000 simulations, and the one that best fitted the dataset was selected under the Akaike information criterion (AIC). The maximum number of chromosomes was set to 500 higher than the highest number found in the empirical data, and the minimum was set to 1.

## RESULTS

Fifty-four accessions of 25 species of Iridaceae were collected in southern Brazil (Table 1). Chromosome numbers for 21 species belonging to ten genera, together with genome size for 22 species (Table 2) and meiotic behaviour/pollen viability for 14 species were determined (Table 3). Chromosome numbers were determined for nine species for the first time.

### CHROMOSOME NUMBERS, KARYOTYPES AND C-VALUE DATA

#### *Tigridieae* species

Clade A (from Chauveau *et al.*, 2012)

All species of *Tigridieae* analysed by mitotic and/or meiotic chromosome counts had  $n = 7$ . The GS values obtained here varied four-fold, ranging from  $2C = 3.90$

to 15.43 pg, and the monoploid genome size ( $1Cx$ ) varied 2.2-fold, ranging from 1.93 to 4.17 pg (Table 2).

The chromosome numbers of five *Calydorea* spp. are reported here for the first time, with all having  $2n = 14/n = 7$ : *C. alba* Roitman & J.A.Castillo, *C. approximata* R.C.Foster (Fig. 2A), *C. basaltica* Ravenna (Fig. 3A), *C. campestris* (Fig. 2B) and *C. crocoides* (Fig. 3B). *Calydorea approximata* had a bimodal karyotype with two pairs of large metacentric/submetacentric chromosomes and five pairs of small submetacentrics, one of them with a satellite (Fig. 2A). In agreement with observations of mitotic chromosomes, the bivalent configurations observed in *C. campestris* suggest that the karyotype is composed of submetacentric and metacentric chromosome pairs (Fig. 2B). By contrast, *C. crocoides* had six ring bivalents and one rod bivalent, indicating a pair of telocentric chromosomes in addition to the metacentrics and submetacentrics (Fig. 3B). Although all *Calydorea* spp. had the same diploid chromosome number and similar karyotypes, their GS varied 1.82-fold. *Calydorea approximata* had a  $2C$  value of 4.58 pg, whereas *C. alba* and *C. basaltica* had approximately twice this DNA content (8.34 and 7.72 pg, respectively); *C. campestris* and *C. crocoides* presented intermediate GS values, 6.96 and 6.90 pg, respectively (Table 2). Chromosome number was not determined for *Catila amabilis* in the present work, but its GS was  $2C = 4.08$  pg.

The chromosome number was  $2n = 14$  for all *Cypella* spp.: *C. hauthalii* (Kuntze) R.C.Foster subsp. *opalina* Ravenna (Fig. 2C), *C. fucata* Ravenna (Fig. 2D), *C. aff. fucata* and *C. herbertii* Herb. (Fig. 2E). *Cypella* spp., much like *Calydorea*, usually had two pairs of large chromosomes and five smaller ones, with one satellited pair. *Cypella hauthalii* subsp. *opalina* and *C. herbertii* had  $2C$  values of c. 4 pg (Table 2), whereas *C. laxa* Ravenna had a slightly higher DNA content (4.83 pg).

In the present work, six *Herbertia* spp. were investigated, and four ploidies ( $2x$ ,  $4x$ ,  $6x$  and  $8x$ ) were found. *Herbertia crosae* Roitman & J.A.Castillo and *H. darwinii* Roitman & J.A.Castillo are diploids ( $2n = 14$ ; Fig. 2I), whereas *H. quareimana* Ravenna (Fig. 2J) and *H. pulchella* Sweet (Fig. 3C, D) are tetraploids ( $2n = 28$  and  $n = 14$ , respectively). Two cytotypes were found in *H. lahue* (Molina) Goldblatt; two populations were hexaploid ( $2n = 42$ ) and another six were octoploid ( $2n = 56$ ; Fig. 2K). *Herbertia zebrina* had its chromosome number determined here for the first time ( $2n = 14$ ; Fig. 2L). *Herbertia* chromosomes were medium to large in size, with diploids presenting both large and small chromosome pairs, whereas polyploids presented a more gradual size decrease among chromosome pairs (compare Fig. 2I–L). *Herbertia darwinii* had two pairs of large



**Table 3.** Haploid chromosome number, meiotic normality, meiotic indexes and pollen fertility in analysed species of Iridaceae

Taxa	N	Meiosis I and II		Meiotic index		Pollen viability	
		N*	%†	N*	%†	N*	%†
<b>Tigridieae</b>							
<b>Clade A</b>							
<b><i>Calydorea</i></b>							
<i>C. alba</i>	7					5 (2500)	98.96
<i>C. approximata</i>	7	2 (160)	96.25			5 (2500)	98.52
<i>C. basaltica</i>	7	1 (500)	98.90			5 (2500)	87.48
<i>C. campestris</i>	7	4 (2000)	98.75	12 (2400)	99.80	20 (10.000)	91.06
<i>C. crocoides</i>	7	2 (1000)	99.00	4 (800)	98.70	10 (5000)	93.58
<b><i>Cypella</i></b>							
<i>C. herbertii</i>	7	4 (144)	91.70	2 (200)	99.00	5 (2500)	98.70
<b><i>Herbertia</i></b>							
<i>H. lahue</i>	28					3 (1500)	98.07
<i>H. pulchella</i>	14	3 (300)	99.30	8 (1600)	100	10 (5000)	99.02
<b><i>Kelissa</i></b>							
<i>K. brasiliensis</i>	7	1 (500)	99.20	5 (1000)	100	5 (2500)	97.90
<b><i>Onira</i></b>							
<i>O. unguiculata</i>	7			5 (1000)	100	5 (2500)	98.40
<b>Clade B</b>							
<b><i>Gelasine</i></b>							
<i>G. caerulea</i>	7					10 (5000)	98.88
<b>Trimezieae</b>							
<b><i>Neomarica</i></b>							
<i>N. candida</i>	9	6 (3000)	99.55	6 (1200)	99.75	10 (5000)	72.30
<i>Neomarica</i> sp	14	4 (2000)	98.00	3 (600)	99.8	5 (2500)	94.36
<b><i>Trimezia</i></b>							
<i>T. spathata</i>	14	7 (4500)	99.60	17 (3400)	99.77	20 (10000)	90.26

\*N, number of individuals analysed (number of cells).

†Percentage of normal cells.

chromosomes, one medium and other small with a satellited pair (Fig. 2I). The tetraploid *H. quareimana* had satellites on two pairs (Fig. 2J). Concerning DNA content, the 2C values in *Herbertia* increased with increasing ploidy: approximately 4.0 pg in diploids, 8.6 pg in tetraploids, 11.6 pg in the hexaploid and 15.4 pg in the octoploid. However, 1Cx decreased with ploidy, ranging from 1.93 to 2.34 pg, with the lowest content observed in the hexaploid and octoploid cytotypes of *H. lahue*. The Brazilian specimens of *Kelissa brasiliensis* and *Onira unguiculata* (Baker) Ravenna analysed here were diploids with  $2n = 14$  (Fig. 2G, H) and  $2C = 4.73$  and  $4.57$  pg, respectively.

#### Clade B

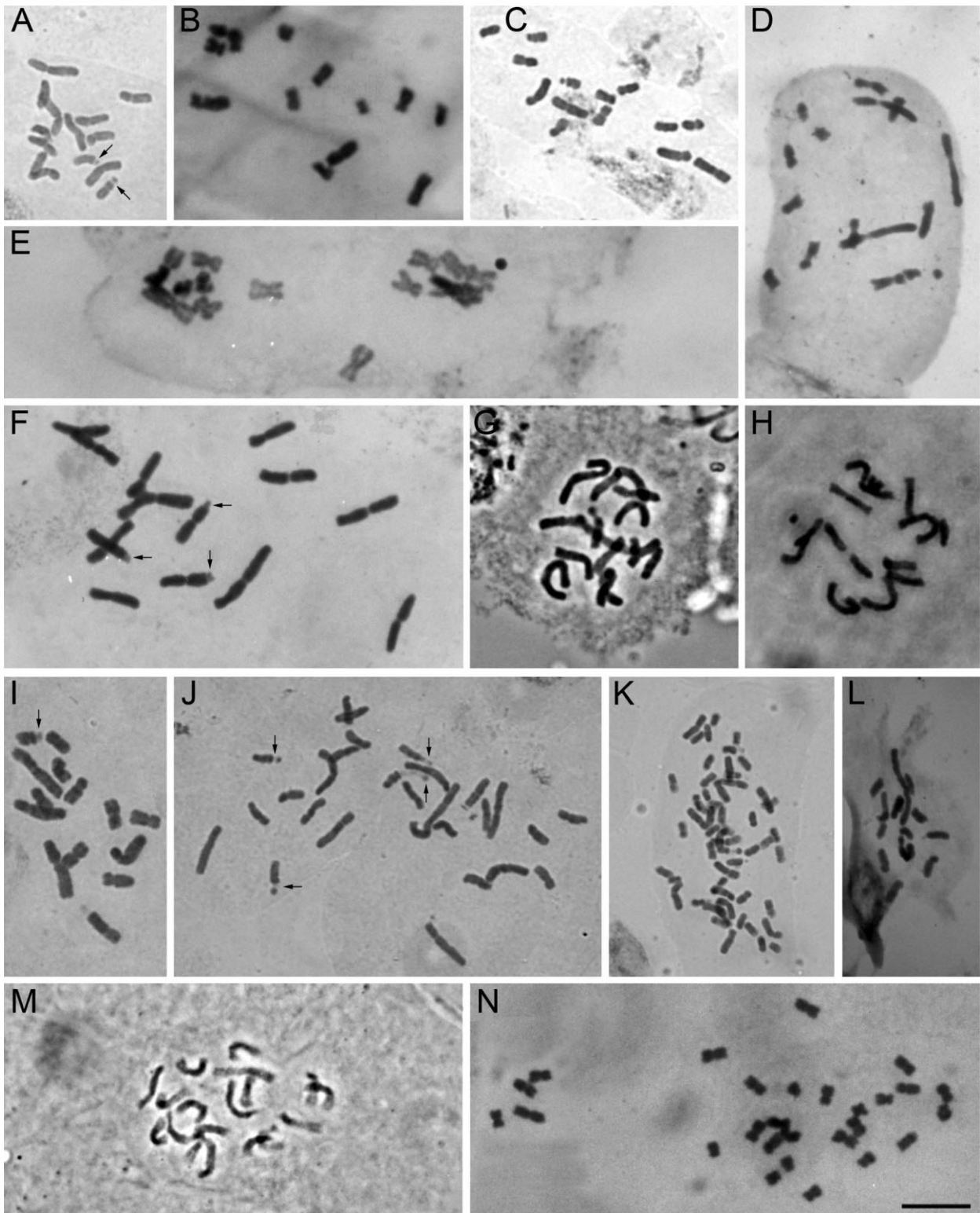
The first count for *Gelasine caerulea* (Vell.) Ravenna is presented here ( $2n = 14$ ; Fig. 2F). The karyotype showed two large meta- and submetacentric chromosome pairs and up to three satellited chromosomes; hence, two chromosome pairs seem to bear satellites.

The DNA content was estimated at  $2C = 5.05$  pg but with a high coefficient of variation. Chromosome counting was not performed for *Phalocallis coelestis*, and the GS was determined to be  $2C = 7.21$  pg.

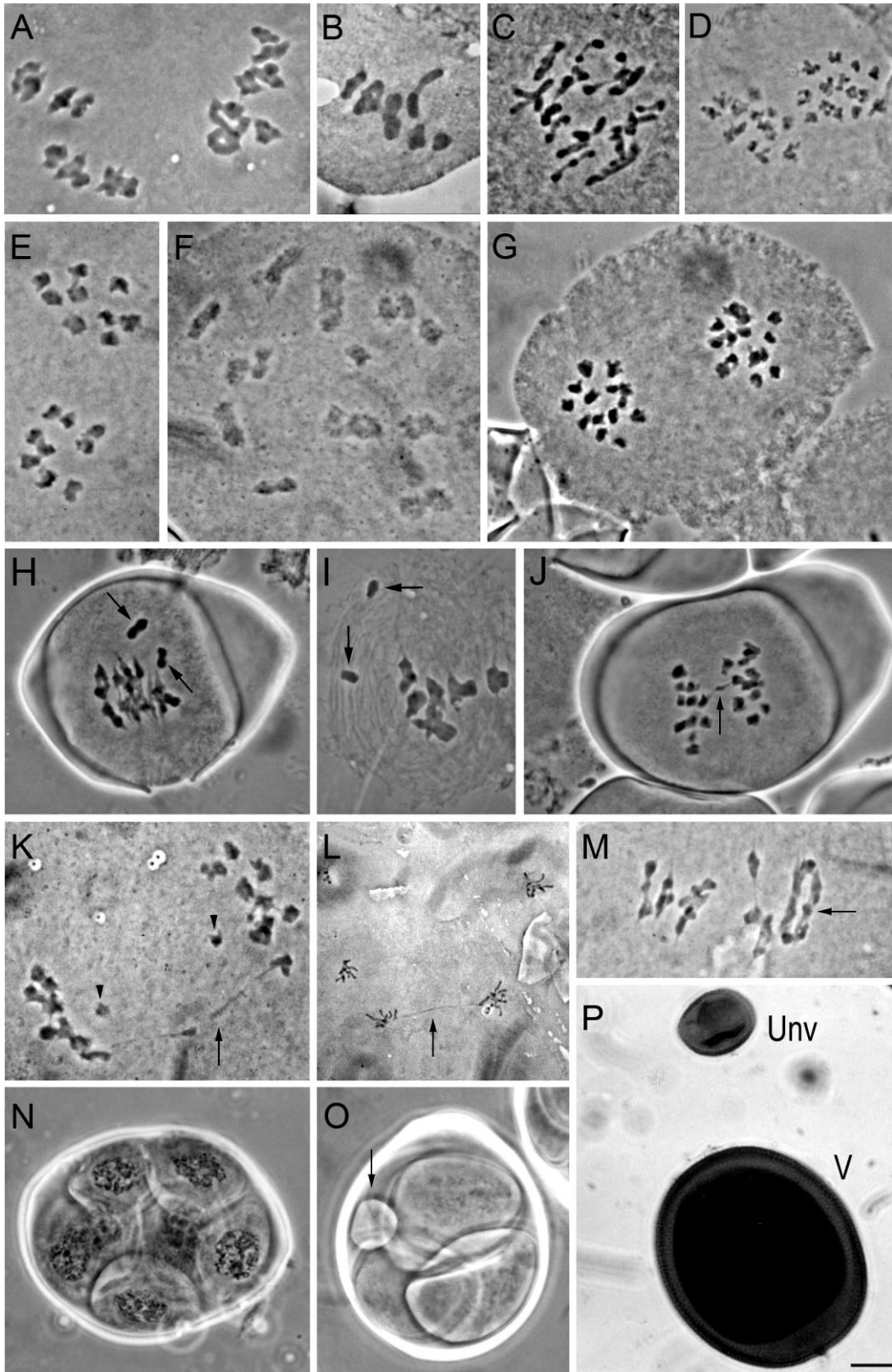
#### *Trimezieae* species

Three species were analysed in Trimezieae, and two haploid chromosome numbers ( $n = 9$  and  $n = 14$ ) were found. The 2C DNA values ranged from 7.6 to 8.7 pg, and 1Cx from 3.80 to 4.35 pg (Table 2).

The chromosome numbers of two *Neomarica* spp. were determined: *Neomarica candida* had  $2n = 18/n = 9$  (Figs 2M, 3E), whereas the other species (*Neomarica* sp.) had  $n = 14$  (Fig. 3F). Genome size was estimated for *N. caerulea* and *N. candida* at  $2C = 7.92$  pg and  $2C = 7.60$  pg, respectively. The last species of Trimezieae investigated was *Trimezia spathata* Baker, with  $2n = 28/n = 14$  (Figs 2N, 3G, H). This species had a symmetrical karyotype with



**Figure 2.** Mitotic metaphases of Iridaceae species. A, *Calydorea approximata*,  $2n = 14$ ; B, *C. campestris*,  $2n = 14$ ; C, *Cypella hauthalii* subsp. *opalina*,  $2n = 14$ ; D, *C. fucata*,  $2n = 14$ ; E, *C. herbertii*,  $2n = 14$ ; F, *Gelasine coerulea*,  $2n = 14$ ; G, *Kelissa brasiliensis*,  $2n = 14$ ; H, *Onira unguiculata*,  $2n = 14$ ; I, *Herbertia darwinii*,  $2n = 14$ ; J, *H. quareimana*,  $2n = 28$ ; K, *H. lahue*,  $2n = 56$ ; L, *H. zebrina*,  $2n = 14$ ; M, *Neomarica candida*,  $2n = 18$ ; N, *Trimezia spathata*,  $2n = 28$ . Arrows in A, F, I and J indicate secondary constrictions. Bar in N represents 10  $\mu\text{m}$ .



**Figure 3.** See caption on next page.



**Figure 3.** Meiotic analysis of Iridaceae species – haploid chromosome numbers and observed meiotic abnormalities. A, *Calydorea basaltica*,  $n = 7$  (metaphase I); B, *C. crocoides*,  $n = 7$  (metaphase I); C, *Herbertia pulchella*,  $n = 14$  (metaphase I); D, *H. pulchella*  $n = 14$  (prophase II); E, *Neomarica candida*,  $n = 9$  (metaphase II); F, *Neomarica* sp.,  $n = 14$  (prophase I – diakinesis I); G, *Trimezia spathata*,  $n = 14$  (prophase II); H, *T. spathata* in metaphase I with non-orientated bivalents (arrows); I, *C. campestris* in metaphase I with univalents (arrows); J, *T. spathata* in anaphase I with bridge (arrow); K, *N. candida* in anaphase I with bridge (arrow) and two laggard chromosomes (arrowheads); L, *Calydorea basaltica* in anaphase II with bridge (arrow); M, *N. candida* in metaphase I with tetravalent ring (arrow); N, *C. crocoides* showing ‘tetrad’ with seven microspores; O, *N. candida* showing tetrad with microcyte (arrow); P, *N. candida* showing viable pollen grain (V) and an unviable micro pollen grain (Unv). Bar in P represents 10  $\mu\text{m}$ .



metacentric and submetacentric chromosomes and a DNA content of  $2C = 8.7$  pg.

#### MEIOTIC BEHAVIOUR AND POLLEN VIABILITY

Species from eight genera were analysed for meiotic chromosome pairing and segregation, tetrad normality (meiotic index) and pollen grain stainability as an indicator of pollen grain viability (Table 3; Fig. 3).

The chromosome numbers of three species (*Calydorea crocoides*, *Herbertia pulchella* and *Neomarica* sp.) could be determined through just meiotic analysis (Fig. 3B, C, F); for the remaining species, meiotic analysis confirmed the somatic chromosome number. All of the species presented highly regular meiosis (91.7–99.6%; Table 3), usually with bivalent (II) formation at diakinesis and metaphase I and regular disjunction (anaphase and telophase I and II). The most common abnormalities were non-orientated bivalents (Fig. 3H, I) and anaphase bridges (Fig. 3K), particularly in *Calydorea basaltica*, in either anaphase I or anaphase II, sometimes with fragments or laggards (Fig. 3L). In some cases, it was possible to observe multivalents in *Neomarica candida* (Fig. 3M).

Nine species showed meiotic indexes  $> 98\%$  (Fig. 3N–P), and 12 had at least 90% viable pollen grains (Table 3); only *Calydorea basaltica* and *Neomarica candida* had  $< 90\%$  fully stained pollen grains (Fig. 3P). Although *Calydorea alba*, *Gelasine coerulea*, *Herbertia lahue* and *Onira unguiculata* underwent no meiotic analysis, their high pollen stainability ( $> 98.8\%$ ) indicates regular meiosis for all.

#### RECONSTRUCTION OF ANCESTRAL CHROMOSOME NUMBERS IN IRIDOIDEAE

Chromosome numbers were recovered for 142 species of Iridoideae (151 species including the present work), and haploid and base chromosome numbers were determined for each genus and plotted against the phylogenetic tree of Chauveau *et al.* (2012) (Figs 4 and 5). Chromosome counts for *Dietes* Salisbury ex Klatt (Irideae) and *Diplarrena* Labill. (Diplarreneae),

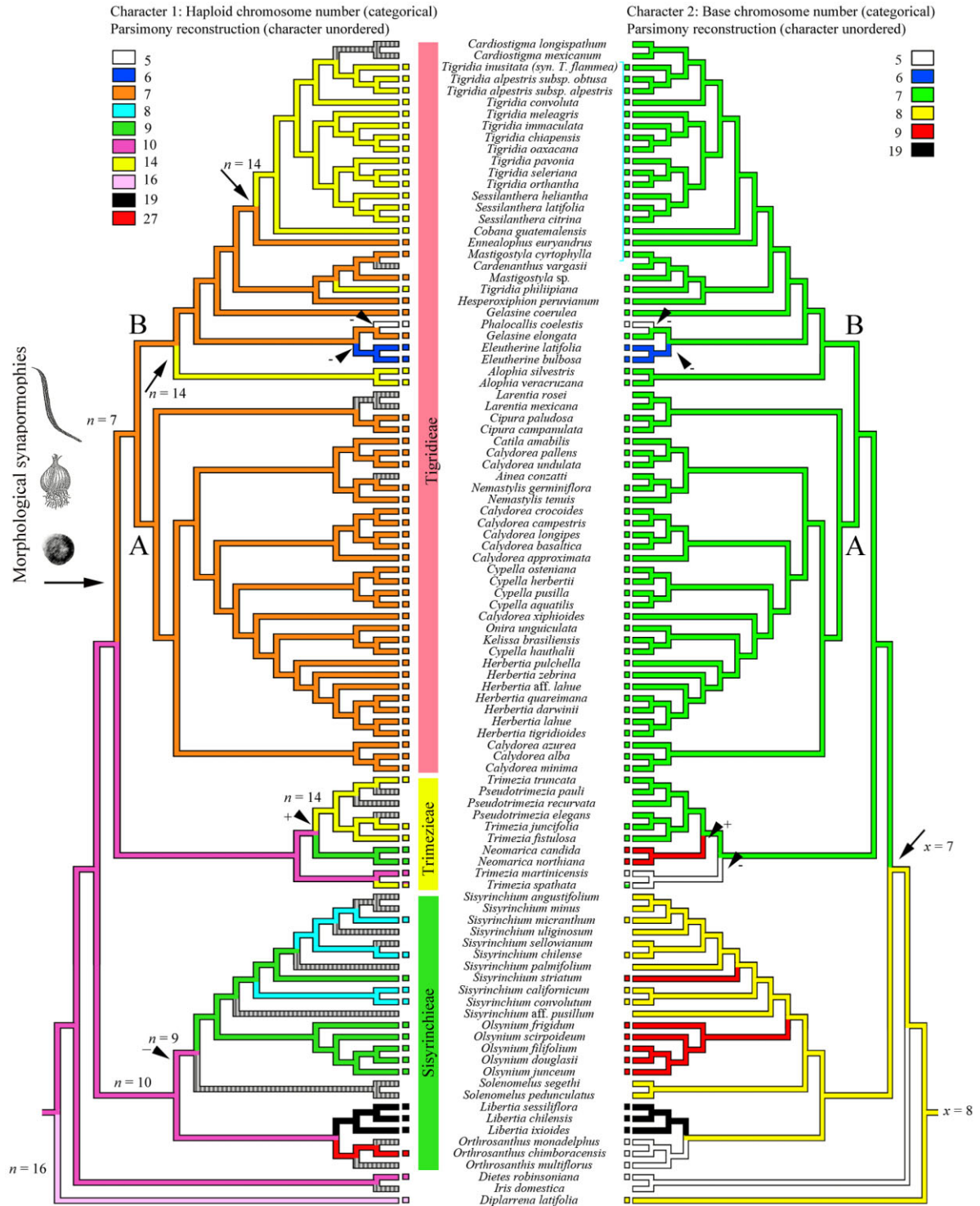
used as outgroups, were also recovered from the literature.

Haploid and base chromosome numbers reconstructed under MP (Mesquite; Fig. 4) and base chromosome numbers reconstructed under MLE (ChromEvol; Fig. 5) were broadly similar across simulations. For haploid number, an unordered MP analysis recovered 680 equally parsimonious trees, all with 14 steps, whereas for base chromosome number, 264 equally parsimonious trees with 11 steps were recovered. ChromEvol performed 10 000 simulations, and the best model, according to the AIC, was constant rate *demi\_est* (Table 4; Fig. 5). Based on these results, polyploidy and descending dysploidy seem to have played important roles during the diversification of Iridoideae (Table 4). The base chromosome number reconstructed for Iridoideae was  $x = 8$ . Some derived base numbers, such as  $x = 5, 6, 7$  and  $9$ , probably arose through dysploidy, whereas  $x = 14, 18$  and  $19$  were probably generated via polyploidy (Figs 4 and 5).

A base number of  $x = 7$  for Tigridaeae was supported by both the MP and the MLE analyses. From the MLE analysis (Fig. 5), the number  $x_2 = 14$  appears to have originated twice by polyploidy in clade B: first in the clade formed by *Alophia* Herb., which is a sister to the rest of clade B, and second, in the large derived clade comprising *Cardiostigma* Baker/*Tigridia* Juss./*Sessilanthera* Molseed & Cruden/*Cobana* Ravenna (Fig. 5). MP also predicted these two polyploid events, but only when haploid chromosome numbers were reconstructed (i.e. in a shift from  $n = 7$  to  $n = 14$ ; Fig. 4).

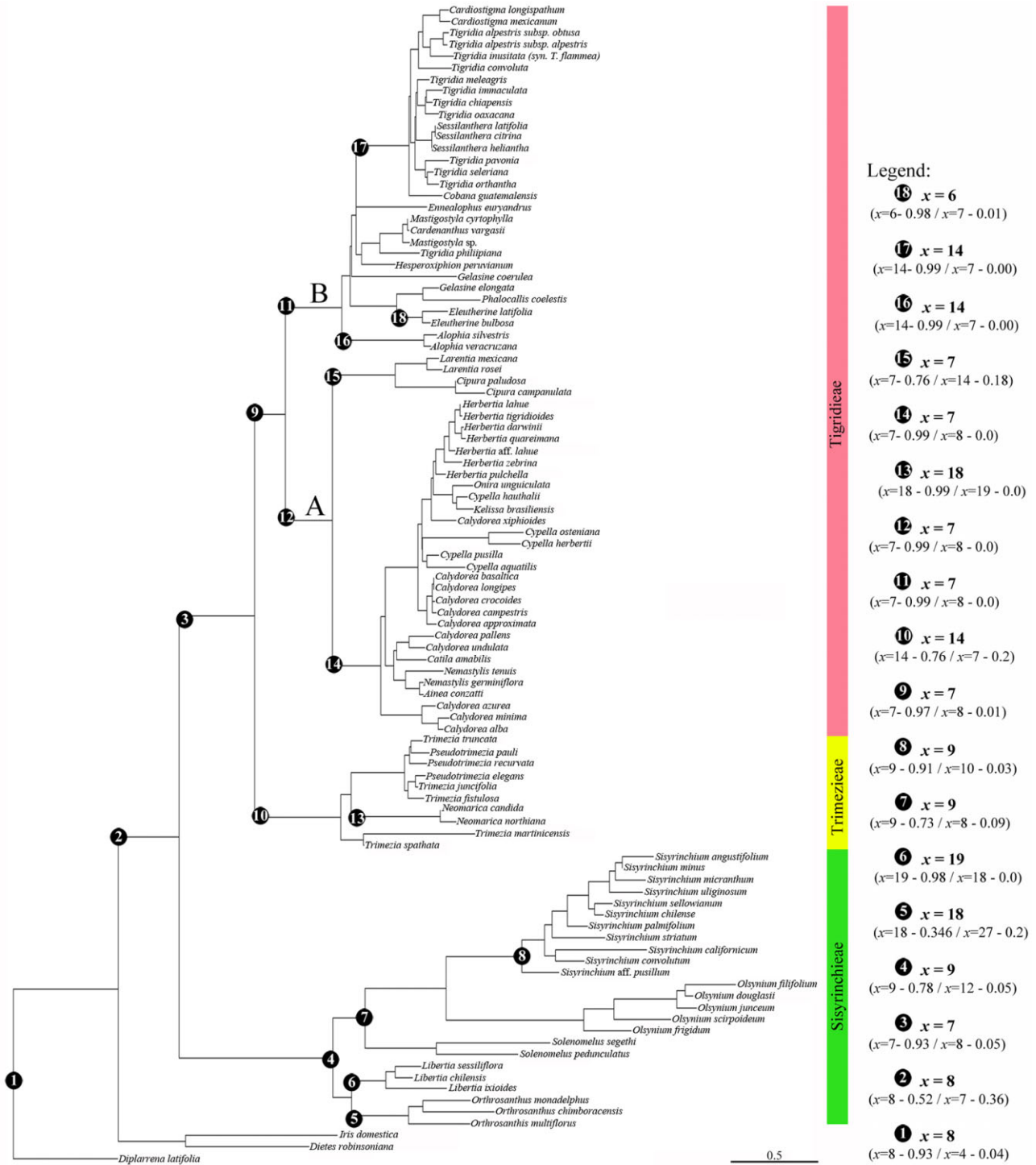
Trimezieae showed considerable variation in chromosome number (Table 2), but due to the limited chromosome data available, contrasting results were obtained from the different analyses. The MP analysis using haploid number suggested an ancestral state of  $n = 10$ , but  $x = 7$  was suggested when using the base chromosome number (Fig. 4). The MLE analysis indicated  $x = 14$  for the tribe, double the ancestral haploid chromosome number suggested by MP (Fig. 5).

Sisyrrinchieae also presented variability in haploid/base chromosome number, as reconstructed by MP



**Figure 4.** Haploid chromosome number ( $n$ ) and base chromosome number ( $x$ ) evolution in Iridoideae, focused on Tigridaeae and Trimezieae. For species with polyploidy series, the lower haploid chromosome number was applied. Reconstructions of character ancestral states were conducted under MP following the phylogeny hypothesis proposed by Chauveau *et al.* (2012). For tree 1 ( $n$ ) arrows indicate events of polyploidization, while for tree 2 ( $x$ ), arrows indicate the probably ancestral state in the origin of Tigridaeae/Trimezieae tribes. Arrowhead/plus signal ascendant dispolyploidy, while arrowhead/minus signal descendant dispolyploidy events. Morphological synapomorphies for Tigridaeae are represented (plicate leaves, bulb and monosulcate pollen grain). For data sources and codification details, see Appendix S1.





**Figure 5.** Base chromosome number estimate and evolution in Iridoideae inferred under Bayesian optimization. For species with polyploidy series, different haploid numbers were plotted considering the frequency of each number. The phylogeny hypothesis used was proposed by Chauveau *et al.* (2012) and simulation of base chromosome number followed the constant rate *demi\_est* model. Base number and posterior probabilities are mainly indicated at nodes. For data sources and codification details, see Appendix S2.

**Table 4.** Log-likelihood and AIC estimates for the data set analysed for the constant rate model implemented by ChromEvol software

Model	Log-likelihood	AIC	Gain	Loss	Duplication	Demi-duplication
Constant gain, loss and duplication	-176	358.1				
Constant gain, loss and demi-duplication	-154.9	315.7				
Constant gain, loss, duplication and demi-duplication*	-151.8	311.7	8.21	18.01	26.91	7.85
Constant gain and loss	-294	592.1				

\*Best-fit model.

and MLE, with an ancestral haploid number of  $n = 10$  using MP and ancestral base numbers of  $x = 8$  (MP, Fig. 4) or  $x = 9$  (MLE, Fig. 5). A range of secondary base numbers, i.e.  $x_2 = 5, 9$  and  $19$  (MP) and  $18$  and  $19$  (MLE), was also reconstructed for some derived lineages. Even in Sisyrinchieae, polyploidy was important, underlying  $x_2 = 19$  in *Libertia* Raf. and  $x_2 = 18$  in *Orthrosanthus* Sweet (MLE, Fig. 5), although both were probably also associated with dysploidy. However, it is important to note the discrepancy between models relating to *Orthrosanthus*. An important difference was detected when using haploid number ( $n = 27$ , MP in Fig. 4) from MLE ( $x_2 = 18$ ; Fig. 5) and the base chromosome number from MP ( $x = 5$ ; Fig. 4). However, these differences are probably the consequence of a paucity of data.

## DISCUSSION

The data obtained herein demonstrate the large variability in chromosome numbers, karyotype features and DNA content among species of Iridoideae. The ancestral base chromosome number suggested for the subfamily,  $x = 8$ , was concordant among analyses. Chromosome rearrangements gave rise by ascending dysploidy to  $x = 9$  (MLE) or  $x = 8$  (MP) in Sisyrinchieae or by descending dysploidy to  $x = 7$  in Tigridaeae (MP and MLE), which in turn, via polyploidy, gave rise to  $x = 14$  in Trimezieae (MLE only). The MP haploid chromosome number analysis further suggested that polyploidy has been important in the evolution of Tigridaeae, as both  $n = 7$  and  $n = 14$  were common. This trend was also reflected in the MLE statistical analysis. However, dysploidy is also clearly an important evolutionary mechanism, and reconstruction models suggest that it is operating in all three tribes.

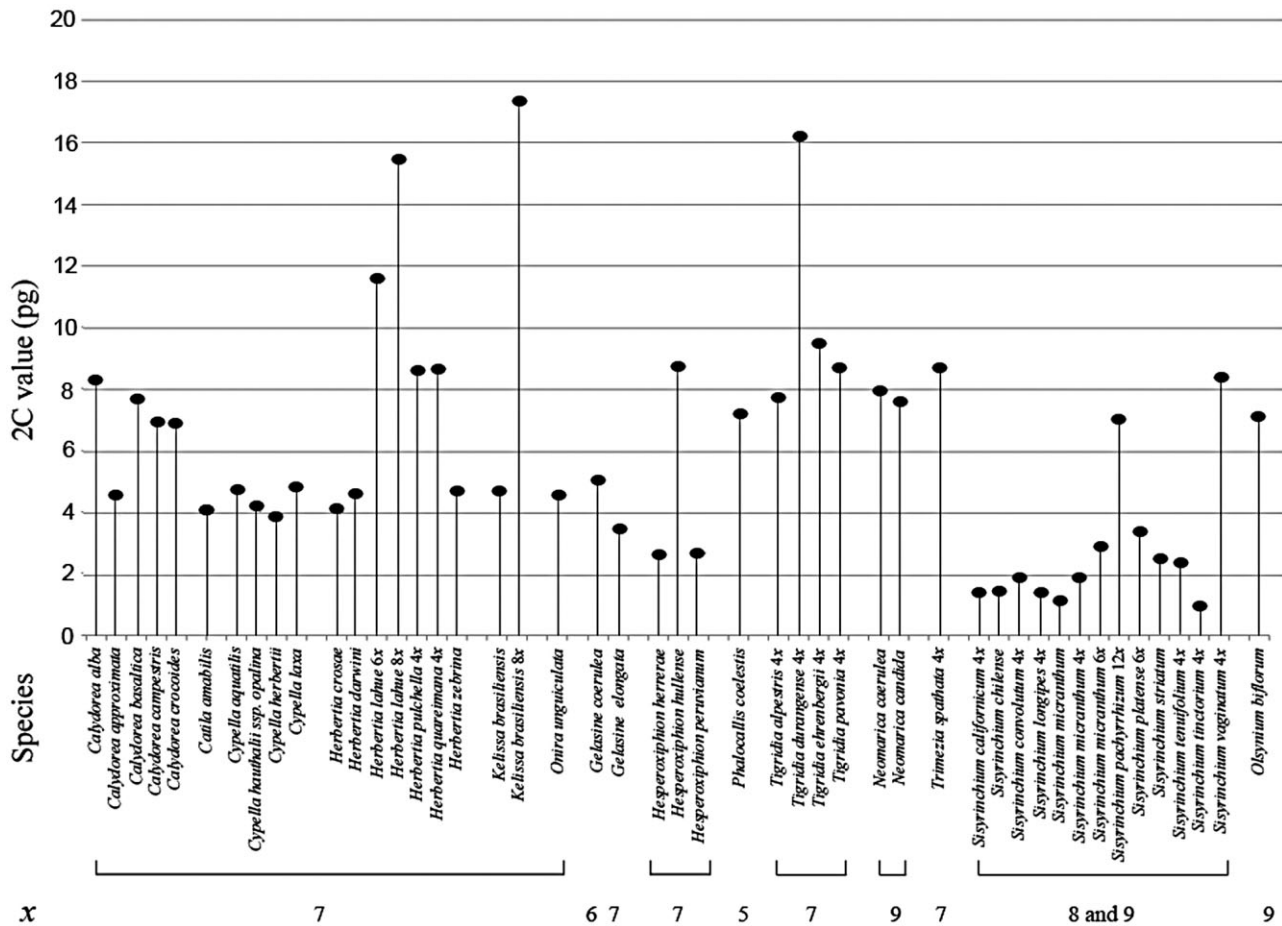
### TIGRIDIEAE SPECIES

The conserved base chromosome number for Tigridaeae,  $x = 7$ , was supported by all analyses, reinforcing the Tigridaeae monophyly hypothesis, as did the presence of a bimodal karyotype composed of two long

and five short chromosome pairs (Goldblatt, 1982; Goldblatt & Takei, 1997). Based on morphological and cytogenetic criteria, Goldblatt (1982) divided Tigridaeae into two subtribes, Cipurinae and Tigridiinae, both exclusively from the New World. Such a division is partly supported by Chauveau *et al.* (2012): Clade A corresponds to Cipurinae and Clade B to Tigridiinae. Some discrepancies were observed between these two proposals. *Ainea conzattii* (R.C.Foster) Ravenna, previously associated with *Tigridia* (Tigridiinae; Goldblatt *et al.*, 2008), was grouped with *Calydorea undulata* Ravenna, *C. pallens* Griseb. and *Catila amabilis/Nemastylis* Nutt. by Chauveau *et al.* (2012), consistent with the present analysis. Secondly, *Phalocallis coelestis*, commonly associated with *Cypella* (Cipurinae, Clade A; Goldblatt *et al.*, 2008), was grouped with *Gelasine elongata* (Graham) Ravenna and *Eleutherine* Herb. (Tigridiinae, Clade B in Chauveau *et al.*, 2012).

Considering the clades within Tigridaeae, the base chromosome number suggested for Cipurinae/Clade A was  $x = 7$ , in accordance with previous authors, presenting a ploidy series without dysploidy events (Goldblatt, 1982; Kenton *et al.*, 1990; Alves *et al.*, 2011). On the other hand, Tigridiinae/Clade B presents more variable haploid numbers, which probably arose from both polyploidy and descending dysploidy events, two important events suggested by both the MP and the MLE analyses. Goldblatt (1982) suggested a palaeopolyploidy event at the base of Tigridiinae, giving rise to  $n = x_2 = 14$ . However, our data suggest two independent polyploidy events across the clade (see circles 16 and 17 in Fig. 5), in addition to two descending dysploidy events [ $x_2 = 6$  in *Eleutherine* (circle 18 in Fig. 5) and  $x_2 = 5$  in *Phalocallis*]. These results suggest that a secondary base number of  $x_2 = 14$  is a derived rather than an ancestral state in Tigridiinae/Clade B.

Ploidy series were commonly observed among genera of Tigridaeae, e.g. *Calydorea* [*C. azurea* Klatt and *C. pallens*,  $2n = 4x = 28$ , and *C. xiphoides* (Poepp.) Espinosa,  $2n = 6x = 42$  (Goldblatt, 1982; Goldblatt & Takei, 1997; De Tullio *et al.*, 2008)] and *Herbertia* in particular. In accordance with the pat-



**Figure 6.** Genome size (2C) and base chromosome number (x) variation in Iridoideae. Filled circles represent the 2C value described for species and the number below species indicates the base chromosome number. Genera are organized according to the phylogeny hypothesis proposed by Chauveau *et al.* (2012). For detailed information see Appendix S1.

terns found here, four ploidy levels (2x, 4x, 6x and 8x) have been reported for *Herbertia*: 2x, 4x and 6x individuals in *H. pulchella* (4x was found here; also see Winge, 1959; Kenton & Heywood, 1984; Goldblatt & Takei, 1997) and 2x, 4x, 6x and 8x individuals in *H. lahue* (6x and 8x were found here; also see Winge, 1959; Kenton & Heywood, 1984; Goldblatt & Takei, 1997; Moreno *et al.*, 2009). The other three *Herbertia* spp. studied here (*H. crosae*, *H. darwinii* and *H. quareimana*) showed just one ploidy. As expected, in *Herbertia*, the GS ranged according to ploidy, with diploids and octoploids presenting lower and higher contents, respectively (Fig. 6). However, considering the 1Cx values, *Herbertia* seems to have undergone the well-known genome downsizing phenomenon following polyploidy (Leitch & Bennett, 2004; Leitch & Leitch, 2008; Bennett & Leitch, 2011), with hexaploids and octoploids having lower 1Cx values than their diploid relatives. Similar results were found in *Kelissa brasiliensis* (Fig. 6): diploid,  $2n = 2x = 14$ , had  $2C = 4.73$  pg/

$1Cx = 2.36$  pg, whereas the octoploid reported in the literature,  $2n = 8x = 56$ , had  $2C = 17.36$  pg/  $1Cx = 2.16$  pg (Bennett & Leitch, 2012).

Despite the recent improvements in understanding the phylogenetic relationships in Iridoideae, some taxonomic uncertainties still persist in Tigridieae. The alliance formed among *Calydorea*, *Cypella*, *Herbertia*, *Kelissa* and *Onira* needs more attention in view of the phylogenetic principles of monophyly. The reconstruction performed by Chauveau *et al.* (2012) revealed that *Cypella* and *Calydorea* were not monophyletic but rather split into unrelated lineages and grouped with species of different genera. No morphological apomorphy has been described for *Cypella*, and, further, *Cypella*, *Herbertia*, *Kelissa* and *Onira* do not have contrasting karyotypes. These observations highlight the need to identify and employ other markers to elucidate such relationships and thus increase our understanding of the diversification of Iridaceae in South America.

On the other hand, chromosome and GS data did support the taxonomic separation of *Phalocallis* from *Cypella* (Figs 4–6), *contra* Goldblatt & Manning (2008). *Cypella coelestis* and *C. plumbea* were previously considered synonyms of *Phalocallis coelestis* ( $2n = 10$ ;  $2C = 7.21$  pg) by Ravenna (1977) and the divergence of chromosome data between this taxon and other Brazilian *Cypella* spp. ( $2n = 14$ ;  $2C =$  about 4 pg) reinforces the position of this species in the genus *Phalocallis*, as also accepted by Barker & Govaerts (2014) and Eggers *et al.* (2014).

#### TRIMEZIEAE SPECIES

Species of Trimezieae show a large range of base numbers ( $x = 8, 9, 10, 13, 14$ ; see Table 2). Such dynamic chromosome evolution is probably a result of cycles of polyploidy followed by increasing or decreasing dysploidy events (Goldblatt & Takei, 1997; Goldblatt & Manning, 2008). The molecular evidence strongly supports Trimezieae as monophyletic and being composed of four major clades (Lovo, Winkworth & Mello-Silva, 2012), but, morphologically, the tribe lacks robust synapomorphies (Goldblatt *et al.*, 2008; Chauveau *et al.*, 2012).

Concerning *Neomarica*, chromosome numbers and karyotype features have been obtained for ten species, seven of which have been taxonomically well determined (Table 2). Despite the chromosome number variation, based on counts for 22 *Neomarica* spp.,  $2n = 2x = 18$  is suggested for the genus (A. Gil, unpubl. data). If so, then some species with alternative chromosome numbers may need to be transferred to new genera. *Neomarica* sp.,  $2n = 2x = 28$ , despite lacking taxonomic definition, showed the same chromosome number as *Neomarica* cf. *paradoxa*,  $n = x_2 = 14$ , and it is possibly the same species as that analysed by Alves *et al.* (2011). However, taking into account such discordant base numbers and some morphological differences in *N.* cf. *paradoxa*, the revision of the generic taxonomy of this genus is important. With respect to *Trimezia spathata*, Chukr & Giuletta (2008) reported two chromosome numbers:  $2n = 28$  and 60 ( $x = 7$  and 5; Table 2). Nevertheless, Kenton & Heywood (1984) considered *T. spathata* (cited as *T. martii*) as a diploid ( $x_2 = 14$ ) because 14 bivalents are formed with high chiasma frequency, in agreement with our observations (Table 3). In this case, *T. spathata*, with  $2n = 28$  and 60, is most probably a diploidized palaeopolyploid. The polyphyletic *Trimezia* varies greatly in chromosome numbers ( $2n = 26, 28, 40, 52, 54, 60, 80, 82$ ; Table 2), making it difficult to establish a relationship between chromosome number and species and to define the ancestral base number.

The MP and MLE analyses performed here suggested  $x = 7/14$  for Trimezieae, with recurrent dys-

ploidy within the tribe. The  $x = 14$  suggested by MLE is probably a result of a polyploidy event prior to the diversification of the tribe. However, considering the haploid number of  $n = 10$  reconstructed by MP, this incongruence is most probably a result of the diverse chromosome numbers reported for the tribe. Morphologically, Trimezieae are diverse, and no morphological character has so far been defined, even for the four molecular clades defined in the tribe (Lovo *et al.*, 2012).

The large chromosome number variation detected in Trimezieae could not be fully represented in our MP and MLE analyses, particularly given the underrepresentation of species in the phylogenetic tree. Additionally, no chromosome data were available for *Pseudotrimezia*, resulting in a gap of data for the Trimezieae. In addition, molecular studies by Lovo *et al.* (2012) revealed a complex organization of Trimezieae, with four clades that do not correspond to specific genera. Furthermore, *Neomarica* and *Trimezia* were both split into three different clades, suggesting that they are not monophyletic (see also Gil, Bittrich & Amaral, 2009). Without a clear taxonomic description with defined autapomorphies for each genus and a well-supported and sampled phylogenetic tree, such diverse chromosome data created by successive cycles of polyploidy and dysploidy are difficult to analyse, even using statistical models, and the mechanisms underlying the chromosome number variations in Trimezieae remain unknown.

#### CONCLUDING REMARKS

Species of Iridaceae show broad variation in morphological features, which has resulted in taxonomic controversies. Different approaches are therefore needed to provide additional clues about species relationships. Despite the large variability in chromosome number and size in this family, cytological research and GS estimations have been useful tools in understanding the evolution of taxa at different taxonomic levels and groups with different geographical origins or ecological specializations (Šmarda *et al.*, 2007; Goldblatt & Manning, 2008; Sonnleitner *et al.*, 2010; Pellicer *et al.*, 2013, 2014).

In light of the paucity of chromosome and genome size data for South American Iridaceae, this study provides new data and corroborates some published data. Above all, the present study has brought together, in a phylogenetic context, all of the new and previous chromosome data related to Iridoideae. The diversity of ploidy and haploid and base chromosome numbers, analysed using two independent statistical modelling approaches, demonstrates the complexity of cytogenetic character evolution in this family, underlining the importance of polyploidy and



descending dysploidy as primary mechanisms of chromosome evolution, but with different events occurring in different tribes. Polyploidy events are frequent in Iridoideae, and the occurrence of intrageneric/intraspecific polyploidy suggests that polyploid cytotypes are common, particularly in Tigridieae/Clade A. However, the dysploidy events usually associated with Iridaceae appear to be especially important in Tigridieae/Clade B and in Trimezieae.

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#### SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Haploid ( $n$ ) and base chromosome number ( $x$ ), both codified as categorical data, mapped along the phylogeny.

Appendix S2. Haploid chromosome number ( $n$ ) mapped along the phylogeny.