

V. Chromosome Counts for the Caricaceae Reveal Unexpected Dysploidy*

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Introduction

The family Caricaceae has six genera with together 34 species and one hybrid (Carvalho 2013). Besides the economically important crop, *Carica papaya*, other species in the family also produce edible fruits that are sold in local markets. The sister group of *C. papaya*, which originated in Central America, consists of a clade of four species, three herbs in the genus *Jarilla* endemic to Mexico and Guatemala, and the single species of *Horovitzia*, *H. cnidoscoloides*, endemic to cloud forests of Sierra de Juarez in Oaxaca, southern Mexico (Carvalho and Renner 2012). This Central American papaya clade in turn is sister to the mostly Andean *Vasconcellea* and *Jacaratia* group (Fig. 1). Sister to the entire Neotropical clade is the African genus *Cylicomorpha*, which consist of two species distributed in pre-montane forests in East and West Africa.

Despite the economic importance of the Caricaceae, only 10 of their 34 species from three genera have had their chromosomes counted. Heilborn (1921) reported $2n = 18$ for *Carica papaya*, *Vasconcellea pubescens*, and the hybrid *Vasconcellea* × *heilbornii*. The same number was reported for *Jacaratia spinosa* (Kumar and Srinivasan 1944; Silva et al. 2012), *Vasconcellea goudotiana*, *V. microcarpa*, *V. monoica* (de Zerpa, 1959), and *V. quercifolia* (Bernardello et al. 1990). More recent studies confirmed $2n = 18$ for these species (Costa et al. 2008; Damasceno et al. 2009; Silva et al. 2012) and reported the same number for four additional species of *Vasconcellea* (*V. cauliflora*, *V. longiflora*, *V. palandensis*, *V. sphaerocarpa*; Caetano et al. 2008; Costa et al. 2008; Damasceno et al. 2009; Silva et al. 2012). The genera *Jarilla*, *Horovitzia*, and *Cylicomorpha* have never had their chromosomes counted. Chromosomes in Caricaceae are relatively small (1–4.25 μm), and the

chromosomes pairs can not be distinguished morphologically (Datta 1971; Damasceno et al. 2009). A study by Costa et al. (2008) provides the only molecular-cytogenetic data for Caricaceae so far: The number and position of 18S and 5S ribosomal DNA (rDNA) fluorescent *in situ* hybridization (FISH) signals in *Carica papaya*, *Vasconcellea pubescens*, and *V. goudotiana* varied, with the two species of *Vasconcellea* being most similar to each other.

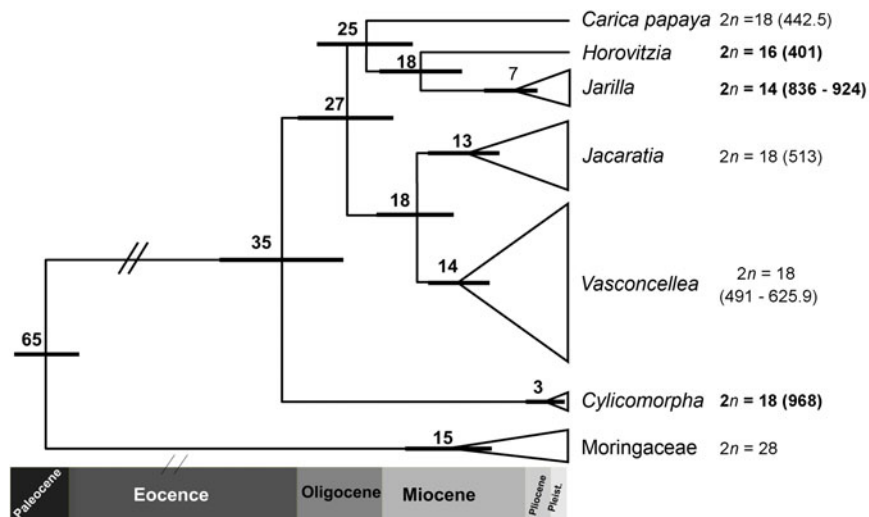


Fig. 1. Evolutionary relationships among the genera of Caricaceae, with branch lengths proportional to time and values at nodes indicating divergence times (modified from Carvalho and Renner 2012). Values in brackets refer to genome size ranges in millions of base pairs (Mbp) per haploid genome (see Table 1 for details). The chromosome number of *Moringa oleifera* is from (Silva et al. 2011). In bold are chromosome numbers and 1C-values first reported here.

Based on wild-collected material brought into cultivation in the greenhouses of the Munich Botanical Garden, we here report chromosomes numbers for *Cylicomorpha parviflora*, *Horovitzia cnidoscoloides*, *Jarilla caudata* and *J. heterophylla*, which have pivotal positions in the family as, respectively, a member of the sister genus to all Neotropical Caricaceae and the sister clade to papaya itself (Fig. 1). We also summarize all C-value measurements so far published for the

Caricaceae, including recently obtained measurements for the four newly counted species. These values are important for calculating the expected coverage in whole-genome sequencing, while chromosome numbers are important to determine expected linkage groups.

Material and Methods

Plant material and pretreatment – Wild-collected seeds of *Cylicomorpha parviflora*, *Jarilla caudata*, *J. heterophylla*, and *Horovitzia cnidoscoloides* were germinated, and since April 2013, seedlings have been growing in the greenhouses of the Munich Botanical Garden. Vouchers have been deposited in the Botanische Staatsammlung (M) and are listed in Table 1. Root tips were collected between 10:45 and 12:00 am and pretreated with 2 mM 8-hydroxyquinoline (8HQ). Roots of *Cylicomorpha* were kept for 20 h at 4°C, while roots of three individuals of *Horovitzia* and *Jarilla* were first kept for 3 h at room temperature and then for an additional 3 h at 4°C. Root tips of both species were then fixed in freshly prepared ethanol: acetic acid (3:1) overnight at room temperature and stored at –20°C.

Chromosomes preparation — The fixed roots tips were washed with dH₂O in three baths of 5 min each, and subsequently digested with 10 μ l of an enzyme mix (0.4% pectolyase, 0.4% cytohelicase, 1% cellulase in citrate buffer) during 5 min at 37°C. After that, the excess of enzyme was removed with a filter paper, and roots were washed and incubated in dH₂O for 30 min. Under a binocular, root meristems were dissected in 45% acetic acid, squashed, and covered with coverslips. The quality of spreads was checked microscopically using phase-contrast, and the best slides were selected for further analysis. The slides were dried on a cold plate at –40°C during 30 min, and then coverslips were removed for further drying at room temperature.

DAPI staining and visualization — Chromosomes were counterstained with 10 μ l of diamidino-2-phenylindol (DAPI, 2 μ g/ml) and mounted in Vectashield (Vector Laboratories, Burlingame, California, USA). The slides were kept dark at room temperature during at least 1 h. Images

were taken with a Leica DMR microscope equipped with a KAPPA-CCD camera, and the KAPPA software.

Results and Discussion

The African species *Cylicomorpha parviflora* has $2n = 18$ small chromosomes of homogeneous size (a count based on 22 metaphases; Fig. 2D). This number was also reported from 11 species in four of the family's six genera (Table 1). Unexpectedly, the closest relatives of *C. papaya* do not share that number. Instead, *Horovitzia cnidoscoloides* has $2n = 16$ (based on 9 metaphases; Fig. 2C), and the two species of *Jarilla* have $2n = 14$ (based on 6 metaphases for *J. heterophylla* and 7 metaphases for *Jarilla caudata*; Figs. 2A and B). Genome sizes so far known in Caricaceae are summarized in Table 1. The genome size of *Cylicomorpha parviflora* is about 968 Mb per haploid genome, and is much larger than that of any other Caricaceae species (Table 1). The two *Jarilla* species also have relatively large genomes, being 924 Mbp in *Jarilla caudata* and 836 Mbp in *Jarilla heterophylla*. The genome size of *H. cnidoscoloides* is 401 Mbp, similar to the genome size of *Carica papaya* (442.5 Mbp; Gschwend et al. 2013).

Based on the available counts, polyploidy plays no role in the Caricaceae. Instead, there is a dysploid reduction in chromosome number that must have begun in the most recent common ancestor of *Horovitzia* and *Jarilla* (Fig. 1), possibly involving reduction a fusion of two chromosomes, which would explain the change from $n = 9$ to $n = 8$. Dysploid reductions in chromosome number have been analyzed in detail in *Arabidopsis* (Yogeeswaran et al. 2005; Lysak et al. 2006; Mandakova and Lysak 2008), Triticeae (Luo et al. 2009), and recently *Cucumis* in fully sequenced genomes of cucumber and its sister species were compared (Yang et al. 2013). In *Arabidopsis*, gradual rearrangements involving inversions, fusions, and translocations led from an ancestral $n = 8$ to $n = 5$ in *A. thaliana* (Lysak et al. 2006).

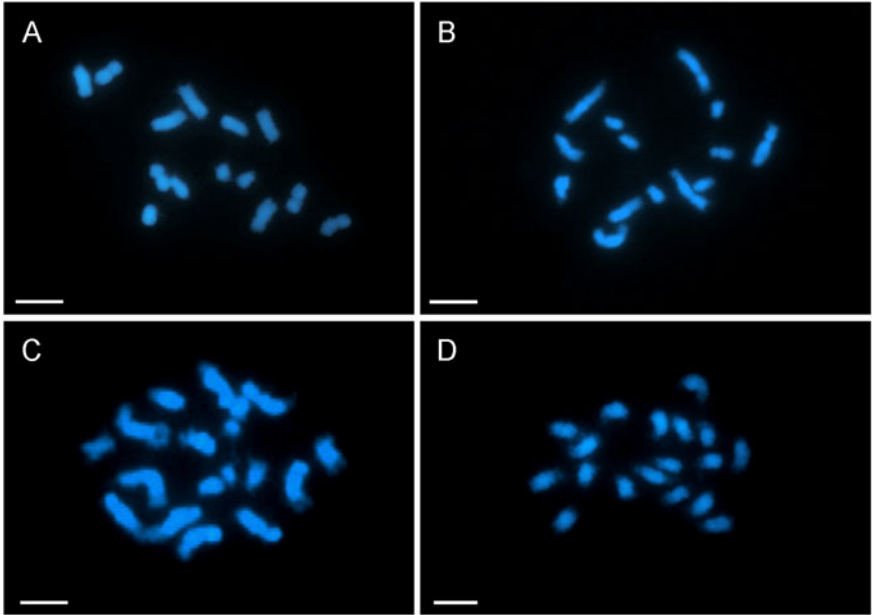


Fig. 2. DAPI-stained metaphase chromosomes. **A**, *Jarilla caudata* ($2n = 14$); **B**, *Jarilla heterophylla* ($2n = 14$); **C**, *Horovitzia cnidoscolooides* ($2n = 16$); and **D**, *Cylicomorpha parviflora* ($2n = 18$). Bar corresponds to $5 \mu\text{m}$

In *Cucumis sativus*, a similar mix of mechanisms led to dysploid chromosome reduction from an $n = 12$ ancestor to the $n = 7$ karyotype of cucumber (Yang et al. 2013). Different from the other genera so far studied, large and small chromosome pairs are identifiable in the karyotype the two *Jarilla* species (Fig. 2A, B). This finding supports our interpretation of the *Jarilla* karyotype having originated from a past fusion event in an ancestral karyotype with $n = 9$. Genomic and cytogenetic analyses of entire syntenic genomes in principle allow inferring the details of such past rearrangements, and the present results make the Caricaceae another family in which the mechanisms of dysploidy could fruitfully be studied.

Table 1. Chromosome counts and genome size for species of Caricaceae with references, including results first reported here. Genome size is expressed in millions of base pairs (Mbp) per haploid genome. Where a species' name has changed due to the taxonomic revision (Carvalho, F.A. 2013 onward), the name used in the original publication is given in brackets. Four C-values were obtained in August 2013 in the lab of Ray Ming at the University of Illinois in Urbana-Champaign from plants grown from the same seed lots and following the methods of Gschwend et al. (2013); other chromosomes numbers come from Tina Kyndt, Dept. of Molecular Biotechnology, Ghent University (personal communications, August and November 2013). RUG refers to E. H Romeijn-Peeters (Kyndt et al. 2005: Table 1). HCAR refers to the accession number of the Clonal Germplasm Repository for Tropical and Subtropical Crops in Hilo, Hawaii, USA.

Species	Voucher	Chromosome number (size)	Genome size	References
<i>Cylicomorpha parviflora</i> Urb.	Carvalho, 2238 (M)	2n = 18		this study
			968 Mbp	R. Ming, from same seed lot
<i>Jarilla caudata</i> (Brandege) Standl.	Carvalho, 2240 (M)	2n = 14		this study
			924 Mbp	R. Ming, from same seed lot
<i>Jarilla heterophylla</i> (Cerv. ex La Llave) Rusby	Carvalho, 2239 (M)	2n = 14		this study
			836 Mbp	R. Ming, from same seed lot
<i>Horovitzia cnidoscoloides</i> (Lorence & Torres Colín, R.) V.M.Badillo	Carvalho, 2242 (M)	2n = 16		this study
			401 Mbp	R. Ming, from same seed lot
<i>Carica papaya</i> L.	Not cited	2n = 18		Heilborn 1921; Simmonds 1954; Joshi & Ranjekar 1982
	Not cited	2n = 18 (1-4.23 µm)		Datta, 1971
	Not cited		372 Mbp	Arumuganathan and Earle 1991
	Not cited Not cited	2n = 18 2n = 18 (1.52-2.29 µm)		Costa et al. 2008 Damasceno et al. 2009
	Not cited	2n = 18	318 Mbp	Araújo et al. 2010
	HCAR 320		442.5 Mbp	Gschwend et al. 2013
	RUG 57 (GENT)	2n = 18		T. Kyndt
<i>Jacaratia spinosa</i> (Aubl.) A.DC. (<i>Carica dodecaphylla</i> Vell.)	Not cited	2n = 18		Kumar & Srinivasan 1944
	Not cited	2n = 18		Silva et al. 2012
	HCAR 227		513.6 Mbp	Gschwend et al. 2013

<i>Vasconcellea candicans</i> A. DC.	RUG113 (GENT)	2n = 18		T. Kyndt
<i>Vasconcellea cauliflora</i> (Jacq.) A.DC.	Not cited	2n = 18		Caetano et al. 2008
	RUG 284 (GENT)	2n = 18		T. Kyndt
<i>Vasconcellea glandulosa</i> A.DC.	HCAR 300		534.9 Mbp	Gschwend et al. 2013
<i>Vasconcellea goudotiana</i> Triana & Planch. (<i>Carica goudotiana</i> (Triana & Planch.) Solms)	Not cited	2n = 18		de Zerpa 1959
	Not cited	2n = 18		Costa et al. 2008
	Not cited	2n = 18		Caetano et al. 2008
	Not cited	2n = 18		Silva et al. 2012
	HCAR 167		607 Mbp	Gschwend et al. 2013
	RUG285 (GENT)	2n = 18		T. Kyndt
<i>Vasconcellea longiflora</i> (V.M.Badillo) V.M.Badillo	Not cited	2n = 18		Caetano et al. 2008
	RUG228 (GENT)	2n = 18		T. Kyndt
<i>Vasconcellea microcarpa</i> (Jacq.) A.DC. (<i>Carica microcarpa</i> Jacq.)	Not cited	2n = 18		de Zerpa 1959
<i>Vasoncellea monoica</i> (Desf.) A.DC. (<i>Carica monoica</i> Desf.)	Not cited	2n = 18		de Zerpa 1959
	Not cited	2n = 18 (1.35-2.49 µm)		Damasceno et al. 2009
	HCAR 171		625.9 Mbp	Gschwend et al. 2013
	RUG58 (GENT)	2n = 18		T. Kyndt
<i>Vasconcellea pubescens</i> A.DC. (<i>Vasconcellea cundinamaricensis</i> V.M. Badillo)	Not cited	2n = 18		Heilborn 1921; Costa et al. 2008; Caetano et al. 2008
	Not cited	2n = 18 (1.66-2.45 µm)		Damasceno et al. 2009
	HCAR 46		566.7 Mbp	Gschwend et al. 2013
	RUG161 (GENT)	2n = 18		T. Kyndt
<i>Vasconcellea palandensis</i> (V.M.Badillo, Van den Eynden & Van Damme) V.M.Badillo	Not cited	2n = 18		Caetano et al., 2008
<i>Vasconcellea quercifolia</i> A.St.-Hil.	Not cited	2n = 18		Silva et al. 2012
	RS3586	2n = 18		Bernadello et al. 1990
	HCAR 226		516.1 Mbp	Gschwend et al. 2013

<i>Vasconcellea horovitziana</i> (V.M. Badillo) V.M.Badillo	HCAR 305		557.7 Mbp	Gschwend et al. 2013
<i>Vasconcellea parviflora</i> A.DC.	HCAR 180/179		491.5 Mbp	Gschwend et al. 2013
<i>Vasconcellea sphaerocarpa</i> (García-Barr. & Hern.Cam.) V.M.Badillo	Not cited	2n = 18		Caetano et al. 2008
<i>Vasconcellea stipulata</i> (V.M.Badillo) V.M.Badillo	HCAR 177 RUG 55(GENT)	2n = 18	520.1 Mbp	Gschwend et al. 2013 T. Kyndt
<i>Vasconcellea pulchra</i> (V.M.Badillo) V.M. Badillo	HCAR 267		554.6 Mbp	Gschwend et al. 2013
<i>Vasconcellea weberbaueri</i> (Harms) V.M.Badillo	RUG10 (GENT)	2n = 18		T. Kyndt
<i>Vasconcellea</i> × <i>heilbornii</i> (V.M. Badillo) V.M.Badillo	Not cited	2n = 18		Heilborn 1921
	RUG198 (GENT)	2n = 18		T. Kyndt

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