Annals of Botany **100**: 505–526, 2007
doi:10.1093/aob/mcm144, available online at www.aob.oxfordjournals.org

OXFORD JOURNALS
OXFORD UNIVERSITY PRESS

Chromosome Numbers and Genome Size Variation in Indian Species of *Curcuma* (Zingiberaceae)

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Received: 1 February 2007 Returned for revision: 5 March 2007 Accepted: 15 May 2007 Published electronically: 8 August 2007

• *Background and Aims* Genome size and chromosome numbers are important cytological characters that significantly influence various organismal traits. However, geographical representation of these data is seriously unbalanced, with tropical and subtropical regions being largely neglected. In the present study, an investigation was made of chromosomal and genome size variation in the majority of *Curcuma* species from the Indian subcontinent, and an assessment was made of the value of these data for taxonomic purposes.
• *Methods* Genome size of 161 homogeneously cultivated plant samples classified into 51 taxonomic entities was determined by propidium iodide flow cytometry. Chromosome numbers were counted in actively growing root tips using conventional rapid squash techniques.
• *Key Results* Six different chromosome counts ($2n = 22$, 42, 63, >70, 77 and 105) were found, the last two representing new generic records. The 2C-values varied from 1·66 pg in *C. vamana* to 4·76 pg in *C. oligantha*, representing a 2·87-fold range. Three groups of taxa with significantly different homoploid genome sizes (Cx-values) and distinct geographical distribution were identified. Five species exhibited intraspecific variation in nuclear DNA content, reaching up to 15·1 % in cultivated *C. longa*. Chromosome counts and genome sizes of three *Curcuma*-like species (*Hitchenia caulina*, *Kaempferia scaposa* and *Paracautleya bhatii*) corresponded well with typical hexaploid ($2n = 6x = 42$) *Curcuma* spp.
• *Conclusions* The basic chromosome number in the majority of Indian taxa (belonging to subgenus *Curcuma*) is $x = 7$; published counts correspond to $6x$, $9x$, $11x$, $12x$ and $15x$ ploidy levels. Only a few species-specific C-values were found, but karyological and/or flow cytometric data may support taxonomic decisions in some species alliances with morphological similarities. Close evolutionary relationships among some cytotypes are suggested based on the similarity in homoploid genome sizes and geographical grouping. A new species combination, *Curcuma scaposa* (Nimmo) Škorničk. & M. Sabu, comb. nov., is proposed.

Key words: Chromosome number, *Curcuma*, cytology, DNA C-value, flow cytometry, genome size, India, intraspecific variation, polyploidy, taxonomy.

INTRODUCTION

The genus *Curcuma* L. (Zingiberaceae) contains many taxa of economic, medicinal, ornamental and cultural importance, turmeric (*C. longa* L.) probably being the best known. It is found throughout south and south-east Asia with a few species extending to China, Australia and the South Pacific. The highest diversity is concentrated in India and Thailand, with at least 40 species in each area, followed by Burma, Bangladesh, Indonesia and Vietnam. Due to the lack of a comprehensive taxonomic revision, there is still little consensus on the number of species that should be recognized. Recent estimates vary from about 50 (Smith, 1981) to 80 (Larsen *et al.*, 1998) and 100 species (Sirirugsa, 1996), although Škorničková *et al.* (2004) suggested that their number will probably reach 120 in the near future in connection with detailed botanical exploration of India and south-east Asia.

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Several taxonomic and biological problems have hindered satisfactory systematic treatment of the genus. Original descriptions of many *Curcuma* species are vague and inaccurate, and type specimens are often lacking or fragmentary. Proper preservation of *Curcuma* specimens is extremely difficult, exacerbating the limited amount of type material, leading to ambiguous name assignment and usage. In addition, high intra- and interpopulation variation has led to debate concerning species concepts and boundaries. As a result, one species has often been described repeatedly under different names whereas the same name has been applied to different taxonomic entities.

Some species may hybridize in the wild and the crosses may become naturalized (Škorničková and Sabu, 2005*b*; Škorničková *et al.*, 2007). Frequent cultivation of *Curcuma* spp. and targeted selection of peculiar morphotypes have further contributed to taxonomic complexity of the group. Moreover, polyploidy has played a significant role in evolution and diversification of various members of Zingiberaceae (e.g. Mukherjee, 1970; Lim, 1972*a*,*b*; Poulsen, 1993; Chen and Chen, 1984; Takano, 2001;

Takano and Okada, 2002), including *Curcuma* (Prana *et al.*, 1978; Apavatjrut *et al.*, 1996; Joseph *et al.*, 1999; Ardiyani, 2002; Sirisawad *et al.*, 2003). It is well documented, in both plants and animals, that an increase in ploidy level is commonly associated with blurring of morphological boundaries between taxa (see Stace, 2000).

The occurrence of different ploidy levels in *Curcuma* was highlighted in early cytological studies (e.g. Suguira, 1931, 1936; Raghavan and Venkatasubban, 1943; Venkatasubban, 1946; Chakravorti, 1948; Sharma and Bhattacharya, 1959), and 20 different somatic chromosome numbers have been reported (Table 1). Considering the widely accepted basic chromosome number $x = 21$ (Ramachandran, 1961; Prana, 1977; Islam, 2004), this chromosomal variation roughly corresponds to three euploid levels ($2x$, $3x$ and $4x$) plus several aneuploids. However, many of the records should be treated with caution due to potential errors in chromosome counting as well as taxonomic ambiguity of the analysed material, which is rarely documented by herbarium vouchers. Such a high basic chromosome number is likely to be secondary, further complicating accurate inference of ploidy level and its evolution within the genus.

In addition to chromosome numbers and ploidy levels, genome size (nuclear DNA content) data also provide information useful in various fields of plant biology, including systematics, evolution and conservation (Bennett and Leitch, 2005*b*). In plants holoploid genome sizes (or 1C-values) vary strikingly, ranging from about 0·065 to 127·4 pg. Genome size variation has significant consequences at cellular, tissue and organismal levels and also influences phenological and ecological behaviour. Despite its usefulness in understanding plant evolution and diversification, genome size variation in *Curcuma* is not well documented, and estimates for only a few species have been published (Table 1). Bharathan *et al.* (1994) determined by flow cytometry 1C = 1·30 pg in *C. zanthorrhiza* and Das *et al.* (1999) used cytophotometry to study genome size in *C. amada* (4C = 3·120 pg), *C. caesia* (4C = 4·234 pg) and *C. longa* (4C = 5·100–5·263 pg). *Curcuma longa* was also investigated by Nayak *et al.* (2006), who observed 4C-values ranging from 4·30 to 8·84 pg in 17 varieties. In addition, flow-cytometric nuclear DNA amounts for 16 taxa (including the above-mentioned species and one undetermined sample) from Bangladesh are given in the unpublished PhD thesis of Islam (2004).

As a part of ongoing comprehensive taxonomic revision of *Curcuma* in India (see Škorničková *et al.*, 2003*a*,*b*, 2004, 2007; Škorničková *and* Sabu, 2005*a*,*b*,*c*), the present study aimed to provide a detailed survey of chromosomal and genome size variation in the majority of known Indian species. In particular, we address whether genome size and chromosome numbers can be used as taxonomically informative markers for species delimitation and whether they can elucidate the taxonomic position of four species with *Curcuma*-like morphological traits often placed in separate genera (*Hitchenia caulina*, *Kaempferia scaposa*, *Paracautleya bhatii* and *Stahlianthus involucratus*). The issue of basic chromosome number and the origin of the polyploid taxa are also discussed in the light of the findings.

The infrageneric classification (subgenera *Curcuma* and *Hitcheniopsis*) generally followed the treatment of Schumann (1904), with some modifications. In particular, *C. petiolata* and *C. roscoeana* were included in subgenus *Curcuma* based on the presence of two floral epigynous glands (derived from gynopleural nectaries). This hitherto neglected character (well developed in subgenus *Curcuma* but absent in subgenus *Hitcheniopsis*) seems to be pivotal for the updated subgeneric delimitation, better reflecting the current state of knowledge than did previous taxonomic concepts (J. Leong-Škorničková *et al.*, unpubl. res.).

MATERIAL AND METHODS

Plant materials

In total, 161 individual plants belonging to 51 taxonomic entities were included in the study, 46 *Curcuma* species (31 assigned to species, 15 determined only tentatively or undetermined), one natural hybrid and four species often classified into separate but related genera: *Hitchenia caulina*, *Kaempferia scaposa*, *Paracautleya bhatii* and *Stahlianthus involucratus*. The number of individuals per species varied from one to 16. Multiple samples were available for 26 species, whereas 25 taxa (12 *Curcuma* species, eight undetermined taxa, four species from related genera and one hybrid) were represented by a single plant accession.

Owing to difficulties surrounding systematic treatment of the genus, specific names were assigned only to plant samples perfectly matching the species description. The remaining samples were left unnamed in order to avoid misleading information resulting from unambiguous identification. All plants were collected in the wild on the Indian peninsula, often at or near the *locus classicus*, during 2000–2004 (Table 2) and are being grown at the Calicut University Botanical Garden, Kerala, India (11 °35′N, 70 °45′E, 50 m a.s.l.). Geographical positions of the collection localities are shown in Fig. 1. Herbarium vouchers are deposited in CALI, with duplicates in MH and SING; incomplete sets are also kept in CAL, K and PR; vouchers of *C. oligantha* from Sri Lanka are deposited in PDA and SING. In addition, a large collection of photographic documentation of living material (including details of flower morphology) is available for each accession (see Fig. 5).

Genome size estimation

Nuclear DNA C-values (= holoploid genome sizes) and Cx-values (= monoploid genome sizes) were estimated using propidium iodide flow cytometry (FCM). Sample preparation generally followed the two-step procedure originally described by Otto (1990). Plants were cultivated for at least 1 year under homogeneous conditions. About 1 cm^2 of young and intact fresh leaf tissue and internal standard was co-chopped in a sandwich-like arrangement with a sharp razor blade in 1 mL of ice-cold Otto I buffer (0·1 M citric acid, 0·5 % Tween 20). The nuclear suspension was filtered through a nylon mesh (42-μm pore size) and centrifuged at 150*g* for 5 min. The supernatant was then removed

TABLE 1. *A synopsis of published chromosome counts and genome sizes in the genus* Curcuma

Species	No. of chromosomes		Genome size (pg)*	Origin of plant material	Reference
	n	2*n*			
subgen. *Curcuma* K.Schum.					
C. aeruginosa Roxb.		63		Indonesia	Prana (1977, 1978)
		63		Thailand	Apavatjrut *et al.* (1996)
		63		India	Joseph *et al.* (1999)
		63		Thailand	Paisooksantivatana and Thepsen (2001)
		63		Indonesia	Ardiyani (2002)
	28–35	63		Thailand	Sirisawad *et al.* (2003)
		63, 84	3·203–5·302	Bangladesh	Islam (2004)
C. amada Roxb		42		India	Raghavan and Venkat. (1943)
		42		India	Chakravorti (1948)
		42		India	Raghavan and Arora (1958)
		42		India	Sharma and Bhattacharya (1959)
		42		India	Ramachandran (1961, 1969)
		40	4·234/4C	India	Das *et al.* (1999)
		42	2·132	Bangladesh	Islam (2004)
C. amarissima Roscoe		63	3·289	Bangladesh	Islam (2004)
C. angustifolia Roxb.		42		India	Chakravorti (1948)
		42		India	Sharma and Bhattacharya (1959)
		42	2·121–2·141	Bangladesh	Islam (2004)
C. aromatica Salisb.		42		India	Raghavan and Venkat (1943)
		42		India	Chakravorti (1948)
		63, 86		India	Ramachandran (1961, 1969)
		84		India	Nambiar *et al.* (1982)
		63		China	Chen and Chen (1984)
		42		India	Sarkar (1990)
		63		Thailand	Paisooksantivatana and Thepsen (2001)
		63	3·184	Bangladesh	Islam (2004)
C. attenuata Wall. ex Baker	42	84		Thailand	Apavatjrut *et al.* (1996)
	42	84		Thailand	Sirisawad *et al.* (2003)
C. australasica Hook.f			2·153–1·181	Bangladesh	Islam (2004)
C. aurantiaca Zijp		42		Indonesia	Prana (1977, 1978)
	21			Pen. Malaysia	Beltran and Kam (1984)
	21	42		Thailand	Sirisawad *et al.* (2003)
C. brog Valeton		63, 64, 70		Indonesia	Prana (1977)
		63, 64		Indonesia	Prana (1978)
C. caesia Roxb.		22	3·12/4C	India	Das *et al.* (1999)
		63		India	Joseph *et al.* (1999)
		63	3·333	Bangladesh	Islam (2004)
C. colorata Valeton		62, 63		Indonesia	Prana (1977, 1978)
C. comosa Craib		42		India	Joseph *et al.* (1999)
C. cf. *comosa* Roxb.		63		Thailand	Paisooksantivatana and Thepsen (2001)
C. decipiens Dalzell	21	42		India	Ramachandran (1961, 1969)
C. elata Roxb.		63		Thailand	Apavatjrut *et al.* (1996)
	28–35	63		Thailand	Sirisawad *et al.* (2003)
		63	3·181	Bangladesh	Islam (2004)
C. haritha Mangaly & M. Sabu		42		India	Joseph *et al.* (1999)

Continued

TABLE 1. *Continued*

Species	No. of chromosomes		Genome size (pg)*	Origin of plant material	Reference
	n	2*n*			
C. heyneana Valeton & Zijp		63		Indonesia	Prana (1977, 1978)
		63		Indonesia	Ardyiani (2002)
C. kwangsiensis S.G.Lee & C.F.Liang	42	84		China	Chen *et al.* (1988)
C. latifolia Roscoe		63	3·435	Bangladesh	Islam (2004)
C. longa L.		64		Unknown	Sugiura (1931, 1936)
		62		India	Raghavan and Venkat (1943)
		62, 63, 64		India	Chakravorti (1948)
		32		Unknown	Sato (1948)
		62, 93		India	Sharma and Bhattacharya (1959)
		63		India	Ramachandran (1961, 1969)
		63		Indonesia	Prana (1977, 1978)
		48	5·1–5·26/4C	India	Das *et al.* (1999)
		63		Thailand	Paisooksantivatana and Thepsen (2001)
		63	3·275	Bangladesh	Islam (2004)
		48	4·30–8·84/4C	India	Nayak *et al.* (2006)
C. malabarica Velay., Mural. & Amalraj		42		India	Joseph *et al.* (1999)
C. mangga Valeton & Zijp		42		Indonesia	Prana (1977, 1978)
		63		Indonesia	Ardyiani (2002)
C. neilgherrensis Wight		42		India	Chakravorti (1948)
		42		India	Ramachandran (1961, 1969)
C. aff. *oligantha* Trimen		42		Thailand	Eksomtramage *et al.* (2002)
		40		Thailand	Saensouk and Chantaranothai (2003)
C. petiolata Roxb.		64		India	Venkatasubban (1946)
		42		Indonesia	Prana (1977, 1978)
		42		Thailand	Apavatjrut *et al.* (1996)
	21	42		Thailand	Sirisawad *et al.* (2003)
		42	2·142	Bangladesh	Islam (2004)
C. cf. *petiolata* Roxb.		42		Thailand	Paisooksantivatana and Thepsen (2001)
C. phaeocaulis Valeton		62, 63, 64		Indonesia	Prana (1977, 1978)
C. purpurascens Blume		63		Indonesia	Prana (1977, 1978)
C. raktakanta Mangaly & M.Sabu		63		India	Joseph *et al.* (1999)
	21	42		Thailand	Sirisawad *et al.* (2003)
C. roscoeana Wall.	21	42		Thailand	Apavatjrut *et al.* (1996)
		42		Thailand	Eksomtramage *et al.* (1996*a*,*b*)
		42		Thailand	Paisooksantivatana and Thepsen (2001)
C. rubescens Roxb.	28–35	63		Thailand	Sirisawad *et al.* (2003)
		63	2·204	Bangladesh	Islam (2004)
		42		Bangladesh	Islam (2004)
C. sessilis Gage		84		Thailand	Saensouk *et al.* (1998)
		46, 92		Thailand	Saensouk and Chantaranothai (2003)
C. soloensis Valeton		63		Indonesia	Prana (1977, 1978)
C. viridiflora Roxb.		42	2·164–2·173	Bangladesh	Islam (2004)
C. wenyujin Y.H.Chen & C.Ling		63		China	Chen and Chen (1984)
C. zanthorrhiza Roxb.		63		Indonesia	Prana (1977, 1978)
		63		China	Chen and Chen (1984)
		63		Thailand	Apavatjrut *et al.* (1996)
		63		Indonesia	Ardiyani (2002)
	28–35	63		Thailand	Sirisawad *et al.* (2003)

		63	3·285	Bangladesh	Islam (2004)
			1·30/1C	Origin unknown	Bharathan *et al.* (1994)
C. zedoaria (Christm.) Roscoe		64		India	Venkatasubban (1946)
		63, 64		India	Chakravorti (1948)
		63		India	Ramachandran (1961, 1969)
		66		India	Sharma (1970)
		63, 64, 66		Indonesia	Prana (1977, 1978)
		66		India	Chatterjee *et al.* (1989)
		63		Thailand	Apavatjrut *et al.* (1996)
		42		Thailand	Paisooksantivatana and Thepsen (2001)
		63		Indonesia	Ardiyani (2002)
	28–35	63		Thailand	Sirisawad *et al.* (2003)
		63	3·321	Bangladesh	Islam (2004)
subgen. *Hitcheniopsis* (Baker) K.Schum.					
C. alismatifolia Gagnep.	16	32		Thailand	Apavatjrut *et al.* (1996)
		32		Thailand	Saensouk *et al.* (1998)
		32		Thailand	Paisooksantivatana and Thepsen (2001)
		32		Thailand	Saensouk and Chantaranothai (2003)
	16	32		Thailand	Sirisawad *et al.* (2003)
C. gracillima Gagnep.		24		Thailand	Saensouk *et al.* (1998)
		24		Thailand	Saensouk and Chantaranothai (2003)
	16	32		Thailand	Sirisawad *et al.* (2003)
C. cf. *gracillima* Gagnep.		40		Thailand	Paisooksantivatana and Thepsen (2001)
C. harmandii Gagnep.	10	20		Thailand	Eksomtramage *et al.* (1996*a*,*b*)
		20		Thailand	Paisooksantivatana and Thepsen (2001)
	10	20		Thailand	Sirisawad *et al.* (2003)
C. parviflora Wall.	14, 17, 18, 28	28, 34, 36		Thailand	Apavatjrut *et al.* (1996)
		32		Thailand	Weerapakdee and Krasaechai (1997)
		30		Thailand	Saensouk *et al.* (1998)
		42		Thailand	Paisooksantivatana and Thepsen (2001)
		32		Thailand	Eksomtramage *et al.* (2002)
		30		Thaialnd	Saensouk and Chantaranothai (2003)
	16	32		Thailand	Sirisawad *et al.* (2003)
	12, 14, 17, 18, 28	24, 28, 34, 36, 56		Thailand	Sirisawad *et al.* (2003)
C. rhabdota Sirirugsa & M.F.Newman		24		Thailand	Eksomtramage *et al.* (2002)
	12	24		Thailand	Sirisawad *et al.* (2003)
C. thorelii Gagnep.		36		Thailand	Eksomtramage and Boontum (1995)
	17	34		Thailand	Apavajrut *et al.* (1996)
		36		Thailand	Paisooksantivatana and Thepsen (2001)
		38?		Thailand	Ardiyani (2002)
		34		Thailand	Saensouk and Chantaranothai (2003)
	17	34		Thailand	Sirisawad *et al.* (2003)

* Genome sizes expressed in 2C-values unless indicated otherwise.

TABLE 2. *2C nuclear DNA content with standard error, mean value for a species, intraspecific variation, 1C-value expressed in DNA picograms and megabase pairs (1 pg = 978 Mbp), somatic chromosome number (2n), ploidy level, homoploid genome size (Cx-value, determined as 2C DNA amount/ploidy level) and its mean for a species, internal standard used, locality, species distribution pattern, and a field accession number for 161 Indian plants belonging to 51 taxa of* Curcuma *and related genera.*

Group/species	Field no.	2C-value (pg) ± s.e.	Mean 2C-value (pg)*	Intraspecific variation (%)	1C-value (pg)	1C-value (Mbp)	$2n$[†]	Ploidy level (x) or DNA ploidy level	1Cx-value (pg)	Mean Cx-value (pg)*	Internal standard[‡]	Locality	Distribution pattern[§]
DIPLOID ($x = 11$)													
C. vamana M. Sabu & Mangaly	84156	1·663 ± 0·008	1·66[u]		0·83	813	22!	2	0·83	0·83[b]	L	Kerala, Trichur Dt.	SW
HEXAPLOIDS–GROUP I													
C. amada Roxb.	71421	1·882 ± 0·002	1·86[st]	3·6	0·94	920		6	0·31	0·31[lmnopqrs]	G	W. Bengal, Kolkata	NE & E
	71472	1·854 ± 0·007			0·93	907	42	6	0·31		L	W. Bengal, Darjeeling Dt.	
	73440	1·816 ± 0·002			0·91	888		6	0·30		L	Bihar, Bhagalpur Dt.	
	73482	1·873 ± 0·004			0·94	916		6	0·31		G	W. Bengal, Kolkata	
	73484	1·875 ± 0·006			0·94	917		6	0·31		G	W. Bengal, Kolkata	
C. aromatica Salisb. *s.l.*–sp. 1	73423	1·900 ± 0·008	1·86[st]	3·5	0·95	929	42	6	0·32	0·31[lmnopqrs]	L	Sri Lanka, Kegalle Dt.	SW & SL
	84109	1·846 ± 0·006			0·92	903	42	6	0·31		L	Kerala, Kollam Dt.	
	84114	1·880 ± 0·006			0·94	919		6	0·31		G	Kerala, Kollam Dt.	
	84123	1·876 ± 0·001			0·94	917		6	0·31		G	Kerala, Wynad Dt.	
	84123-II	1·881 ± 0·004			0·94	920		6	0·31		G	Kerala, Wynad Dt.	
	84170	1·865 ± 0·006			0·93	912		6	0·31		G	Kerala, Idukki Dt.	
	84183	1·841 ± 0·001			0·92	900		6	0·31		G	Kerala, Idukki Dt.	
	84183A	1·836 ± 0·009			0·92	898		6	0·31		G	Kerala, Idukki Dt.	
	84183B	1·838 ± 0·002			0·92	899		6	0·31		G	Kerala, Idukki Dt.	
C. mangga Valeton & Zijp	84101	1·807 ± 0·001	1·83[t]	3·2	0·90	884		6	0·30	0·31[mnopqrs]	G	Kerala, Ernakulam Dt.	SW
	84115	1·862 ± 0·008			0·93	911		6	0·31		L	Kerala, Kollam Dt.	
	84149	1·851 ± 0·002			0·93	905		6	0·31		G	Kerala, Trichur Dt.	
	84150	1·804 ± 0·002			0·90	882	42	6	0·30		G	Kerala, Trichur Dt.	

C. montana Roxb.	71484	1·816 ± 0·006	1·79[tu]	6·1	0·91	888	42!	6	0·30	0·30[rs]	L	Jharkhand, Ranchi Dt.	E & C
	73419	1·854 ± 0·008			0·93	907	42!	6	0·31		L	Jharkhand, Paschim Singhbum Dt.	
	73425	1·823 ± 0·004			0·91	891		6	0·30		G	Orissa, Koraput Dt.	
	73430	1·786 ± 0·008			0·89	873		6	0·30		L	Orissa, Koraput Dt.	
	73433	1·806 ± 0·004			0·90	883		6	0·30		G	Jharkhand, Paschim Singhbum Dt.	
	73433-II	1·778 ± 0·002			0·89	869		6	0·30		G	Jharkhand, Paschim Singhbum Dt.	
	73437	1·774 ± 0·001			0·89	867		6	0·30		G	Jharkhand, Paschim Singhbum Dt	
	73456	1·763 ± 0·004			0·88	862		6	0·29		G	Jharkhand, Ranchi Dt.	
	73471	1·767 ± 0·004			0·88	864		6	0·29		G	Chhattisgarh, Jagdalpur Dt.	
	73473	1·782 ± 0·004			0·89	871		6	0·30		G	Chhattisgarh, Jagdalpur Dt.	
	73474	1·774 ± 0·008			0·89	867		6	0·30		G	Chhattisgarh, Jagdalpur Dt.	
	73479	1·748 ± 0·002			0·87	855		6	0·29		G	Chhattisgarh, Bilaspur Dt.	
C. prakasha S. Tripathi	71441	1·877 ± 0·003	1·87[st]	4·5	0·94	918	ca 42!	6	0·31	0·31[klmnopqr]	L	Meghalaya, Ribhoi Dt.	NE
	71442	1·847 ± 0·005			0·92	903		6	0·31		L	Meghalaya, Ribhoi Dt.	
	71450	1·918 ± 0·009			0·96	938		6	0·32		G	Meghalaya, S. Garo Hills Dt.	
	71462	1·893 ± 0·002			0·95	926	42!	6	0·32		G	Meghalaya, E. Garo Hills Dt.	
	71463	1·835 ± 0·007			0·92	897		6	0·31		G	Meghalaya, E. Garo Hills Dt.	
	73406	1·872 ± 0·001			0·94	915		6	0·31		G	Meghalaya, E. Khasi Hills Dt.	
C. roscoeana Wall.	73309	1·962 ± 0·005	1·96[s]		0·98	959	42	6	0·33	0·33[k]	G	Andaman Isls. M. Andaman	ANI
C. rubescens Roxb.	71454	1·889 ± 0·005	1·87[st]	2·5	0·94	924		6	0·31	0·31[lmnopqrs]	L	Meghalaya, E. Garo Hills Dt.	NE
	71457	1·843 ± 0·004			0·92	901	ca 42	6	0·31		L	Meghalaya, E. Garo Hills Dt.	
C. rubrobracteata Škorničk., M. Sabu & Prasanthk.	86241	1·836 ± 0·008	1·84[st]		0·92	898	42!	6	0·31	0·31[mnopqrs]	L	Mizoram, Lawngtlai Dt.	NE
C. sp. 'sulphurea'	86231	1·853 ± 0·002	1·85[st]		0·93	906	42!	6	0·31	0·31[lmnopqrs]	G	Mizoram, Lunglei Dt.	NE
C. sp. 'repens'	71456	1·834 ± 0·007	1·83[st]		0·92	897	42!	6	0·31	0·31[mnopqrs]	L	Meghalaya, E. Garo Hills Dt.	NE

Continued

TABLE 2. *Continued*

Group/species	Field no.	2C-value (pg) ± s.e.	Mean 2C-value (pg)*	Intraspecific variation (%)	1C-value (pg)	1C-value (Mbp)	$2n$[†]	Ploidy level (x) or DNA ploidy level	1Cx-value (pg)	Mean Cx-value (pg)*	Internal standard[‡]	Locality	Distribution pattern[§]
HEXAPLOIDS–GROUP II													
C. angustifolia Roxb.	73449	2·148 ± 0·003	2·15[r]	2·3	1·07	1050	ca 42	6	0·36	0·36[j]	G	Uttaranchal, Dehra Dun Dt.	N & E
	73452	2·158 ± 0·007			1·08	1055	42	6	0·36		G	Uttaranchal, Tehri Gharwal Dt.	
	73452-II	2·162 ± 0·007			1·08	1057		6	0·36		G	Uttaranchal, Tehri Gharwal Dt.	
	73454	2·145 ± 0·001			1·07	1049		6	0·36		G	Uttaranchal, Tehri Gharwal Dt.	
	73465	2·114 ± 0·004			1·06	1034		6	0·35		G	Jharkhand, Sahibganj Dt.	
	73480	2·153 ± 0·006			1·08	1053		6	0·36		G	Chhattisgarh, Bilaspur Dt.	
C. aurantiaca Zijp	73455	2·223 ± 0·008	2·20[qr]	3·1	1·11	1087		6	0·37	0·37[hij]	G	Kerala, Kozhikode Dt.	SW
	84108	2·156 ± 0·007			1·08	1054	42	6	0·36		G	Kerala, Kollam Dt.	
	77019	2·206 ± 0·004			1·10	1079	42	6	0·37		G	Kerala, Kozhikode Dt.	
C. cannanorensis R. Ansari, V. J. Nair & N. C. Nair	84144	2·330 ± 0·007	2·33[op]	0·0	1·17	1139	42!	6	0·39	0·39[efg]	G	Kerala, Kannur Dt.	SW
	84164	2·331 ± 0·005			1·17	1140	42!	6	0·39		B	Karnataka, Udupi Dt.	
C. decipiens Dalzell	73445	2·363 ± 0·010	2·35[op]	2·8	1·18	1156		6	0·39	0·39[e]	B	Maharashtra, Sindudurg Dt.	W
	84179	2·305 ± 0·003			1·15	1127		6	0·38		G	Maharashtra, Sindudurg Dt.	
	84179	2·370 ± 0·007			1·19	1159	42	6	0·40		B	Maharashtra, Sindudurg Dt.	
C. inodora Blatt.	73403	2·290 ± 0·006	2·29[pq]		1·15	1120	42!	6	0·38	0·38[efg]	G	Maharashtra, Thane Dt.	W
C. karnatakensis Amalraj, Velay. & Mural	84163	2·340 ± 0·012	2·34[op]		1·17	1144	42!	6	0·39	0·39[ef]	B	Karnataka, Uttar Kannad Dt.	SW
C. kudagensis Velay., V. S. Pillai & Amalraj	84152	2·287 ± 0·007	2·29[pq]		1·14	1118	42!	6	0·38	0·38[efgh]	B	Karnataka, Kodagu Dt.	SW
C. neilgherrensis Wt.	73490	2·251 ± 0·011	2·29[pq]	3·8	1·13	1101		6	0·38	0·38[efg]	G	Tamil Nadu, Nilgiris Dt.	S & SW
	84157	2·336 ± 0·001			1·17	1142		6	0·39		G	Kerala, Wyanad Dt.	
	84174	2·297 ± 0·005			1·15	1123	42	6	0·38		B	Tamil Nadu, Nilgiris Dt.	
	84181	2·273 ± 0·001			1·14	1111		6	0·38		G	Kerala, Wyanad Dt.	
C. pseudomontana J. Graham	73401	2·225 ± 0·005	2·25[pqr]	2·4	1·11	1088		6	0·37	0·38[fghi]	G	Maharashtra, Pune Dt.	W
	73402	2·279 ± 0·010			1·14	1114	42!	6	0·38		B	Maharashtra, Pune Dt.	

C. reclinata Roxb.	73477	2·313 ± 0·002	2·29^{pq}	3·9	1·16	1131	42!	6	0·39	0·38^{efgh}	G	Chhattisgarh, Bilaspur Dt.	C
	73467	2·226 ± 0·008			1·11	1089		6	0·37		G	Madhya Pradesh, Hoshangabad Dt.	
	73469	2·292 ± 0·003			1·15	1121		6	0·38		G	Madhya Pradesh, Satna Dt.	
	73469	2·309 ± 0·007			1·15	1129		6	0·38		G	Madhya Pradesh, Satna Dt.	
HEXAPLOIDS–GROUP III													
C. coriacea Mangaly & M. Sabu	73447	2·603 ± 0·006	2·60^{lm}		1·30	1273	42!	6	0·43	0·43^{c}	B	Kerala, Idukki Dt.	SW
C. mutabilis Škorničk., M. Sabu & Prasanthk.	84145	2·492 ± 0·004	2·49^{mn}		1·25	1219	42!	6	0·42	0·42^{d}	B	Kerala, Malappuram Dt.	SW
C. sp. 'aff. prakasha'	71443	2·446 ± 0·005	2·45no		1·22	1196	42!	6	0·41	0·41^{d}	B	Meghalaya, Ribhoi Dt.	NE
C. sp.	73417	2·501 ± 0·001	2·50^{mn}		1·25	1223	42!	6	0·42	0·42^{d}	L	Assam, Bongaigaon Dt.	NE
NONAPLOIDS													
C. aeruginosa Roxb.	71431	2·825 ± 0·013	2·86^{efg}	3·2	1·41	1381	63	9	0·31	0·32^{klmn}	L	Assam, Bongaigaon Dt.	S
	84119	2·808 ± 0·012			1·40	1373		9	0·31		L	Kerala, Ernakulam Dt.	
	84130	2·898 ± 0·003			1·45	1417		9	0·32		G	Kerala, Kozhikode Dt.	
	86102	2·876 ± 0·005			1·44	1406		9	0·32		G	Kerala, Kottayam Dt.	
	86354	2·889 ± 0·005			1·44	1413		9	0·32		G	Andaman Isls., S. Andaman	
C. aromatica Salisb. *s.l.*–sp. 2	71460	2·863 ± 0·014	2·83^{efghi}	1·7	1·43	1400		9	0·32	0·31^{klmnopq}	L	Meghalaya, E. Garo Hills Dt.	NE
	73410	2·844 ± 0·001			1·42	1391		9	0·32		G	Meghalaya, E. Khasi Hills Dt.	
	71445	2·815 ± 0·012			1·41	1377		9	0·31		L	Meghalaya, E. Khasi Hills Dt.	
	71447	2·822 ± 0·013			1·41	1380	63	9	0·31		L	Meghalaya, E. Khasi Hills Dt.	
	71453	2·820 ± 0·012			1·41	1379		9	0·31		L	Meghalaya, E. Garo Hills Dt.	
C. aromatica Salisb. *s.l.*–sp. 3	71486	2·694 ± 0·007	2·68^{jkl}	0·9	1·35	1317		9	0·30	0·30^{rs}	G	Jharkhand, Devghar Dt.	E
	71488	2·686 ± 0·002			1·34	1313		9	0·30		G	Jharkhand, Dumka Dt.	
	71491	2·678 ± 0·007			1·34	1310		9	0·30		G	Jharkhand, Dumka Dt.	
	71492	2·671 ± 0·012			1·34	1306		9	0·30		G	Jharkhand, Dumka Dt.	
C. caesia Roxb.	71418	2·825 ± 0·011	2·82^{efghi}	4·9	1·41	1381		9	0·31	0·31^{klmnopq}	G	Unknown, cultivated at CUBG	NE & E
	71439	2·823 ± 0·016			1·41	1380	ca 63	9	0·31		G	Meghalaya, Ribhoi Dt.	
	71439A	2·830 ± 0·011			1·42	1384		9	0·31		L	Meghalaya, Ribhoi Dt.	

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TABLE 2. *Continued*

Group/species	Field no.	2C-value (pg) ± s.e.	Mean 2C-value (pg)*	Intraspecific variation (%)	1C-value (pg)	1C-value (Mbp)	$2n$†	Ploidy level (x) or DNA ploidy level	1Cx-value (pg)	Mean Cx-value (pg)*	Internal standard‡	Locality	Distribution pattern§
	71439B	2·782 ± 0·007			1·39	1360		9	0·31		L	Meghalaya, Ribhoi Dt.	
	71451	2·893 ± 0·008			1·45	1415		9	0·32		G	Meghalaya, S. Garo Hills Dt.	
	71459	2·892 ± 0·005			1·45	1414		9	0·32		L	Meghalaya, E. Garo Hills Dt.	
	71469	2·759 ± 0·008			1·38	1349		9	0·31		L	W. Bengal, Darjeeling Dt.	
	71490	2·825 ± 0·004			1·41	1381		9	0·31		G	Jharkhand, Pakur Dt.	
	86222	2·788 ± 0·011			1·39	1363		9	0·31		G	Mizoram, Lunglei Dt.	
C. codonantha Škorničk., M. Sabu & Prasanthk.	73319	2·838 ± 0·010	2·84^{efgh}		1·42	1388	63!	9	0·32	0·32^{klmnop}	G	Andaman Isls., N. Andaman	ANI
C. elata complex													
C. elata Roxb.	86321	2·853 ± 0·014	2·85^{efg}		1·43	1395		9	0·32	0·32^{klmno}	L	Andaman Isls., S. Andaman	ANI
C. latifolia Roscoe	73321	2·801 ± 0·005	2·80^{efghij}		1·40	1370		9	0·31	0·31^{lmnopqrs}	G	Andaman Isls., M. Andaman	ANI
C. sp. 'elata-latifolia'	71419	2·853 ± 0·010	2·91^{e}	3·1	1·43	1395		9	0·32	0·32^{kl}	L	Uttaranchal, Dehra Dun Dt.	N, E & NE
	71423	2·941 ± 0·012			1·47	1438	63	9	0·33		L	W. Bengal, Jalpaiguri Dt.	
	71440	2·905 ± 0·006			1·45	1421		9	0·32		G	Meghalaya, Ribhoi Dt.	
	71448	2·889 ± 0·012			1·44	1413		9	0·32		L	Meghalaya, S. Garo Hills Dt.	
	71461	2·884 ± 0·008			1·44	1410		9	0·32		G	Meghalaya, E. Garo Hills Dt.	
	71471	2·925 ± 0·008			1·46	1430		9	0·33		L	W. Bengal, Darjeeling Dt.	
	71477	2·940 ± 0·004			1·47	1438		9	0·33		G	W. Bengal, Darjeeling Dt.	
	71483	2·930 ± 0·008			1·47	1433		9	0·33		G	Uttaranchal, Dehra Dun Dt.	
	73412	2·942 ± 0·005			1·47	1439		9	0·33		G	W. Bengal, Jalpaiguri Dt.	
C. ferruginea Roxb.	71479	2·805 ± 0·005	2·80^{efghijk}	2·5	1·40	1372		9	0·31	0·31^{lmnopqrs}	G	W. Bengal, S. 24-Prghanas Dt.	E & ANI
	73320	2·814 ± 0·001			1·41	1376		9	0·31		G	Andaman Isls., N. Andaman	
	73320-II	2·818 ± 0·008			1·41	1378		9	0·31		G	Andaman Isls., N. Andaman	
	86334B	2·749 ± 0·006			1·37	1344		9	0·31		G	Andaman Isls., M. Andaman	

C. leucorhiza Roxb.	71489	2·714 ± 0·013	2·70^{ijkl}	1·0	1·36	1327		9	0·30	0·30^{qrs}	L	Jharkhand, Dumka Dt.	E
	71493	2·688 ± 0·005			1·34	1314		9	0·30		G	Jharkhand, Pakur Dt.	
	73441	2·692 ± 0·015			1·35	1316	63!	9	0·30		L	Bihar, Bhagalpur Dt.	
C. longa L.	71420	2·621 ± 0·011	2·71^{ijkl}	15·1	1·31	1282		9	0·29	0·30^{pqrs}	L	W. Bengal, Kolkata	S, C, E & NE
	71422	2·580 ± 0·007			1·29	1262	63	9	0·29		L	W. Bengal, Kolkata	
	71433	2·817 ± 0·008			1·41	1378	63	9	0·31		L	Assam, Bongaigaon Dt.	
	71436	2·603 ± 0·012			1·30	1273		9	0·29		L	Assam, Dispur Dt.	
	71473	2·603 ± 0·011			1·30	1273		9	0·29		L	W. Bengal, Darjeeling Dt.	
	71480	2·653 ± 0·004			1·33	1297		9	0·29		G	W. Bengal, S. 24-Parghanas Dt.	
	71487	2·969 ± 0·005			1·48	1452		9	0·33		G	Jharkhand, Devgar, Dt.	
	73303	2·751 ± 0·004			1·38	1345		9	0·31		G	Andaman Isls., N. Andaman	
	73411	2·715 ± 0·002			1·36	1328		9	0·30		G	Meghalaya, Ribhoi Dt.	
	73478	2·766 ± 0·001			1·38	1353		9	0·31		G	Chhattisgarh, Bilaspur Dt.	
	84127	2·677 ± 0·002			1·34	1309		9	0·30		G	Kerala, Wyanad Dt.	
	84154	2·665 ± 0·004			1·33	1303		9	0·30		G	Kerala, Palghat Dt.	
	84160	2·656 ± 0·007			1·33	1299		9	0·30		G	Kerala, Wyanad Dt.	
	86221	2·841 ± 0·006			1·42	1389		9	0·32		G	Mizoram, Lunglei Dt.	
	86221-II	2·846 ± 0·006			1·42	1392		9	0·32		G	Mizoram, Lunglei Dt.	
	86221-III	2·829 ± 0·004			1·41	1383		9	0·31		G	Mizoram, Lunglei Dt.	
C. zanthorrhiza Roxb.	73302	2·896 ± 0·003	2·88^{ef}	2·9	1·45	1416		9	0·32	0·32^{klm}	G	Andaman Isls., N. Andaman	S & ANI
	84107	2·901 ± 0·007			1·45	1419		9	0·32		G	Kerala, Kollam Dt.	
	84166	2·895 ± 0·003			1·45	1416		9	0·32		G	Kerala, Idukki Dt.	
	84182	2·820 ± 0·015			1·41	1379	63	9	0·31		L	Kerala, Idukki Dt.	
	84182A	2·839 ± 0·003			1·42	1388		9	0·32		G	Kerala, Idukki Dt.	
C. sp. 'fucata'	71430	2·805 ± 0·013	2·80^{efghij}		1·40	1372	63!	9	0·31	0·31^{lmnopqrs}	L	Assam, Bongaigaon Dt.	NE
C. sp. 'man-and'	86306	2·804 ± 0·006	2·76^{fghijk}	3·1	1·40	1371	63!	9	0·31	0·31^{mnopqrs}	L	Andaman Isls., S. Andaman	ANI

Continued

TABLE 2. *Continued*

Group/species	Field no.	2C-value (pg) ± s.e.	Mean 2C-value (pg)*	Intraspecific variation (%)	1C-value (pg)	1C-value (Mbp)	$2n$[†]	Ploidy level (x) or DNA ploidy level	1Cx-value (pg)	Mean Cx-value (pg)*	Internal standard[‡]	Locality	Distribution pattern[§]
	86313	2·721 ± 0·009			1·36	1331		9	0·30		G	Andaman Isls., S. Andaman	
C. sp. 'picta'	71452	2·803 ± 0·010	2·81^{efghijk}	3·7	1·40	1371		9	0·31	0·31^{lmnopqrs}	L	Meghalaya, S. Garo Hills Dt.	NE & SL
	71464	2·782 ± 0·014			1·39	1360		9	0·31		L	Meghalaya, E. Garo Hills Dt.	
	71465	2·753 ± 0·009			1·38	1346		9	0·31		L	Assam, Kokrajar Dt.	
	73422	2·854 ± 0·004			1·43	1396		9	0·32		G	Sri Lanka, Kegalle Dt.	
C. sp. 'roxburgh'	71434	2·717 ± 0·012	2·72^{hijkl}		1·36	1329	ca 63!	9	0·30	0·30^{opqrs}	L	Assam, Bongaigaon Dt.	NE
C. sp. 'tikhur'	73476	2·735 ± 0·001	2·74^{ghijk}		1·37	1337		9	0·30	0·30^{nopqrs}	G	Chhattisgarh, Bilaspur Dt.	C
C. sp. 'aff. zanthorrhiza'	73420	2·672 ± 0·005	2·67^{kl}	0·5	1·34	1307		9	0·30	0·30^{s}	G	Jharkhand, Paschim Singhbum Dt	E
	73420A	2·663 ± 0·013			1·33	1302		9	0·30		G	Jharkhand, Paschim Singhbum Dt	
	73420B	2·676 ± 0·011			1·34	1309		9	0·30		G	Jharkhand, Paschim Singhbum Dt	
11-PLOID													
C. oligantha Trimen	73325	4·755 ± 0·026	4·76[a]		2·38	2325	77!!	11	0·43	0·43[c]	B	Sri Lanka, Badulla Dt.	SL
cf. 12-PLOID													
C. sp. 'ranchi'	71485	3·713 ± 0·005	3·71[c]		1·86	1816	> ca 70!	≤ 12	≥ 0·31	0·31^{lmnopqrs}	G	Jharkhand, Ranchi Dt.	E
15-PLOIDS													
C. raktakanta Mangaly & M. Sabu	73414	4·614 ± 0·010	4·57[b]	5·1	2·31	2256		15	0·31	0·30^{nopqrs}	G	Assam, Bongaigaon Dt.	SW & NE
	71432	4·413 ± 0·017			2·21	2158	105!!	15	0·29		L	Assam, Bongaigaon Dt.	
	84120	4·637 ± 0·012			2·32	2267		15	0·31		L	Kerala, Ernakulam Dt.	
	84120	4·637 ± 0·012			2·32	2267	105!!	15	0·31		G	Kerala, Ernakulam Dt.	
	84148	4·638 ± 0·003			2·32	2268		15	0·31		G	Kerala, Trichur Dt.	
HYBRID													
C. angustifolia × *montana*	73480B	1·903 ± 0·005	1·90[st]		0·95	931		6	0·32	0·32^{klmno}	G	Chhattisgarh, Bilaspur Dt.	C

RELATED GENERA												
Hitchenia caulina (J. Graham) Baker (= *C. caulina* J. Graham)	84178	2·245 ± 0·011	2·24^{pqr}	1·12	1098	42!	6	0·37	0·37^{ghi}	B	Maharashtra, Satara Dt.	W
Kaempferia scaposa (Nimmo) Benth. (= *C. scaposa* (Nimmo) Škorničk. & M. Sabu, comb. nov.)	77029	2·333 ± 0·007	2·33^{op}	1·17	1141	42!	6	0·39	0·39^{efg}	G	Goa, N. Goa Dt.	W
Paracautleya bhatii R. M. Sm. (= *C. bhatii* (R.M.Sm.) Škorničk. & M. Sabu)	73446	2·181 ± 0·003	2·18^{qr}	1·09	1067	42!	6	0·36	0·36^{ij}	G	Karnataka, Udupi Dt.	SW
Stahlianthus involucratus (King ex Baker) Craib ex Loes.	71449	3·114 ± 0·003	3·11^{d}	1·56	1523	(22)	2	1·56	1·56 ^{a}	G	Meghalaya, S. Garo Hills Dt.	NE

* Letters indicate group of taxa that are not significantly different at $\alpha = 0{\cdot}05$.
† Chromosome numbers determined in the present work, ! new species count, !! new count for a genus. Chromosome numbers taken from the literature are given in parentheses.
‡ B, *Bellis perennis* L. (2C = 3·96 pg); G, *Glycine max* 'Polanka' (2C = 2·5 pg, primary reference standard); L, *Solanum lycopersicum* 'Stupnické polní tyčkové rané' (2C = 2·21 pg).
§ ANI, Andaman Islands; C, Central India; E, Eastern India; N, Northern India; S, Southern India; SL, Sri Lanka; W, Western India.

and nuclei were gently resuspended in 100 μL of fresh Otto I buffer. After incubation (15 min at room temperature), 1 mL of Otto II buffer (0·4 M $Na_2HPO_4.12H_2O$) supplemented with propidium iodide (at a final concentration 50 μL mL^{-1}), RNase IIA (50 μL mL^{-1}) and 2-mercaptoethanol (2 μL mL^{-1}) was added. The samples were incubated for 30 min at room temperature, after which fluorescence intensity of 5000 particles was recorded on a Partec Cyflow instrument (Partec GmbH, Münster, Germany) equipped with a 532-nm solid-state laser (Cobolt Samba 100 mW, Cobolt, Sweden). Each plant was re-analysed at least three times on different days and only histograms with peaks of approximately the same height were accepted. If between-day variation (max./min. value) exceeded 2 %, the outlying value was discarded and the sample re-measured. *Glycine max* 'Polanka' (2C = 2·50 pg; Doležel *et al.*, 1994) was selected as a primary internal reference standard. *Solanum lycopersicum* L. 'Stupické polní tyčkové rané' (2C = 2·11 pg) and *Bellis perennis* L. (2C = 3·96 pg) were used as secondary reference standards for *Curcuma* samples with low and high genome sizes, respectively, in order to minimize standard-to-sample peak ratio and thus avoid potential non-linearity of FCM measurements. Genome sizes of secondary reference standards were calibrated against the primary one, based on nine replications on different days. The total number of flow cytometric measurements for *Curcuma* was 678.

Chromosome counts

Chromosome numbers were counted in actively growing root tips of the cultivated plants. Samples were pretreated with a saturated solution of p-dichlorbenzene (3 h, room temperature), fixed in a 3:1 mixture of ethanol and acetic acid (4 h, 4 °C), macerated in 1:1 hydrochloric acid/ethanol (30 s, room temperature) and immediately squashed in a drop of lactopropionic orceine. The number of chromosomes was determined in 5–10 complete well-spread mitotic plates using a Carl-Zeiss Jena NU microscope equipped with an Olympus Camedia C-2000 Z camera.

Statistical analysis

Statistical analyses were performed in the SAS 8·1 statistical package (SAS Institute, Cary, NC, USA). Between-species differences in genome size were tested using GLM (general linear models) because of unbalanced data design, and Tukey's procedure was applied to compare mean values. The Spearman-rank correlation coefficient (CORR procedure) was used to test whether mean genome size of the taxa was related to the geographical location of the populations.

RESULTS

Chromosome counts and ploidy levels

Chromosome numbers together with inferred ploidy levels for the majority of the studied taxa (84 %) are given in Table 2. In total, 50 plants (i.e. nearly one-third of all accessions) were analysed karyologically. Forty-one taxa

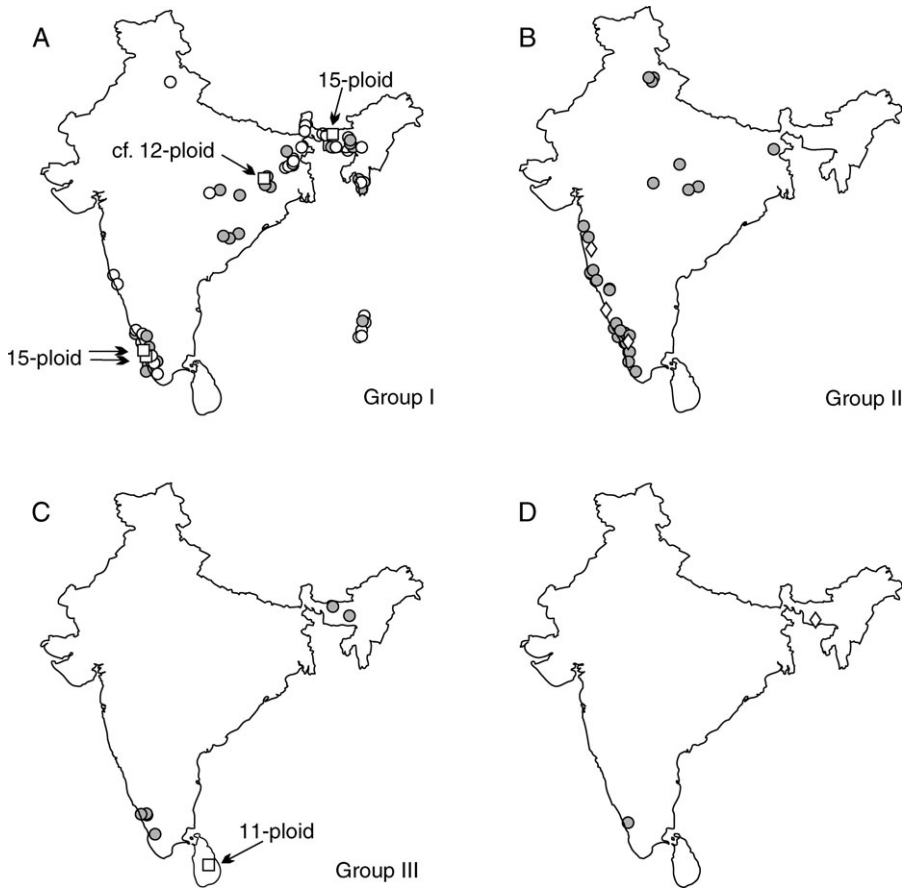

FIG. 1. Geographical origin of the plant samples analysed. (A) Species from genome group I (1Cx = 0·30–0·33 pg) and $x = 7$; (B) species from genome group II (1Cx = 0·36–0·39 pg) and $x = 7$; (C) species from genome group III (1Cx = 0·41–0·43 pg) and $x = 7$; (D) species with 1Cx > 0·83 pg and $x = 11$ (circle, *Curcuma vamana*; diamond, *Stahlianthus involucratus*). Symbol explanation (unless otherwise indicated): closed circles, hexaploids; open circles, nonaploids; open diamonds, *Curcuma*-like species often placed into separate genera; open squares, high polyploids designated by a corresponding ploidy level.

(including undetermined samples) yielded definite chromosome numbers, a preliminary count was obtained for one tentatively determined specimen (*C.* sp. 'ranchi' with $2n > 70$), and a single chromosome record referring to *Stahlianthus involucratus* was taken from the literature (as *Kaempferia involucrata*; Bisson *et al.*, 1968). Six different chromosome numbers were identified (i.e. $2n = 22$, 42, 63, >70, 77 and 105). The majority of these are multiples of $x = 7$, which may be regarded as a genuine basic chromosome number. Consequently, plants with 42, 63, 77 and 105 somatic chromosomes correspond to hexaploids, nonaploids, 11-ploids and 15-ploids, respectively.

New chromosome numbers were found in two species, *C. oligantha* ($2n = 77$) and *C. raktakanta* ($2n = 105$). Both also represent new generic records and the latter is the highest chromosome number so far determined in Zingiberaceae. Micrographs documenting metaphase chromosomes in *C. vamana* ($2n = 22$) and *C. raktakanta* ($2n = 105$) are shown in Fig. 2.

Genome size variation

Table 2 summarizes the results for 161 samples belonging to 51 taxa of *Curcuma* and related genera. The majority of genome size estimates represent novel records; previous C-values were available for only ten taxa.

Flow cytometric analyses yielded high-resolution histograms (Fig. 3). Coefficients of variation (CVs) of G_0/G_1 peaks ranged from 0·89 to 5·93 % (mean 2·60) for *Curcuma* samples and from 1·13 to 6·54 % (mean 2·98) for the reference standard. An arbitrary threshold of 3·0 % was not exceeded in 72 and 62 % of *Curcuma* and internal

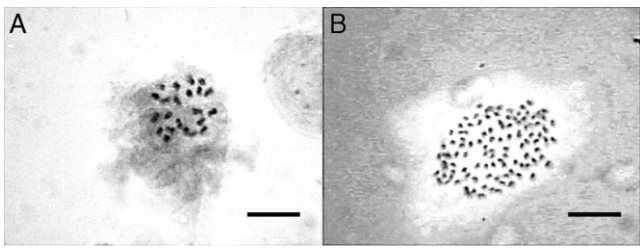

FIG. 2. Chromosome complements of (A) *C. vamana* (species with the smallest number of chromosomes) and (B) *C. raktakanta* (species with the highest number of chromosomes) showing 22 and 105 somatic chromosomes, respectively. Scale bars = 10 μm. In (A), two micrographs were taken at different focal planes and computer-merged in order to achieve sufficient image sharpness.

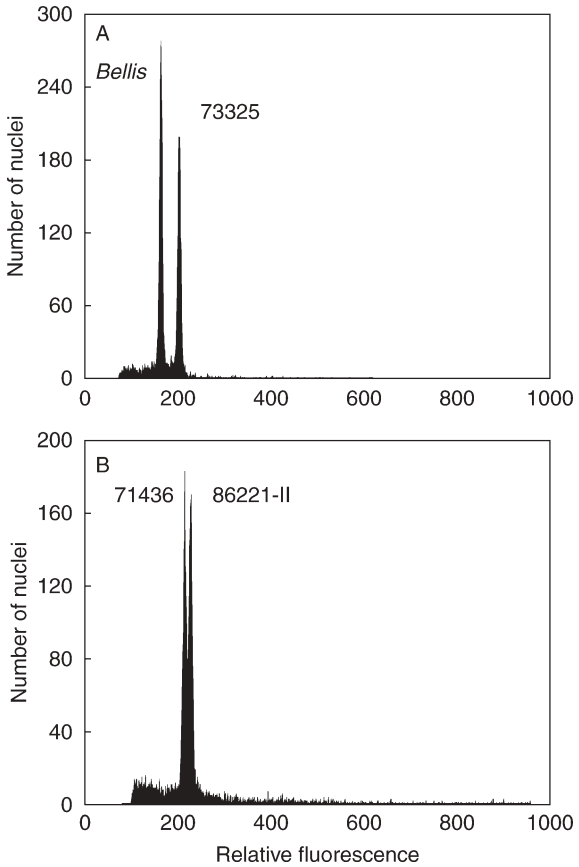

FIG. 3. (A) Representative flow cytometric histogram documenting genome size determination in *Curcuma oligantha* (accession number 73325) using *Bellis perennis* as internal reference standard. (B) Flow cytometric evidence for genome size variation in *Curcuma longa* [simultaneous analysis of accessions 71436 (2C = 2·60 pg) and 86221-II (2C = 2·85 pg)]. Plant nuclei were isolated, stained with propidium iodide and measured simultaneously.

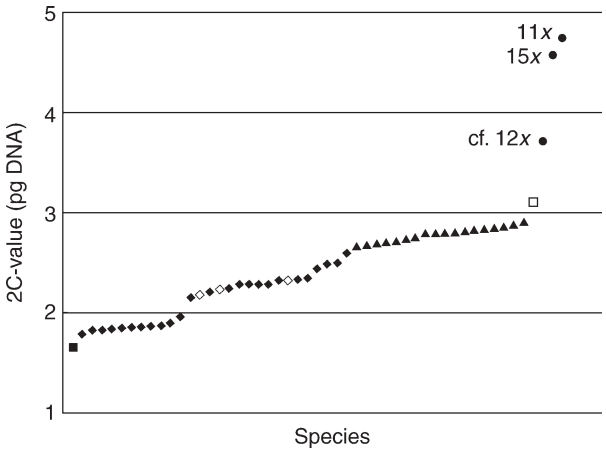

FIG. 4. 2C-value distribution (DNA pg means) for 51 taxa investigated. Symbol explanation: squares, diploids ($2n = 2x = 22$); diamonds, hexaploids ($2n = 6x = 42$); triangles, nonaploids ($2n = 9x = 63$); circles, higher polyploids designated by a corresponding ploidy level. Closed symbols, *Curcuma* species; open symbols, closely related taxa often placed into separate genera.

standard runs, respectively. Between-day fluctuation in FCM measurements due to instrument instability or non-identical sample preparation was negligible, with standard error of the mean ranging from 0·02 to 0·55 % of the estimated 2C-value. Reliability of determined DNA amounts was repeatedly confirmed in simultaneous FCM analyses, which gave two distinct peaks even in *Curcuma* samples with only small differences in genome size (see Fig. 3B).

Mean 2C-values varied from 1·66 pg in diploid *C. vamana* ($2n = 2x = 22$) to 4·76 pg in *C. oligantha* ($2n = 11x = 77$), a 2·87-fold range (Fig. 4). Homoploid genome sizes ranged from 0·30 pg in several species to 1·56 pg in *Stahlianthus involucratus*, a 5·25-fold range. Most of the species with more than one accession showed low intraspecific genome size variation (3·4 % on average). However, differences between maximum and minimum C-values that exceeded 4 % were observed in five species (*C. prakasha*, *C. caesia*, *C. raktakanta*, *C. montana* and *C. longa* – arranged by increasing percentage variation) reaching 15·1 % in *C. longa* (Fig. 3B).

The value of FCM data for taxonomic purposes (species delineation) seems to be rather limited owing to the small number of species-specific genome sizes (see Table 2). Nonetheless, there are some species alliances with phenotypic similarities in which nuclear DNA amounts may provide a clue for accurate determination, *C. cannanorensis* (2C = 2·33 pg) – *C. oligantha* (2C = 4·76 pg) being a representative example (Fig. 5). In addition, genome size reflected morphological variation in the *C. aromatica* complex, in which three taxonomic entities with distinct DNA amounts are currently recognized (Table 2). The genome size of a putative hybrid between *C. angustifolia* and *C. montana* was close to the mean of those of the parental species.

Hexaploid *Curcuma* species showed marked variation in homoploid genome sizes, amounting to 45%. This variation was discontinuous and three groups of taxa with significantly different Cx-values ($P<0{\cdot}0001$, $n = 24$) could be distinguished (Table 3). These groups corresponded well to the geographical origin of the samples (Fig. 1). Higher polyploids mirrored this pattern and split into a genome group I (1Cx = 0·30–0·32 pg) and a genome group III (1Cx > 0·40 pg) cluster. To gain closer insights into the genome size variation, relationships between Cx-values and geographical locations of all samples with $x = 7$ were examined. A significant negative correlation was found for both latitude (Spearman $r = -0{\cdot}50$, $P = 0{\cdot}0004$, $n = 49$) and longitude ($r = -0{\cdot}63$, $P<0{\cdot}0001$, $n = 49$).

As diploids were found to possess a different basic chromosome number than polyploids (i.e. 11 vs. 7), potential changes in homoploid genome size with respect to ploidy level were difficult to assess. Nevertheless, average DNA content per chromosome in two diploid species (*C. vamana* and *Stahlianthus involucratus*) exceeded the corresponding values in polyploid taxa.

With the exception of *Stahlianthus involucratus*, genome sizes of other species often placed in separate genera (*Hitchenia caulina*, *Kaempferia scaposa*, and *Paracautleya bhatii*; Fig. 5) fitted well into the range of C-values of hexaploid curcumas (they also shared the same number of chromosomes).

FIG. 5. Phenotypic diversity of *Curcuma* species included in the study. (A) *Curcuma vamana* (accession number 84156); (B) *C. cannanorensis* (no. 84144); (C) *C. raktakanta* (no. 84120). (D) *C. roscoeana* (no. 73309); (E) *C. oligantha* (no. 73325); (F) *C.* (= *Paracautleya*) *bhatii* (no. 73446); (G) *C.* (= *Hitchenia*) *caulina* (no. 84178); (H) *C. kudagensis* (no. 84152); (I) *C.* (= *Kaempferia*) *scaposa* (no. 77029). Individual plates not to scale.

DISCUSSION

Comparison of chromosome numbers with previous investigations

Our karyological analyses revealed six different chromosome counts, $2n = 22$, 42, 63, >70, 77 and 105. The somatic numbers 42 and 63 predominated and the other counts were each encountered in one taxon only ($2n = 22$ in *C. vamana*, $2n>70$ in an undetermined sample from eastern India, $2n = 77$ in *C. oligantha* and $2n = 105$ in *C. raktakanta*; Figs 2 and 5). The last two records represent new chromosome numbers for the genus *Curcuma*, and $2n = 105$ is the highest somatic count recorded so far for the family Zingiberaceae.

TABLE 3. *Summary of chromosome counts and genome size estimates for species of* Curcuma *and related genera determined in the present study*

Ploidy level or DNA ploidy	No. of chromosomes ($2n$)	No. of taxa/no. of individuals	2C-value range (pg)	1Cx-value range (pg)
Curcuma				
$2x$	22	1/1	1·66	0·83
$6x$, group I	42	10/42	1·79–1·96	0·30–0·33
$6x$, group II	42	10/27	2·15–2·35	0·36–0·39
$6x$, group III	42	4/4	2·45–2·60	0·41–0·43
$9x$	63	18/75	2·67–2·91	0·30–0·32
$11x$	77	1/1	4·76	0·43
cf. $12x$	>70	1/1	3·71	(≥0·31)
$15x$	105	1/5	4·57	0·30
Related genera				
$2x$	22	1/1	3·11	1·56
$6x$	42	3/3	2·18–2·33	0·36–0·39

No intraspecific variation in the chromosome number was detected. Nevertheless, it should be noted that chromosome counts were only analysed in more than one individual for eight taxa only.

A targeted search for karyological data in *Curcuma* showed that chromosome numbers have so far been published for 40 identified species and four taxa of unclear identity (see Table 1), 17 of which were also included in our study. Generally, there was agreement between the data sets, although some discrepancies occurred. In particular, several different chromosome counts for the same species have been reported in some cases, but this was not confirmed in our study. Moreover, previous numbers showed evidence of aneuploidy, a phenomenon not encountered by us. Such incongruities may be explained either by existence of karyological variation not sampled in the present study, species misidentification or a broader species concept adopted in earlier studies; lack of herbarium vouchers, however, precludes verification of the taxonomic identity of previously studied plant material in many cases.

For example, two different numbers had previously been published for *C. rubescens* ($2n = 42$ and $2n = 63$; Islam, 2004), but only the former was confirmed in our study. We believe that the plants with 63 chromosomes belonged to another species because *C. rubescens* was originally described to produce both lateral and central inflorescences by Roxburgh (1810), a feature common in hexaploids but unknown in any nonaploid species. A parallel situation appears to exist in *C. mangga.* Again, we found only 42 chromosomes in this species, but found $2n = 63$ in an undetermined, though closely related, specimen (accession no. 86306). We have some doubts about the degree of chromosomal variation in *C. aromatica* (published $2n = 42$, 63, 84 and 86). Current taxonomic investigations revealed that this is a complex group composed of several species that possess either 42 or 63 chromosomes according to the present results. Despite representative sampling, attempts to find other chromosome numbers within the *C. aromatica* aggregate were unsuccessful, either using direct karyological counting or using indirect FCM measurements. Finally, there are apparent discrepancies in previous chromosome numbers for *C. malabarica* ($2n = 42$) and *C. raktakanta* ($2n = 63$; both published by Joseph *et al.*, 1999). The counts here based on material from the type localities showed uniformly $2n = 105$. These taxa should be treated as one species (*C. raktakanta*) as also indicated by morphology. Considering the discrepancies, it is likely that genuine *C. malabarica* and *C. raktakanta* were not involved in the study of Joseph *et al.* (1999).

In addition to euploid chromosome numbers, several apparently aneuploid counts (i.e. 62, 64, 66, etc.) have been reported (see Table 1). Prana (1977) claimed that this phenomenon may be associated with abnormal mitosis occasionally encountered in Indonesian polyploid curcumas. Daughter cells with unequal number of chromosomes may be formed in this way and lead to incidence of aneuploidy and/or chromosomal chimaeras, especially in plants with predominantly vegetative reproduction. However, no aneuploids were found in the present study. Although we do not *a priori* reject such an option (e.g. intraspecific variation in genome size observed in some *Curcuma* spp. may potentially refer to chromosomal heterogeneity), several records of aneuploidy are suspicious and should be interpreted with caution until reliably confirmed. Karyological investigation of *Curcuma* spp. is rather challenging and errors may easily be introduced due to both relatively high number and small size (mostly 0·5–2 μm) of chromosomes (Ramachandran, 1961; Apavatjrut, 1996; Joseph *et al.*, 1999; Sirisawad *et al.*, 2003). It should also be noted that majority of doubtful counts were published several decades ago and have not been recorded since.

Basic chromosome number and ploidy level variation

Since the pioneering karyological surveys, there has been continuous dispute concerning the basic chromosome number in *Curcuma*. Investigating 25 Zingiberaceae species, including three curcumas, Raghavan and Venkatasubban (1943) were the first to report $x = 21$. Sato (1948) suggested $x = 8$ in his early work on *C. longa*, but later claimed that two basic numbers ($x = 7$ and $x = 8$) occur in the genus (Sato, 1960). Further support for $x = 21$ appeared in the study of Ramachandran (1961), who observed regular bivalent formation during meiosis in *C. decipiens* ($2n = 42$) and a high frequency of trivalents in *C. longa* (under the name *C. domestica*) with $2n = 63$. In line with Venkatasubban (1946), Ramachandran considered $x = 21$ a secondary number possibly derived from a combination of $x = 9$ and $x = 12$, which are common in several genera of Zingiberaceae. Yet another basic chromosome number, $x = 16$, has been proposed and adopted by some researchers (e.g. Sharma and Bhattacharya, 1959). However, $x = 21$ became the most commonly accepted basic chromosome number in *Curcuma* (Ramachandran, 1961; Prana, 1977; Prana *et al.*, 1978; Ardiyani, 2002; Islam, 2004).

It should be noted that the lower alternative, $x = 7$, is not in conflict with the large majority of published somatic

counts. Actually, this value fits better with the range of chromosome numbers currently known (see our prime count $2n = 77$). We therefore believe that $x = 7$ should be considered a primary basic chromosome number, at least for the majority of Indian *Curcuma* species (belonging to the subgenus *Curcuma*). Grant (1982) regarded $x = 7$ and 10 as the most common basic chromosome numbers in monocots so the present findings are in concordance with a large body of evidence. Following this calculation, species investigated here correspond to 6*x*, 9*x*, 11*x* and 15*x* cytotypes. In addition, dodecaploid ($2n = 84$) plants from subgenus *Curcuma* were reported by earlier researchers (Table 1).

It appears that there are some *Curcuma* species in which somatic chromosome numbers ($2n = 20$, 22, 24, 32, 34, 36 and 38) do not fit into the series based on $x = 7$. However, they all belong to the subgenus *Hitcheniopsis*, which encompasses mainly Thai species such as *C. alismatifolia*, *C. gracillima*, *C. harmandii*, *C. parviflora*, *C. rhabdota* and *C. thorelii* (see Table 1), and which differs markedly in morphological traits from the nominate subgenus. From the present species set, only *C. vamana* ($2n = 22$) belongs to subgenus *Hitcheniopsis*. We are convinced that this species is only distantly related to the other investigated taxa (subgenus *Curcuma*), and its basic chromosome number is most plausibly $x = 11$, as also documented for several other genera of Zingiberaceae (e.g. *Kaempferia*, *Zingiber* and *Renealmia*).

It is plausible that both subgenera *Curcuma* and *Hitcheniopsis* represent independent evolutionary units with distinct basic chromosome numbers. In addition, $x = 7$ may be considered a subgenus-specific cytological marker for the former.

Inter- and intraspecific variation in genome size

The taxa involved in the present study differed 2·87-fold in their holoploid genome sizes (2C = 1·66–4·76 pg). This range is slightly below the mean for other plant genera (3·28-fold based on data from the Plant DNA C-values database; Bennett and Leitch, 2005*a*). There was no clear gap in nuclear DNA C-values between hexaploid and nonaploid plants and a major discontinuity in these cytotypes actually occurred between two groups of hexaploids (Fig. 4). These results indicate that DNA content alone is often insufficient to distinguish between 6*x* and 9*x* cytotypes, and FCM measurements should always be accompanied by chromosome counts. The C-values of diploid and high polyploid taxa were more distinct, but again FCM measurements alone may not be an accurate indicator of ploidy level, as illustrated by 11-ploid *C. oligantha* and 15-ploid *C. raktakanta* with reverse total amounts of nuclear DNA (Fig. 4).

By contrast, homoploid genome sizes formed three well-defined clusters with significantly different values, namely a genome group I (1Cx = 0·30–0·33 pg; 29 taxa), a genome group II (1Cx = 0·36–0·39; 13 taxa) and a genome group III (1Cx = 0·41–0·43 pg; five taxa). We assume that these groups may have different evolutionary histories, as also supported by their distinct distribution patterns (see below).

Intraspecific genome size variation was low in most taxa in which multiple individuals were analysed. However, values above 4 % (i.e. beyond potential instrumental fluctuation) were encountered in five species. When *C. longa* with about 1·15-fold divergence in C-value is excluded, the extent of intraspecific variation was comparable in all cytotypes: hexaploids, 0·0–6·1 %; nonaploids, 0·5–4·9 %; and a 15-ploid, 5·1%. Intraspecific variation in nuclear DNA content has been controversial since the early studies on genome size. This debate has been fuelled by numerous early reports of intraspecific variation, many of which were dismissed in subsequent investigations using the best practice methodology (Greilhuber, 2005). Several sources of artefactual variation have been identified: (1) instrumental or methodological errors; (2) disturbing effects of secondary metabolites with potential seasonal fluctuation (e.g. Walker *et al.*, 2006); (3) differences in measurements among different laboratories (Doležel *et al.*, 1998); and (4) taxonomic heterogeneity of the material under investigation (Murray, 2005). As a result, the concept of genome size stability has gained broader support. Examples have nonetheless been accumulated in recent years of some genome size variation in FCM assays despite meticulous methodology (Obermayer and Greilhuber, 2005; Šmarda and Bureš, 2006). Several *Curcuma* species are known to contain, particularly in rhizomes, phenolic secondary metabolites such as curcumins (Lubis, 1968; Prana, 1977). Although phenolics may bias genome size estimates (Greilhuber, 1998; Walker *et al.*, 2006), we believe that our FCM measurements are accurate for several reasons. Low coefficients of variation were achieved, which are not compatible with the presence of interfering metabolites. Nuclear suspensions were clear, lacking any coatings of debris that might indicate a negative effect of metabolites (Loureiro *et al.*, 2006). Repeated measurements of the same sample yielded nearly identical genome size values (average difference 0·8 %) even if particular FCM analyses were performed in different years. Between-plant differences remained stable also in studies using DAPI (an AT-selective fluorochrome less sensitive to secondary metabolites than the intercalating propidium iodide). Also, a narrow species concept was adopted to guarantee taxonomic homogeneity of the plant material. Moreover, co-processed samples with different genome sizes always gave two distinct peaks, which is the most convincing evidence for genuine differences in DNA content (Greilhuber, 2005).

The reasons for intraspecific genome size variation in *Curcuma* remain unknown. Aneuploidy or presence of B-chromosomes may lead to heterogeneity in nuclear DNA amount, but this explanation seems rather unlikely as only euploid numbers were revealed in our study and, more importantly, two accessions of *C. longa* with more than 9 % genome size variation possessed the same number of chromosomes (see Table 2). Plausibly, intraspecific variation may be related to a long-term cultivation and targeted selection of desirable genotypes in several *Curcuma* species, *C. longa* in particular. India is responsible for around 90 % of turmeric production worldwide,

and this species has been widely used in India since Vedic times. Perhaps the variation may have adaptive value, as previously documented in another crop, *Zea mays* (Rayburn and Auger, 1990). Murray (2005) argued that intraspecific genome size variation may indicate incipient speciation; the blurred species boundaries in several *Curcuma* alliances may support such a hypothesis. A third explanation takes into account heterochromatic polymorphism. In vegetatively propagating lines, strains with smaller and larger telomeric or centromeric heterochromatin regions may occur (J. Greilhuber, University of Vienna, Austria, pers. comm.) leading to greater genome sizes.

Comparison of genome sizes in Curcuma *with those in other members of Zingiberaceae and other monocots*

More than three-quarters of the taxa (39 out of 51) in the present study possessed very small genomes defined as 1C≤1·40 pg (Leitch *et al.*, 1998), whereas the remaining 12 taxa had small genomes (i.e. 1C = 1·41–3·50 pg). Low nuclear DNA content in *Curcuma* spp. is in line with previous records for other members of Zingiberaceae and related families. The Plant DNA C-values database (Bennett and Leitch, 2005*a*) includes measurements for three members of Zingiberaceae with 1C-values varying from 1·30 pg in *Curcuma zantorrhiza* (misspelled as *C. xanthorrhiza*) to 6·03 pg in *Zingiber officinale*. Very small genomes were also revealed in all but one (Lowiaceae with 1C = 3·55 pg) families from the order Zingiberales, including Marantaceae (14 species, 1C = 0·33–0·68 pg), Musaceae (six species, 1C = 0·58–0·61 pg), Heliconiaceae (1C = 0·45 pg), Strelitziaceae (1C = 0·58 pg), Cannaceae (1C = 0·72 pg) and Costaceae (1C = 1·00 pg).

Although only one *Curcuma* record is included in the Plant DNA C-values database, more genome size estimates have been published (Table 1). Das *et al.* (1999) used Feulgen densitometry to analyse three *Curcuma* spp.: *C. caesia* ($2n = 22$, 4C = 3·120 pg), *C. amada* ($2n = 40$, 4C = 4·234 pg) and *C. longa* ($2n = 48$, 4C = 5·100–5·263 pg). *Curcuma longa* was also investigated by Nayak *et al.* (2006) who reported 4C-values from 4·30 to 8·84 pg (i.e. 2·06-fold variation) in 17 cultivars. However, both these studies come from the same laboratory and we believe the results should be treated with caution. First, chromosome counts have not been confirmed by other researchers. Moreover, the authors used hot hydrolysis, which is strongly discouraged owing to the very short optimum treatment that is difficult to control (Greilhuber, 2005). No fixative solution was specified, and if a standard acetic acid/alcohol fixation was applied to plant samples rich in phenolics, stoichiometry of the Feulgen staining was likely to be distorted (the so-called 'self-tanning' effect; Greilhuber, 1986, 1988). Feulgen methodology is also not recommended for conventional chromosome counting in *Curcuma* as it does not allow good spreading of the root tips issue on slides (Islam, 2004) and gives rather pale staining. By contrast, the FCM data published for *Curcuma* spp. collected in Bangladesh by Islam (2004) seem to be accurate, at least from the methodological point of view. Unfortunately, genome size estimates were not linked to a particular herbarium voucher, which lessens their value. We had the opportunity to examine several well-prepared herbarium specimens prepared from Islam's living material and encountered some misidentifications (e.g. for *C. aeruginosa* and *C. zedoaria*).

In comparison with Islam's work (Islam, 2004), our estimates for ten species in common were on average 14 % lower, whereas we determined genome size to be about 11 % higher in *C. zantorrhiza* than that reported by Bharathan *et al.* (1994). Plausibly, different reference standards could be responsible for such divergences (i.e. *Raphanus sativus* – Islam, 2004; chicken red blood cells – Bharathan, 1994).

Species distribution and origin of high polyploids with respect to homoploid genome size

Excluding the distantly related *C. vamana*, species with the lowest number of chromosomes ($2n = 6x = 42$) clustered in three groups with significantly different Cx-values (Table 3), which also showed a distinct geographical pattern (Fig. 1). Group I (1Cx = 0·30–0·33 pg) contained ten taxa, the majority of which occurred in north-east India, group II (1Cx = 0·36–0·39 pg) contained ten taxa with a centre of distribution in the Western Ghats (west and south-west India) and Central India and group III (1Cx = 0·41–0·43 pg) contained four taxa occurring either in south-west or north-east India. A link between genome size and geographical location was also confirmed by correlation analysis, which revealed highly significant negative relationships between Cx-values and both latitude and longitude of the localities.

Homoploid genome sizes of all but one (*C. oligantha*) high-polyploid taxa matched perfectly the Cx-values of hexaploids from group I. This allows us to hypothesize that these hexaploids have played a key role in the evolution of polyploidy in Indian *Curcuma* spp. Nonaploid cytotypes probably originated by a fusion of reduced and unreduced gametes of hexaploids, either within or between species, giving rise to auto- or allopolyploids. Within Zingiberaceae, regular production of gametes with the somatic number of chromosomes has been reported in the genus *Globba* (Takano and Okada, 2002), which shows similar ploidy composition to *Curcuma*. Both nonaploid (unreduced gamete) and hexaploid (reduced and unreduced gamete) plants were probably involved in the genesis of rare dodecaploid and 15-ploid taxa. The vast majority of nonaploid species undergo asexual propagation via rhizome branching and produce a high percentage of aborted pollen grains (Prana, 1977; Nasir Uddin, 2000). It is plausible, however, that even a small fraction of viable pollen may eventually lead to the formation of higher polyploids. The origin of 11-ploid *C. oligantha* cannot be explained with certainty given the current stage of knowledge. Homoploid genome size (1Cx = 0·43 pg) of this species fits in the range of a genome group III.

Genome size and chromosome numbers as supportive markers for delimitation of Curcuma *species*

Chromosome numbers and/or ploidy levels have long been utilized as an efficient taxonomic marker, helping to delimit boundaries between various taxonomic categories or reveal cryptic taxa (Stace, 2000). In addition, the last decade has seen significant progress in the application of FCM to exploit differences in nuclear DNA content, which can in some cases be used as a supportive marker for distinguishing between closely related taxa at both heteroploid and homoploid levels (e.g. Mahelka *et al.*, 2005).

As *Curcuma* is a taxonomically challenging group, the usefulness of genome size and chromosome counts as supportive markers for taxon determination was assessed. Although the low C-value variation (2·87-fold) and the small number of species-specific DNA amounts reduce their utility, some alliances with morphological similarities have been identified in which cytogenetic data may nonetheless support taxonomic decisions. For example, *C. oligantha* (described from Sri Lanka) and *C. cannanorensis* (described from Kerala, western coast of South India) have traditionally be merged as one taxon due to their overall phenotypic resemblance (Bhat, 1987; Mangaly and Sabu, 1993; Sabu, 2006). However, the present examination of living plants in natural conditions revealed constant, though minor, morphological divergences (see Fig. 5), along with differences in ecological requirements. Distinctiveness of both species was further corroborated by distinct chromosome numbers ($2n = 77$ in the former, $2n = 42$ in the latter). Similarly, it was found that *C. aromatica* is a species complex comprising three distinct morphotypes with non-overlapping genome sizes, including hexaploids (2C = 1·86 pg) from south India, nonaploids (2C = 2·83 pg) from north-east India and nonaploids (2C = 2·68 pg) from eastern India. Taxon descriptions and clarification of the nomenclatural issues are in progress. *Curcuma reclinata* (2C = 2·29 pg), *C. decipiens* (2C = 2·35 pg) and *C. inodora* (2C = 2·29 pg) are difficult to define morphologically and have more or less overlapping distributions. Cytogenetic data do not provide reliable data for species-level determination and we believe that they should be merged as one taxon (i.e. *C. reclinata* Roxb.).

Taxonomic position of Curcuma*-like species placed into separate genera*

The taxonomic position of some species with a *Curcuma*-like morphology has long been discussed. Traditionally, they have been placed into separate genera such as *Hitchenia*, *Kaempferia*, *Paracautleya* and *Stahlianthus*. However, a phylogenetic analysis of the tribe Zingibereae based on internally transcribed spacers and *trnL–trnF* gene sequences (Ngamriabsakul *et al.*, 2004) did not support their independent generic status as these taxa were nested within the *Curcuma* complex. Our molecular analyses (T. Fér *et al.*, Charles University, Prague, Czech Republic, unpubl. res.) based on *trnL–trnF* gene sequences of a number of Indian taxa, including *Hitchenia caulina*, *Kaempferia scaposa* and *Paracautleya bhatii*, reveal a similar situation.

One aim of the present study was to assess whether ploidy and genome size data provide additional information useful for taxonomic decision-making. Genome sizes of the three controversial species (*Hitchenia caulina*: 2C = 2·25 pg, *Kaempferia scaposa*: 2C = 2·33 pg and *Paracautleya bhatii*: 2C = 2·18 pg) were found to match hexaploid *Curcuma* taxa from the genome group II. In addition, they all had the hexaploid number of chromosomes ($2n = 42$) and similar geographical distribution in the Western Ghats, a biodiversity hotspot for *Curcuma*. These lines of evidence together with a lack of clear-cut morphological traits support inclusion of the taxa in question in a broadly defined *Curcuma* (see Škorničková and Sabu, 2005*a*, for the latest circumscription of the genus).

Although valid name combinations in *Curcuma* already exist for both *Hitchenia caulina* (originally described as *C. caulina*) and *Paracautleya bhatii* (*C. bhatii*, in Škorničková and Sabu, 2005*a*), *Kaempferia scaposa* has never been included in *Curcuma*, perhaps due to the absence of pouches formed by the bracts and its pure white flowers with extremely long floral tubes (Fig. 5). However, the presence of pouches is neither a unique nor a universal feature for the genus *Curcuma* (Kress *et al.*, 2002; Škorničková and Sabu, 2005*a*) and the distinct flower morphology and colour may represent an adaptation to night pollination, as also observed in another ginger species with nocturnal anthesis, *Leptosolena haenkei* (Funakoshi *et al.*, 2005). Transfer of *Kaempferia scaposa* to the genus *Curcuma* requires a new name combination, which is proposed below.

By contrast, *Stahlianthus involucratus* possesses unique holoploid and homoploid genome sizes (1C = 1Cx = 1·56 pg), dissimilar to any other species involved in the current study. The diploid number of chromosomes ($2n = 22$) was shared only with south Indian *C. vamana*, but this species has a different Cx-value and overall morphology. We therefore keep this taxon in a separate genus at this point, but targeted investigation of Thai and south-east Asian members of the genera *Curcuma* and *Kaempferia* is necessary to arrive at a final decision concerning the validity of this genus.

Curcuma scaposa (Nimmo) Škorničk. & M.Sabu, **comb. nov.**

Basionym *Hedychium scaposum* Nimmo, in Graham, Cat. Pl. Bombay 205 (1839);≡*Monolophus scaposus* (Nimmo) Dalzell, Hooker's J. Bot. Kew Gard. Misc. 2: 143 (1850);≡*Kaempferia scaposa* (Nimmo) Benth. Gen. Pl. 3: 642 (1883).

Type: [India, Maharashtra], Western Ghats, Lonavlie, September 1878, G. King s.n. (Neotype: BM!, designated here. Isoneotype: K!).

ACKNOWLEDGEMENTS

A long-term stay of J.L.-Š. at the Calicut University, Kerala, India, was supported by fellowships from the Indian Council of Cultural Relationships and the Ministry

of Education, Youth and Sport of the Czech Republic. We thank the authorities of the Ministry of Environment and Forests, Government of India, and the Botanical Survey of India for granting collecting permits and permits to access Indian herbaria. We are also grateful to Johann Greilhuber (Vienna) for critical comments on intraspecific genome size variation, and Ilia Leitch (Kew) for providing some literature. We thank the handling editor Michael Fay (Kew) and two anonymous reviewers for useful remarks on the manuscript. This project was supported by the Grant Agency of the Academy of Sciences of the Czech Republic (project no. B6407401), the Department of Science and Technology, Government of India (Order No. SP/SO/A-20/99 dt. 9·11·2001), the European Commission's Research Infrastructure Action via the SYNTHESYS Project (GB-TAF-606), the Ministry of Education, Youth and Sport of the Czech Republic (MSM 0021620828) and the Academy of Sciences of the Czech Republic (AV0Z60050516).

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