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A critical review on cytogenetics of Cucurbitaceae with updates on Indian taxa

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Abstract

The cytogenetic relationships in the species of Cucurbitaceae are becoming immensely important to answer questions pertaining to genome evolution. Here, a simplified and updated data resource on cytogenetics of Cucurbitaceae is presented on the basis of foundational parameters (basic, zygotic and gametic chromosome numbers, ploidy, genome size, karyotype) and molecular cytogenetics. We have revised and collated our own findings on seven agriculturally important Indian cucurbit species in a comparative account with the globally published reports. Chromosome count (of around 19% species) shows nearly three-fold differences while genome size (of nearly 5% species) shows 5.84-fold differences across the species. There is no significant correlation between chromosome numbers and nuclear genome sizes. The possible trend of evolution is discussed here based on molecular cytogenetics data, especially the types and distribution of nucleolus organizer regions (NORs). The review supersedes the scopes of general chromosome databases and invites scopes for continuous updates. The offline resource serves as an exclusive toolkit for research and breeding communities across the globe and also opens scope for future establishment of web-database on Cucurbitaceae cytogenetics.

Keywords

chromosome, genome size, karyotype, NORs, ploidy

Introduction

The family Cucurbitaceae contains an extensive range of diversity consisting of about 1000 species spread over 96 genera (Renner and Schaefer 2017). The diversity of plant families is associated with variation in genome sizes and chromosome numbers as a result of enormous adaptive radiation (Soltis et al. 2004; Lysák and Schubert 2013). The viewpoint of evolution has been changed with the understanding of whole genome duplication (WGD) (Soltis et al. 2014) followed by core-eudicot hexaploidy (Wang et al. 2018). A cytogenetic database is essential to gain insights into evolution by supplementing phylogeny trees with chromosome number information (Mota et al. 2016) to upgrade knowledge on plant systematics (Soltis et al. 2014; Viruel et al. 2021). Cucurbitaceae, being the fourth most important and one of the earliest consumed vegetables yielding family, has coped with extreme climates, extensive human intervention and a huge domestication syndrome (Chomicki et al. 2020). Considerable advances have been made in molecular phylogeny (Renner and Schaefer 2016; Bellot et al. 2020; Chomicki et al. 2020; Guo et al. 2020) and genomics (CuGenDB, http://cucurbitgenomics.org) (Zheng et al. 2019). We had previously discussed about the gaps in cytogenetic studies (Bhowmick and Jha 2015b) which has been surmounted with the advent of molecular cytogenetics.

Currently, we have collated the cytogenetic reports of Cucurbitaceae globally and integrated our own findings for a collective interpretation. The review attempts to address i) the trend of chromosome evolution in specific tribes and species based on available information, ii) correlation between chromosome numbers and ploidy or genome size in the studied taxa and iii) the requirement of an exclusive cytogenetic catalogue for genome researchers, taxonomists and breeders working on Cucurbitaceae.

Methodological approaches

Data compilation

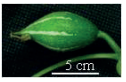
The data have been collated as per Schaefer and Renner (2011) after consultation of books, Chromosome atlases, research articles and public resources like Chromosome Counts Database (CCDB; http://ccdb.tau.ac.il/) (Rice et al. 2015), The Index to Plant Chromosome Numbers (IPCN, http://legacy.tropicos.org/Project/IPCN) (Goldblatt and Lowry 2011) and The Plant DNA C-values database (Pellicer and Leitch 2020) (https://cvalues.science.kew.org/).

Chromosome analysis in the Cucurbit species ocurring in India

Presently an enzymatic maceration and air drying (EMA) method followed by flurochrome banding has been employed as per our previous protocols (Bhowmick et al. 2012, 2016; Bhowmick and Jha 2015a, 2019, 2021) to represent fresh karyotypes of seven agriculturally important cucurbit species (Table 1) belonging to Benincaseae and

Sicyoeae. Fresh and healthy roots were used from different sources (like germinating seeds, seedlings and underground root stocks). Roots were pretreated with 0.002 M hydroxyquinoline and fixed in 1:3 aceto-methanol solution. The standardization of EMA- fluorescence banding was conducted for the different species. In brief, fixed roots were digested in enzyme mixture [1% Cellulase (Onozuka RS), 0.75% Macerozyme (R-10), 0.15% Pectolyase (Y-23), 1 mM EDTA] for 40–45 min at 37 °C, macerated on slides, air-dried, stained with 2% Giemsa solution (Merck, Germany) and plates selected for karyotyping. After de-staining, slides were kept in McIlvaine buffer, stained with 0.1 μg mL^{-1} DAPI for 15–20 min in darkness. For CMA staining, slides were incubated in 0.1 mg mL^{-1} CMA for 15–25 min in darkness. For meiotic chromosomes, fixed anthers were digested in enzyme mixture for 5–8 min, macerated on slides and DAPI staining protocol was followed with minor modifications. All slides

Table 1. Chromosome numbers and nature of fluorescent bands in some cucurbit species occurring in India.

Tribes	Species (common name, status of cultivation/ wild)	Collection site, Latitude/ Longitude	Fruit image	2n	CMA bands		DAPI bands (Non-nucleolar)
					Nucleolar	Non-nucleolar	
Sicyoeae	*Luffa acutangula* Linnaeus, 1753 (ridged gourd, cultivated)	Bhubaneswar, Odisha, 20.2960°N, 85.8245°E		26	11^{th} , 12^{th}, 13^{th}	12^{th} (centromeric)	1^{st} to 13^{th} (distal)
	Luffa cylindrica aegyptiaca Miller, 1768 (sponge gourd, cultivated)	Imphal, Manipur, 24.6637°N, 93.906°E		26	12^{th} , 13^{th}	1^{st} , 2^{nd} (distal)	0
	Luffa echinata Roxburgh, 1814 (wild)	Pantnagar, Uttarakhand, 30.0667°N, 79.019°E		26	11^{th} , 12^{th} , 13^{th}	0	1^{st} to 13^{th} (distal)
	Trichosanthes cucumerina Linnaeus, 1753 (wild)	NBPGR, Thrissur, Kerala, 10.5276°N, 76.2144°E		22	10^{th} , 11^{th}	0	1^{st} to 11^{th} (distal)
	Trichosanthes cucumerina ssp. *cucumerina* Anguina (snake gourd, cultivated)	Bengaluru, 12.9716°N, 77.5946°E		22	10^{th} , 11^{th}	2^{nd} (distal)	0
	Trichosanthes dioica Roxburgh, 1832 (pointed gourd, cultivated)	Bhagalpur, Bihar, 25.2414°N, 86.9924°E		22 (female)	0	7^{th} , 8^{th} , 10^{th} (distal)	1^{st} to 11^{th}
				22 (male)	0	0	1^{st} to 11^{th}
Benincaseae	*Benincasa hispida* Thunberg, 1784 (ash gourd, cultivated)	Imphal, Manipur 24.6637°N, 93.906°E		24	12^{th}	9^{th} (distal)	0
	Coccinia grandis Linnaeus, 1767 (ivy gourd, restricted cultivation)	Nagpur, Maharashtra, 21.1458°N, 79.0881°E		24 (female)	8^{th}, 12^{th} *	1^{st} to 5^{th}, 8^{th} to 12^{th} (centromeric)	0
				24 (male)	8^{th}, 12^{th} *	1^{st} to 5^{th}, 8^{th}, 10^{th} to 12^{th} (centromeric)	0

were mounted in non-fluorescent glycerol and chromosome plates were observed under a Zeiss Axioscop 2 fluorescence microscope (using UV and BV filter cassettes for DAPI and CMA stains, respectively). Images were captured using the attached ProgRes MFscan Jenoptik D07739 camera and ProgRes CapturePro 2.8.8 software.

Statistical analyses

Statistical analysis involving foundational cytogenetic parameters have been demonstrated to imply significant knowledge on chromosomal evolution within a group (Winterfeld et al. 2020). Considering the lack of hypotheses, we have tested for correlation between the dependent variables (2C genome size, MCL and HCL) and predictor variables [chromosome number (2n) and ploidy level (pl)] and also calculated linear models for regression analysis using IBM SPSS (v23, free).

The modern cytogenetic catalogue of cucurbitaceae

Along with the global review, fresh EMA based somatic plates and idiograms (Figs 1–3) of Indian species are presented here. We retain the previous designation of 10 tribes as 'understudied' (Bhowmick and Jha 2015b), excluding Indofevilleeae, having no cytological reports.

Chromosome numbers

Currently, chromosome counts are available for 188 species (~19%) belonging to about 44 genera (~46%) of the 15 tribes, including the less attended 'understudied tribes'. Within the 'understudied tribes', chromosome counts are available for only 42 species (out of almost 310) belonging to 17 genera (out of nearly 44). The basal number ranges from x/n = 5 (*Thladiantha* Bunge, 1833) to x/n = 15 (*Zanonia* Linnaeus, 1753) in these tribes (Table 2). Polyploidy has been abundantly reported in Gomphogyneae. Momordiceae have almost 60 species (Schaefer and Renner 2011) of which reports are known in nearly 11 species. The dibasic condition is noticed in *Momordica* Linnaeus, 1753 (x = 11 and 14) (Table 3) while polyploidy is detected in *M. charantia* Linnaeus, 1753 and *M. dioica* Willdenow, 1805 (2n = 56). *M. cymbalaria* Hooker, 1871, has the lowest count (2n = 18). In Bryonieae the X-Y sex determination system has been analysed in *Bryonia* Linnaeus, 1753 as the model along with *Ecballium* Richard, 1824 (Bhowmick and Jha 2015a). Chromosome counts are reported so far in 10 species of *Bryonia* (x = 10) and its sister genus *Ecballium* (x = 12 or x = 9, Table 4). Polyploidy is frequent in *Bryonia*. Sicyoeae is largest in terms of species (~264–266 species) (Schaefer and Renner 2011) of which cytological reports are known in around 14% species belonging to 9 genera (Table 5). Sicyoeae species range from x = 8 to x = 14 (Table 5). *Trichosanthes* Linnaeus, 1753 and *Luffa* Miller, 1754 have x = 11 and x = 13, respectively (Table 1). The less prevalent numbers include x = 12, x = 8 and x = 9 (Table 5). The possibility of multiple base number is noted in *Frantzia* Pittier, 1910 (x = 12/14) and *Sicyos* Lin-

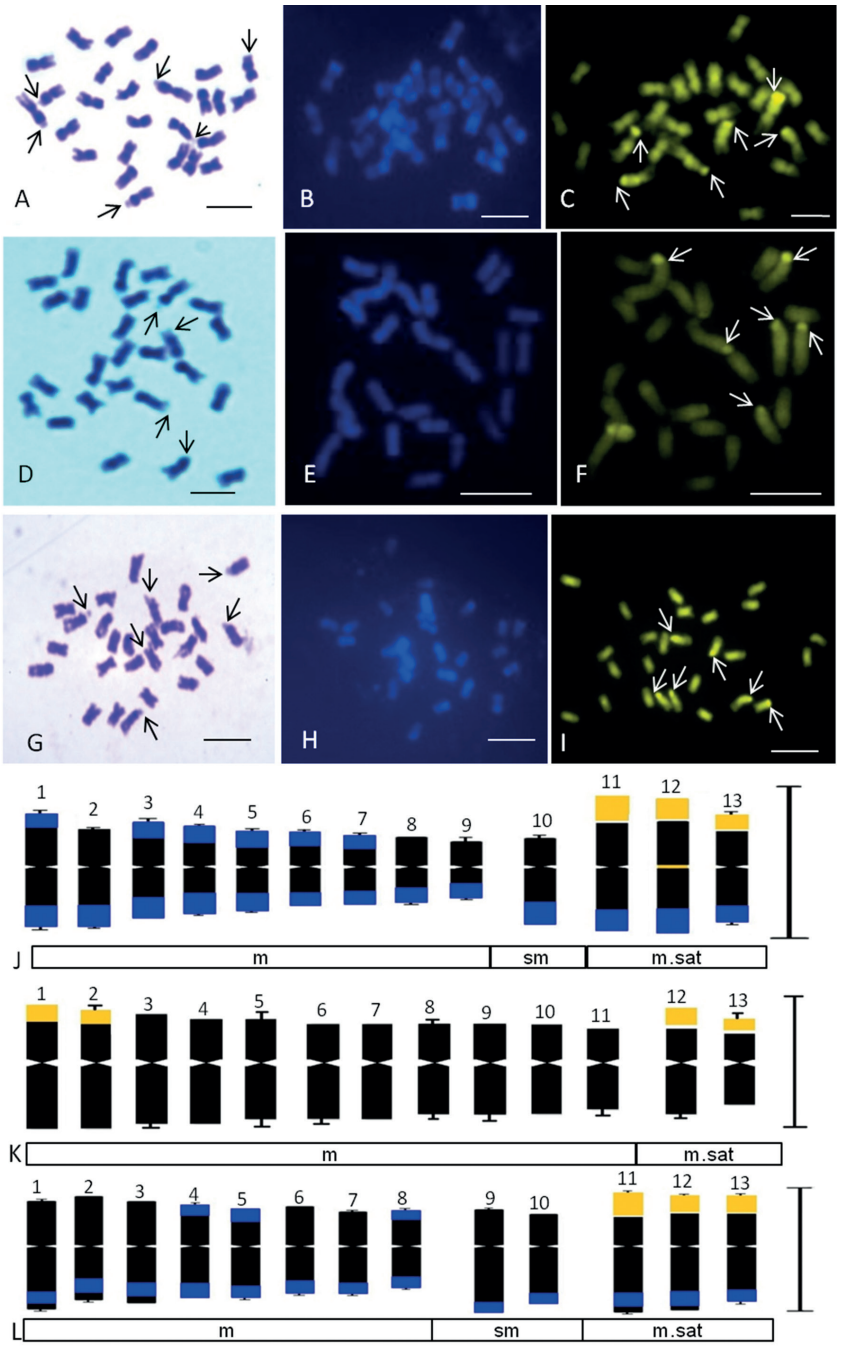


Figure 1. Somatic metaphase chromosomes and idiograms of *Luffa* species (2n = 26) stained with Giemsa (**A, D, G**), DAPI (**B, E, H**) and CMA3 (**C, F, I**) **A–C** *L. acutangula* **D–F** *L. aegyptiaca cylindrica* **G–I** *L. echinata*. Arrows indicate satellited chromosomes in Giemsa plates and CMA^{+ve} signals in **C, F, I**. Corresponding somatic idiograms (haploid set) of: **J** *L. acutangula* **K** *L. aegyptiaca* **L** *L. echinata*, showing DAPI^{+ve} (blue) and CMA^{+ve} (golden yellow) bands. Scale Bars: 5 µm

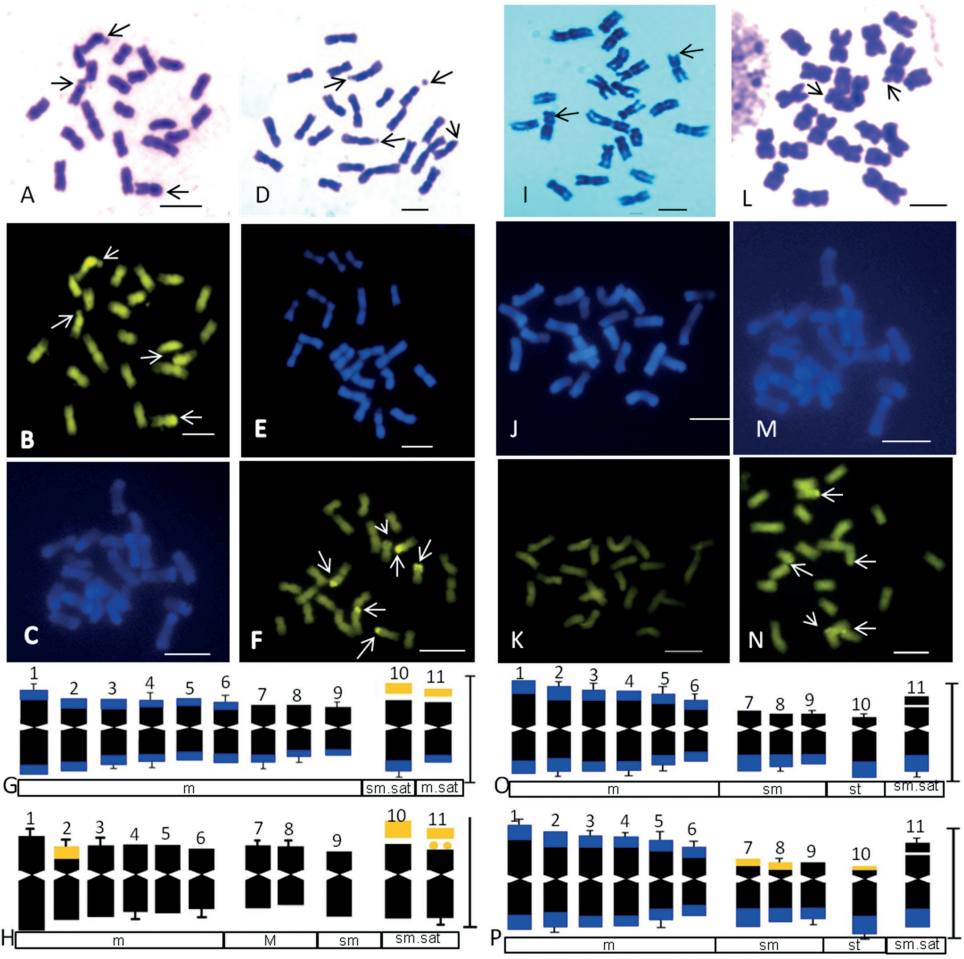


Figure 2. Somatic metaphase chromosomes and idiograms of *Trichosanthes* species stained with Giemsa (**A, D, I, L**), DAPI (**C, E, J, M**) and CMA3 (**B, F, K, N**) **A–C** *T. cucumerina* ssp. *cucumerina* (2n = 22), **D–F** *Trichosanthes cucumerina* ssp. *cucumerina* 'Anguina' (2n = 22) **I–K** *T. dioica* (male, 2n = 22) **L–N** *T. dioica* (female, 2n = 22). Arrows indicate satellited chromosomes in Giemsa plates and CMA^{+ve} signals in **B, F, K, N**. Corresponding somatic idiograms (haploid set) of: **G** *T. cucumerina* ssp. *cucumerina* **H** *Trichosanthes cucumerina* ssp. *cucumerina* 'Anguina' **O** *T. dioica* male plant **P** *T. dioica* female plant. Blue and golden yellow bands in idiograms indicate DAPI^{+ve} and CMA^{+ve} signals, respectively. Scale Bars: 5 µm

naeus, 1753 (x = 12/13/14). Natural tetraploids are known in two species of *Trichosanthes* while the majority are diploids. Benincaseae is the second largest tribe comprising of 204–214 species in 24 genera (Schaefer and Renner 2011). Cytological reports are known in around 35% species (76 species of which 41 belong to *Cucumis* Linnaeus, 1753) of 12 genera (Tables 6, 7). x = 12 is the prevalent condition in Benincaseae (Tables 1, 6, 7). Dual base numbers are noted in the widely studied *Cucumis* (x = 7, 12). *Coccinia* Wight et Arnott, 1834 (x = 12) may also possess dual base numbers (x = 10 in *C. trilobata* Cogniaux, 1895). Molecular cytogenetics of *Cucumis sativus* Linnaeus, 1753

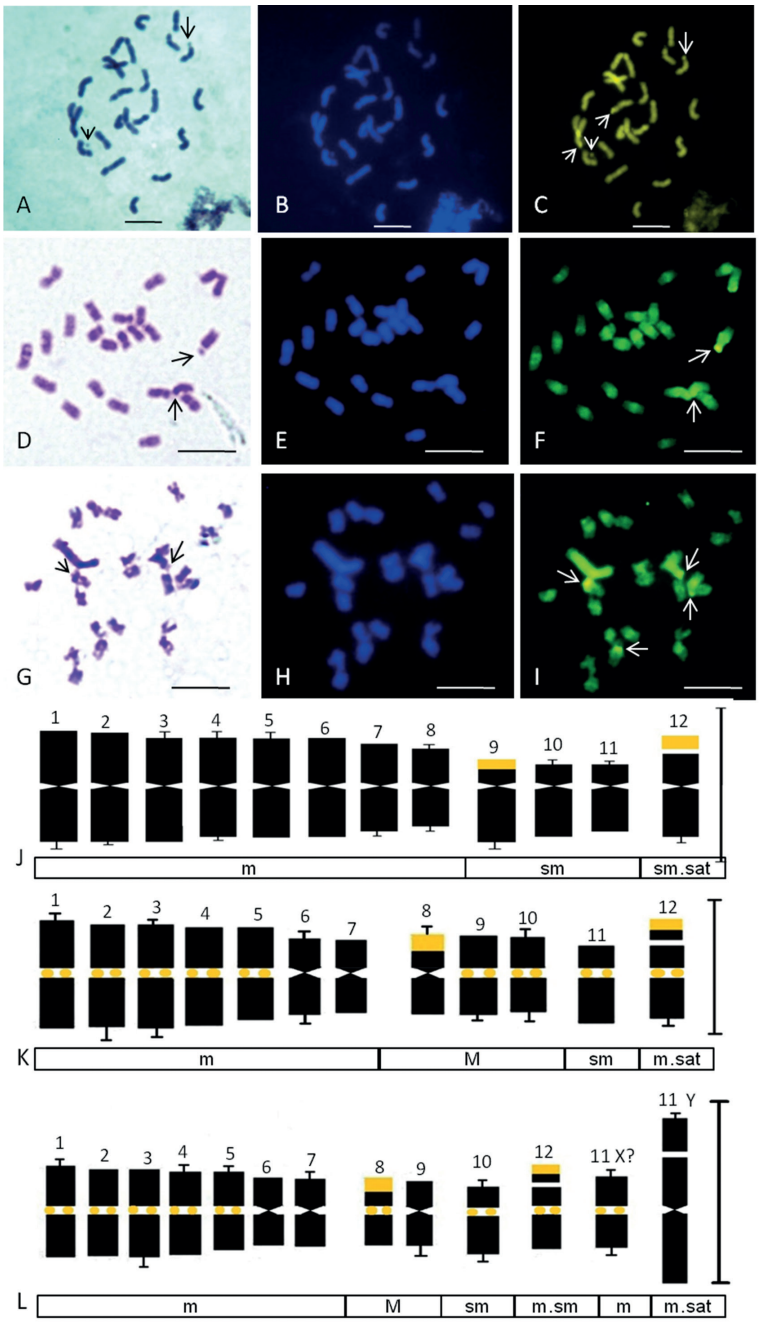


Figure 3. Somatic metaphase chromosomes and idiograms of two Benincaseae species (2n = 24) stained with Giemsa (**A, D, G**), DAPI (**B, E, H**) and CMA3 (**C, F, I**) **A–C** *Benincasa hispida* **D–F** *Coccinia grandis* (female plant) **G–I** *Coccinia grandis* (male plant). Arrows indicate satellited chromosomes in Giemsa plates and distal CMA^{+ve} signals in **C, F, I**. Note the longest Y chromosome without any CMA band in **G–I** and centromeric CMA^{+ve} signals in **F, I**. Corresponding somatic idiograms (haploid set) of: **J** *Benincasa hispida* **K** *Coccinia grandis* (female plant) **L** *Coccinia grandis* (male plant) with CMA^{+ve} (golden yellow) bands. Note the X chromosome remaining indistinguishable in **L**. Scale Bars: 5 µm

Table 2. Cytogenetic reports in the understudied tribes of Cucurbitaceae #.

Tribe and Genera	Species studied	Chromosome no.			Ploidy, Genome size, Chromosome features	References
		x	2n	n		
Gomphogyneae *Gomphogyne* Griffith, 1845	*G. cissiformis* Griffith, 1837		32[a]	16[b]	Tetraploid[c], autopolyploid[d]; 10 secondary constrictions, one pair satellited[e]; II, III, IV in meiosis[f]	CCDB[b]; Kumar and Subramaniam (1987)[a], Singh (1990)[a,d], Roy et al. (1991)[a,c,e,f]
Hemsleya F.B. Forbes et Hemsley, 1888	*H. amabilis* Diels, 1912, *H. carnosiflora* Wu et Chen, 1985, *H. chinensis* Forbes et Hemsley, 1888, *H. emeiensis* Shen et Chang, 1983, *H. graciliflora* Cogniaux, 1916, *H. heterosperma* Wallich, 1831, *H. macrocarpa* Cogniaux, 1916, *H. panacis-scandens* Wu et Chen, 1985, *H. sphaerocarpa* Kuang et Lu, 1982	7[a]	28[b], 22[c], 24[d], 26[e], 32[f], 40[g], 42[h]	14[h]	Tetraploid[i], aneuploids[j]	Samuel et al. (1995)[a-j], Anmin et al. (2011)[b, h]
Gynostemma Blume, 1825	*G. cardiospermum* Oliver, 1892	11[a]	66[b]		Hexaploid[c]	IPCN[a-c]
	G. guangxiense Chen et Qin, 1988		22[a]		Diploid[b]	IPCN[a,b]
	G. laxiflorum Wu et Chen, 1983		22[a]		Diploid[b]	IPCN[a,b]
	G. longipes Wu et Chen, 1983		22[a], 44[b]		Polyploid[c]	IPCN[a-c]
	G. microspermum Wu et Chen, 1983		22[a]		Diploid[b]	IPCN[a,b]
	G. pedatum Blume, 1825	12[a]	24[b]		Diploid[c]	Roy et al. (1991)[a,b,c]
	G. pentagynum Wang, 1989		22[a]		Diploid[b]	IPCN[a,b]
	G. pentaphyllum Thunberg, 1784		22[a], 24[b], 64[c], 66[d]		Diploid[c], triploid[f], hexaploid[g]; 2C (flow cytometry): 3.62pg[h]; 17M+14sm+2st[i]; CSR: 2.16–4.09 μm[j] 5S (8), 45S (10) rDNA and telomeric signals[k]	IPCN[a,b,c,e,f]; Zhang et al. (2013)[h], Pellerin et al. (2018)[d,g,i,j,k]
	G. pentaphyllum var. *dasycarpum* Wu, 1983		22[a], 33[b], 44[c]		Polyploid[d]	IPCN[a-d]
	G. pentaphyllum var. *pentaphyllum* Thunberg, 1784		22[a], 44[b], 66[c], 88[d]		Polyploid[e]	IPCN[a-e]
	G. yixingense Wang et Xie, 1981		88[a]		Polyploid[b]	IPCN[a,b]
Triceratieae *Fevillea* Linnaeus, 1753		8[a]			-	Roy et al. (1991)[a]
Zanonieae *Zanonia* Linnaeus, 1753	*Z. indica* Linnaeus, 1759	15[a]	30[b]	15[c]	Autoploid[d]; Metacentric chromosomes[e]; CSR: 1.10-1.98 μm[f]	Lekhak et al. (2018)[a-f]
Actinostemmateae	*A. lobatum* (Maxim.) Maxim. ex Franch. & Sav.		16[a]		-	IPCN[a]
Actinostemma Griffith, 1841	*A. tenerum* Griffith, 1837		16[a]		Diploid[b]; 7M +1sm[c]; CSR: 2.88–4.02 μm[d]; 45S (1) rDNA and 45S+5S (1) rDNA adjacent signal[e]; telomeric repeat signals[f]	Pellerin et al. (2018)[a-f]
Thladiantheae *Thladiantha* Bunge, 1833	*T. calcarata* Clarke, 1876, *T. cordifolia* Blume, 1826 *T. davidii* Franchet, 1886, *T. dentata* Cogniaux, 1916, *T. lijiangensis* Lu et Zhang, 1981, *T. nudiflora* Hemsley, 1887, *T. pustulata* Léveillé, 1916	3[a], 5[b], 9[c]	18[d]	5[e], 9[f]	Diploid[g]	Darlington and Janaki Ammal (1945) [c]; Roy et al. (1991)[a,b,d,e,g], IPCN[d,f]
	T. dubia Bunge, 1833		18[a], 22[b]		Diploid[c]; 7M+1sm+1st[d]; CSR: 2.60–4.10 μm[e]; 45S (4) and co-localized 45S+5S (1) rDNA signals[f]; telomeric repeat signals[g]	Samuel et al. (1995)[b], Pellerin et al. (2018)[a,c,d,e,f,g]
Baijiania Lu et Li, 1993	*B. yunnanensis* Lu et Zhang, 1984		32[a]		-	IPCN[a]
Siraitieae *Siraitia* Merrill, 1934	*S. grosvenorii* Swingle, 1941		28[a]		45S (6) and 5S (2) rDNA signals[b]	IPCN[a], Li et al. (2007)[b]
Joliffieae *Telfairia* Hooker, 1827	*T. occidentalis* Hooker, 1871		22[a], 33[b], 44[c]		Diploid[d], aneuploid[e], triploid[f], Tetraploid[g]; 1 B[h]	Uguru and Onovo (2011)[a-h]
	T. pedata Sims, 1826		22[a]		-	Bhowmick and Jha (2015)[a]

Tribe and Genera	Species studied	Chromosome no.			Ploidy, Genome size, Chromosome features	References
		x	2n	n		
Schizopeponeae *Herpetospermum* Hooker, 1867	*H. pedunculosum* Seringe, 1828			11[a]	45S (14), 5S (2) rDNA signals[b]	Xie et al. (2019a)[a,b]
Schizopepon Maximowicz, 1859	*S. bryoniifolius* Maximowicz, 1859	10[a]	20[b]		-	Roy et al. (1991)[a], IPCN[b]
Coniandreae *Apodanthera* Arnott, 1841	*A. undulata* Gray, 1853	14[a]			-	IPCN[a]
Corallocarpus Bentham et Hooker, 1867	*C. epigaeus* Rottler, 1803		26[a]	13[b]	-	Beevy and Kuriachan (1996)[a,b]
	C. welwitschii Naudin, 1863		72[a]		-	Singh (1990)[a]
Ibervillea Greene, 1895		11[a], 12[b]			-	Darlington and Janaki Ammal (1945)[a,b]
Kedrostis Medikus, 1791	*K. africana* Linnaeus, 1753		40[a]		2C (feulgen densitometry): 0.8 pg[b]; 2C (flow cytometry): 1674 Mbp[c]	Bennet et al. (1982)[a,b], Plant C DNA Values Database[c]
	K. foetidissima Jacquin, 1788		26[a]	13[b]		Beevy and Kuriachan (1996)[a,b]
	K. rostrata Rottler, 1803	13[a]	26[b]	13[c]	-	IPCN[a-c]
Seyrigia Keraudren, 1960				13[a]	-	IPCN[a]

x: base number; 2n: zygotic number; n: gametic number; CSR: chromosome size range; B: B chromosome; II: bivalents, III: trivalent, IV: tetravalent; superscripts correspond to references.

has demonstrated the evolution of x = 7 from x = 12 in Benincaseae. x = 11 has been confirmed in *Citrullus* Schrader, 1836 and *Lagenaria*. The base number of *Melothria* Linnaeus, 1753, *Solena* Loureiro, 1790 and *Zehneria* Endlicher, 1833 can be x = 11 or x = 12 or both (Table 6). Cases of natural polyploidy are noted only in four species of *Cucumis* (Table 7). Cytogenetic information is available for 17 species in three genera of Cucurbiteae with x = 10 and many polyploids (Table 8). The zygotic chromosome numbers of *Luffa*, *Trichosanthes*, *Benincasa* Savi, 1818 and *Coccinia*, corroborate the previous reports (Figs 1–3, Table 1).

Nuclear genome contents

Nuclear genome sizes are reported in 49 species (~5% of total species) belonging to 15 genera (~16% of total genera) of Cucurbitaceae. Among the understudied tribes, 2C genome content is known for one species each from Gomphogyneae and Coniandreae (Table 2). Within the Momordiceae species of India, significant interspecific genome size differences have been reported (Ghosh et al. 2021). The species differed 5.19-fold in their genome sizes (2C = 0.72-3.74 pg) (Table 3) (Ghosh et al. 2021). Interestingly, the species with lowest chromosome number (*M. cymbalaria*, 2n = 18) contained highest nuclear DNA content among the four *Momordica* species (Table 3). In Bryonieae, flow cytometric genome size of *Bryonia* shows a 2.2-fold increase than *Ecballium* (Table 4). In case of Sicyoeae, flow cytometric 2C DNA content ranges from 1.49–2.32 pg/2C, indicating 1.55-fold differences in genome size. *Echinocystis lobata* Michaux, 1803, in spite of tetraploid condition, shows lowest genome size (Table 5). There is no significant difference in genome size between the genders of *Trichosanthes dioica* Roxburgh, 1832

Table 3. Cytogenetic information in *Momordica* (Momordiceae)#.

Species	Chromosome no.		Ploidy, Genome size, Chromosome features	References
	2n	n		
M. balsamina Linnaeus, 1753	22[a]		Diploid[b]; two chromosomes with double constrictions[c]; CSR:0.65–1.98μm[d]; MCL:1.30μm[c]; TCL: 28.61μm[f]	Bharathi et al. (2011)[a-f]
M. charantia	22[a]	11[b]	Diploid[e], 2C (Feulgen densitometry): 4.10pg [f], 2C (flow cytometry): 1.43pg[g]; chromosomes mostly metacentric, few submetacentric and subtelocentric[h]; 2 chromosomes with satellites[i]; CSR: 1.26-1.81μm[i]; 45S (4) and 5S (2) rDNA signals[k]	Plant DNA C-Values Database[f]; Bharathi et al. (2011)[a,i] ; Barow and Meister (2003)[g]; Lombello and Pinto-Maglio (2007)[a,h,h]; Bharathi et al. (2011); Waminal and Kim (2012)[a,e,h,j,k]; Kausar et al. (2015)[a]; Kido et al. (2016)[a,i]
M. charantia var. *charantia*	22[a]	11[b]	Diploid[c]; 2C (flow cytometry): 0.72pg[d]; NORs: 4[c]; nucleolar and centromeric CMA$^+$ bands[f]; CSR: 1.27-3.07μm[g]; MCL: 1.97 μm[h]; HCL: 21.77μm[i]	Ghosh et al. (2018)[a-f]; Ghosh et al. (2021)[a,c,d,g,h,i]
M. charantia var. *muricata* Chakravarty, 1982	22[a]	11[b]	Diploid[c]; 2C (flow cytometry): 1.16pg[d]; NORs: 6[c]; nucleolar and centromeric CMA$^+$ bands[f]; CSR: 1.64-3.13μm[g]; MCL: 2.19μm[h]; HCL: 24.19μm[i]	Ghosh et al. (2018)[a-f]; Ghosh et al. (2021)[a,c,d,g,h,i]
M. cochinchinensis Loureiro, 1790	28[a]	14[b]	Diploid[c], 2C (flow cytometry): 2.64pg[d], 6[e] chromosomes with secondary constrictions; CSR:1.16–2.03μm[f] /1.71-3.17μm[g] ; MCL: 2.27μm[h]; HCL: 31.86μm[i]; 45S (8) and 5S (2) rDNA signals[j]	IPCN[b]; Xie et al. (2019a)[a,j]; Bharathi et al. (2011)[a,e,f]; Ghosh et al. (2021)[a,c,d,g,h,i]
M. cymbalaria	18[a]	8[b], 9[c], 11[d]	Diploid[e], 2C (flow cytometry): 3.74 pg[f]; 2[g]–4[b] chromosomes with secondary constrictions; CSR: 2.71-4.57μm[i]; MCL:3.75μm[j]; HCL: 33.79μm[k]	IPCN[b]; CCDB[b,d]; Bharathi et al. (2011)[a,c,e,g]; Ghosh et al. (2021)[a,e,f,h,i,j,k]
M. denudata Clarke, 1879		14[a]	-	IPCN[a]
M. dioica Willdenow, 1805	28[a], 56[b]		Diploid[c]; 2C (flow cytometry): 3.36 pg[d], 2[e]-12[f] chromosomes with secondary constrictions; CSR: 2.04-3.58μm[g]; MCL: 2.75μm[h]; HCL: 77.10μm[i]; 45S (4) and 5S (2) rDNA signals[j]	Bharathi et al. (2011)[a,c,e]; Xie et al. (2019a)[a,j]; Ghosh et al. (2021)[b,d,f,g,h,i]
M. foetida Schumacher, 1827	44[a]		-	Behera et al. (2011)[a]
M. rostrata Zimmermann, 1922	22[a]		-	Behera et al. (2011)[a]
M. sahyadrica Kattukunnel et Antony, 2007	28[a]		2 chromosomes with secondary constrictions[b];CSR: 0.73–1.83μm[c]; TCL: 37.53μm[d], MCL: 1.34μm[e]	Behera et al. (2011)[a-e]
M. subangulata Blume, 1826	56[a]		2C (flow cytometry): 3.06pg[b]; 8 chromosomes with secondary constrictions[c]; CSR: 1.52-3.11μm[d]; HCL: 60.30μm[e]	Ghosh et al. (2021)[a-e]
M. subangulata subsp. *renigera* Don, 1834	56[a]		4 chromosomes with secondary constrictions[b]; CSR: 0.52-1.26μm[c]; MCL: 0.93μm[d]; TCL 51.88μm[e]	Bharathi et al. (2011)[a-e]
M. tuberosa Miquel, 1855	22[a]	11[b]	-	IPCN[a,b]; CCDB[a,b]

2n: Zygotic chromosome number; n: gametic chromosome number; I: univalent; II: bivalent; III: trivalent; CSR: chromosome size range; MCL: mean chromosome length; HCL: total length of haploid set of chromosomes; TCL: total length of diploid set of chromosomes; NOR: nucleolar organizing region, superscripts correspond to references.

Table 4. Chromosome number and genome size in Bryonieae#.

Genera	Species studied	Chromosome no.			Genome size	References
		x	2n	n		
Bryonia		10[a]				Darlington and Janaki Ammal (1945)[a]
	B. alba Linnaeus, 1753	10[a]	20[b]	10[c]	2C (flow cytometry): 5827Mbp[d]	CCDB[d] , Volz and Renner (2008) [a,b,c]
	B. aspera Ledebour, 1843	10[a]	40[b], 60[c]	20[d], 10[e]	-	Kumar and Subramaniam (1987)[c], Volz and Renner (2008)[a,b,d,e]
	B . cretica Linnaeus, 1753	10[a]	60[b]	30[c]	-	Volz and Renner (2008) [a,b,c]
	B. dioica Jacquin, 1774	10[a]	20[b]	10[c]	2C (microdensitometry): 4.01pg[d]; 2C (flow cytometry): 5522Mbp[c]	CCDB[d,e], Volz and Renner (2008)[a,b,c]
	B. macrostylis Heilbronn et Bilge, 1954			10[a]	-	IPCN[a]
	B . marmorata Petit, 1889		40[a]	20[b]	-	Volz and Renner (2008)[a,b]
	B . monoica Aitchison et Hemsley, 1886	10[a]	20[b]		-	Volz and Renner (2008)[a,b]
	B. multiflora Boissier et Heldreich, 1849	10[a]			-	Volz and Renner (2008)[a]
	B. syriaca Boissier, 1856	10[a]	20[b]		-	Volz and Renner (2008) [a,b]
	B. verrucosa Aiton, 1789	10[a]	20[b]	10[c]	2C (flow cytometry): 2.09pg[d]; 4504Mbp[c]	CCDB[d,e], Volz and Renner (2008)[a–c]
Ecballium		12[a]				Darlington and Janaki Ammal (1945)[a]
	E. elaterium Linnaeus, 1753		18[a]	12[b]	2C (flow cytometry): 2442Mbp[c]	Veselý (2012)[c] , Volz and Renner (2008)[b]
	E. elaterium subsp. *dioicum* Battandier, 1989		18[a], 24[b]	9[c], 12[d]	-	Volz and Renner (2008)[a–d]
	E. elaterium Linnaeus, 1753 subsp. *elaterium*		18[a]	9[b]	-	Volz and Renner (2008)[a,b]

2n: Zygotic chromosome number; n: gametic chromosome number.

(Table 5). Genome size estimates are known from 24 Benincaseae species of which 17 species belong to *Cucumis* (Tables 6, 7). Highest 2C nuclear genome is known in *Benincasa hispida* Thunberg, 1784 (1.97 pg) (Bhowmick and Jha 2015a) while the lowest is known in *Cucumis melo* var. *inodorus* Harz, 1885 (0.64 pg) (Karimzadeh et al. 2010). In case of *Cucumis*, there is yet no consensus on whether the taxa with different base numbers (x = 7, 12) have correspondingly dissimilar genome sizes since the researchers depended on diverse methods of genome size estimation. Lower 2C genome size was reported in *C. Coccinia grandis* Linnaeus, 1767 (2n = 24) while *C. trilobata* (2n = 20) had higher 2C DNA content (Table 6). The divergence in genome size between genders was found to be highest in dioecious *C. grandis* (Table 6), a sharp contrast to dioecious *Trichosanthes dioica* (Table 5). Benincaseae shows a 3.07-fold overall difference in genome size. Genome sizes are known in eight species of *Cucurbita* Jussieu, 1789. Flow cytometric genome size ranges from 0.686–0.933 pg/2C, indicating a 1.36-fold variation (Table 8). Despite polypoidy, the nuclear DNA content of *Cucurbita* species is comparable to many diploids.

Karyotypes, chromosome banding and molecular cytogenetics

Among the understudied tribes, information on chromosome morphology, size and karyotype are reported in very few taxa (Table 2). In *Gynostemma pentaphyllum* Thunberg, 1784, the number of rDNA loci was suggested to reduce during polyploidization (Pellerin et al. 2018). The *Actinostemma tenerum* Griffith, 1837, genome contained in-

Table 5. Cytogenetic information in Sicyoeae#.

Genera studied	Species studied	Chromosome no.			Ploidy, Genome size, Chromosome features	References
		x	2n	n		
Cyclanthera Lilja, 1870		8[a]				Darlington and Janaki Ammal (1945)[a]
	C. pedata (L.) Schrader, 1831		16[a], 32[b]	8[c]	Diploid[e]	Roy et al. (1991)[a,c,e], Samuel et al. (1995)[b]
Echinocystis Torrey et Gray, 1840		8[a]		16[b]		Bhowmick and Jha (2015b)[b], Darlington and Janaki Ammal (1945)[a]
	E. lobata Michaux, 1803		16[a], 32[b]		Tetraploid[c]; 2C (flow cytometry):1.49pg[d]	IPCN[a,b], Plant DNA C-Values Database[c,d]
	E. macrocarpa Greene, 1885		32[a]		-	Whitaker (1950)[a]
Echinopepon Naudin, 1866	*E. wrightii* Gray, 1853			12[a]	-	IPCN[a], CCDB[a]
Frantzia				12[a], 14[b]	-	Schaefer and Renner (2011)[a,b]
Hodgsonia Persson, 1953	*H. macrocarpa* var. *capniocarpa* Ridley, 1920		18[a]		-	IPCN[a], CCDB[a]
Luffa		13[a]				Darlington and Janaki Ammal (1945)[a]
	L. acutangula	13[a]	26[b]	13[c]	Diploid[d]; CSR:1.39–3.20µm[e]; 18m+2sm+6m.st[f]; NORs:6[g]; distal DAPI and nucleolar CMA signals[h]	Kumar and Subramaniam (1987)[a,b], IPCN[c], Bhowmick and Jha (2021)[b,d-h]
	L. acutangula var. *acutangula*			13[a]	-	Beevy and Kuriachan (1996)[a]
	L. acutangula var. *amara* Clarke, 1879			13[a]	-	Beevy and Kuriachan (1996)[a]
	L. aegyptiaca (syn *L. cylindrica* Roemer, 1846)	13[a]	26[b]	13[c]	Diploid[d], 2C (flow cytometry): 1.56 pg[e]; 2C (Feulgen densitometry): 1.7pg[f]; CSR: 1.60–2.06µm[g]; 24M+1sm[h]; 22m+ 4m.st[i]; NORs:2[j]; nucleolar and distal CMA signals[k]; 45S (10) and 5S (2) rDNA signals[l]	Bennet et al. (1982)[b,d,f], Kumar and Subramaniam (1987)[a,b], Waminal and Kim (2012)[b,d,g,h,l], Bhowmick and Jha (2015a)[b,c,d,e,i,j,k]
	L. echinata		26[a], 39[b], 52[c]	13[d]	Diploid[c]; CSR 2.44–3.96 µm[f]; 16m+4sm+6m.st[g]; NORs: 6[h]; Distal and intercalary DAPI and nucleolar CMA signals[i]	Kumar and Subramaniam (1987)[a-c], Bhowmick and Jha (2021)[a,e,i]
	L. graveolens Roxburgh, 1832	13[a]			-	Kumar and Subramaniam (1987)[a]
	L. hermaphrodita Singh et Bhandari, 1963			13[a]	-	IPCN[a]
	L. operculata Linnaeus, 1759	13[a]	26[b]	13[c]	-	Kumar and Subramaniam (1987)[a,b], IPCN[c]
Sicyos (75, includes *Sechium*, *Microsechium*)		12[a]	24[b]		-	Darlington and Janaki Ammal (1945)[a,b]
	S. angulatus	12[a]	24[b]		Diploid[c]; CSR: 1.9-4.6µm[d]; 4 adjacent 45S+5S rDNA signals[e]	Waminal and Kim (2015)[a-e]; IPCN[b]
	S. australis Endlicher, 1833		24[a], 26[b]		12II[c], 13II[d]	IPCN[a-d], CCDB[a-d]
	S. edulis Jacquin, 1760 (syn of *Sechium edule*)	13[a]	26[b], 28[c]	12[d], 13[c]	Diploid[f]; metacentric and submetaccentric chromosomes[g]; CSR: 2.69–5.38µm[h]; 45S (6), 5S (2) rDNA and telomeric repeat signals (28)[i]	Beevy and Kuriachan (1996)[a,b,e], Pellerin et al. (2018)[c,f,g,h,i], Ting et al. (2019)[c], IPCN[d], CCDB[d]
	S. nihoaensis St. John, 1970			12[a]	-	IPCN[a], CCDB[a]
	Sechium compositum Smith, 1903 (syn. *Microsechium compositum*)			14[a]	-	IPCN[a], CCDB[a]
	S. hintonii Wilson, 1958 (syn *Microsechium hintonii*)			14[a]	-	IPCN[a], CCDB[a]

Genera studied	Species studied	Chromosome no.			Ploidy, Genome size, Chromosome features	References
		x	2n	n		
Trichosanthes (100)		11[a]				Darlington and Janaki Ammal (1945)[a]
	T. anaimalaiensis Beddome, 1864		22[a]	11[b]	-	Beevy and Kuriachan (1996)[a,b]
	T. boninensis Nakai et Tuyama, 1928		22[a]		-	IPCN[a]
	T. bracteata Lamarck, 1797	11[a]	22[b], 44[c], 66[d]		-	Kumar and Subramaniam (1987)[a,b], Roy et al. (1991)[c,d]
	T. bracteata var. *bracteata*			11[a], 22[b]	-	Beevy and Kuriachan (1996)[a,b]
	T. chingiana Handel-Mazzetti, 1936		22[a]		-	IPCN[a]
	T. costata Blume, 1826 (syn *Gymnopetalum chinense* Loureiro, 1790)		22[a]		Diploid[b]; 45S (6) and 5S (4) rDNA signals[c]	Kumar and Subramaniam (1987)[a], Xie et al. (2019a)[b,c]
	T. cucumerina		22[a]	11[b]	Diploid[c]; 12m+4M+2sm+4sm.st[d]; CSR: 2.26–4.99µm[e]; 6 chromosomes with double constrictions[f]; NORs: 4[g]; nucleolar CMA and distal DAPI bands[h]	Bhowmick and Jha (2019)[a-h]
	T. cucumerina ssp. *cucumerina* Anguina	11[a]	22[b]	11[c], 22[d], 32[e], 33[f]	Diploid[c]; 2C (Feulgen densitometry): 2.2pg[f]; CSR: 2.77–5.01µm[g]; 12m+4M+2sm+4sm.st[h]; 6 chromosomes with double constrictions[i]; NORs: 4[j]; nucleolar and distal CMA bands[k]; 45S (6) and 5S (2) rDNA signals[l]	Kumar and Subramaniam (1987)[a], Bhowmick and Jha (2019)[b,c,e,g,h,i,j,k], Xie et al. (2019a)[b,l], IPCN[e-f]
	T. dioica	11[a]	22[b]	11[c]	Diploid[d]; 2C (flow cytometry): male- 2.27pg, female- 2.32 pg[e]; 12m+6Sm +2St+2Sm.t[f]; distal DAPI bands[g]; distal CMA bands in females[h]; 1 rod bivalent in meiosis[i]	Kumar and Subramaniam (1987)[a], Guha et al. (2004)[b,d,f,h], Bhowmick and Jha (2015a)[b,c,d,e,f,g,h,i]
	T. dunniana Léveillé, 1911		22[a]		Diploid[b]; 45S (6) and 5S (2) rDNA signals[c]	Xie et al. (2019a)[a-c]
	T. himalensis Clarke, 1879			11[a]	-	Roy et al. (1991)[a]
	T. hupehensis Cheng et Yueh, 1974		22[a]		-	IPCN[a], CCDB[a]
	T. kirilowii Maximowicz, 1859		60[a], 66[b], 88[c], 110[d]		Hexa-, octa-, decaploid[e];CSR: 2.3-3.5µm[f]; 45S (4), 5S (4) and 45S +5S (6) adjacent rDNA signals[g]	IPCN[a], CCDB[a], Waminal and Kim (2015)[b,c,d,e-g]
	T. kirilowii var. *japonica*			11[a]	-	Roy and Saran (1990)[a]
	T. lepiniana Naudin, 1868		44[a]	11[b]	1 B[c]	Roy et al. (1991)[b,c], IPCN[a], CCDB[a]
	T. lobata Roxburgh, 1832	11[a]		11[b]	-	Kumar and Subramaniam (1987)[a], Beevy and Kuriachan (1996)[b]
	T. mianyangensis Yueh et. Liao, 1992		88[a]		-	IPCN[a], CCDB[a]
	T. nervifolia Linnaeus, 1753			11[a]	-	Beevy and Kuriachan (1996)[a]
	T. ovigera Blume, 1826		22[a]		Diploid[b]; 45S (10) and 5S (2) rDNA signals[c]	Xie et al. (2019a)[a-c]
	T. palmata Linnaeus, 1759		22[a], 44[b], 66[c]	11[d]		IPCN[a-d]
	T. pedata Merril et Chun, 1934		22[a]		-	IPCN[a], CCDB[a]
	T. truncata Clarke, 1879		22[a]		-	IPCN[a], CCDB[a]
	T. wallichiana Wight, 1840	11[a]	22[b]		-	Kumar and Subramaniam (1987)[a,b]

#x: base number; 2n: zygotic number; n: gametic number; NOR: nucleolar organizing region; B: B chromosome; II: bivalents; superscripts correspond to references.

Table 6. Cytogenetic information on Benincaseae#.

Genera studied	Species studied	Chromosome no.			Ploidy, Genome size, Chromosome features	References
		x	2n	n		
Benincasa	*B. fistulosa*		24[a]		Diploid[b]; 45S (4) and 5S (4) signals[c]	Li et al. (2016)[a,b,c]
	B. hispida	12[a]	24[b]	12[c]	Diploid[d]; 2C (flow cytometry):1.97pg[e], 2C (feulgen densitometry):2.1pg[f]; CSR 2.54-4.59µm[g]; 16m+6Sm+2Sm.t[h]; NORs:2[i]; distal CMA signals[j]; 45S (2) and 45S+5S (2) adjacent rDNA signals[k]	Plant DNA C-Values Database[e], Waminal et al. (2011)[b,d,g,k], Bhowmick and Jha (2015a)[h,c,d,e,h,i,j]
Citrullus		11[a]				Darlington and Janaki Ammal (1945)[a]
	C. amarus (syn. *C. lanatus* var. *citroides*)	11[a]	22[b]		Diploid[c]; CSR: 3.1–4.7µm[d]; 45S (2) and 5S (4) rDNA signals[e]	Reddy et al. (2013)[b-d], Waminal and Kim (2015)[a-e], Renner et al. (2017)[b]
	C. colocynthis		22[a]	11[b]	Diploid[c]; 45S (2) and 45S+5S (2) adjacent rDNA signals[d]	Beevy and Kuriachan (1996)[b], Reddy et al. (2013)[a,c,d], Li et al. (2016)[a,c,d]
	C. ecirrhosus		22[a]	11[b]	Diploid[c]; 2 satellites detected in meiosis[d]; 45S (2) and 5S (4) rDNA signals[e]; regular meiosis[f]	Li et al. (2016)[a,c,e], Renner et al. (2017)[a,b,c,d,f]
	C. lanatus		22[a]	11[b]	Diploid[c]; CSR: 1.09µm-1.72µm[d]; 14m+8sm[e]; 45S (2) and 45S+5S (2) adjacent rDNA signals[f]; linkage groups hybridized to chromosomes[g]	Beevy and Kuriachan (1996)[b], Waminal et al. (2011)[a,c,d,e,f], Ren et al. (2012)[a,c,g]
	C. lanatus subsp. *lanatus*		22[a]		Diploid[b]; 45S (2) and 5S (4) rDNA signals[c]	Li et al. (2016)[a-c]
	C. lanatus subsp. *mucosospermus* Fursa, 1972		22[a]		Diploid[b]; 45S (2) and 45S+5S (2) adjacent rDNA signals[c]	Li et al. (2016)[a-c]
	C. lanatus subsp. *vulgaris* Schrader, 1836		22[a]		Diploid[b]; 45S (2) and 45S+5S (2) adjacent rDNA signals[c]	Li et al. (2016)[a-c]
	C. lanatus var. *lanatus*		22[a]		Diploid[b]; 45S (2) and 45S+5S (2) adjacent rDNA signals[c]	Reddy et al. (2013)[a-c]
	C. naudinianus (syn *Acanthosicyos naudinianus*)		24[a]		Diploid[b]; 45S (2) and co-localized 45S+5S (2) rDNA signals[c]	Li et al. (2016)[a,b,c]
	C. rehmii		22[a]		Diploid[b]; 45S (2) and 5S (2) rDNA signals[c]	Reddy et al. (2013)[a-c], Li et al. (2016)[a-c]
	C. vulgaris Schrader, 1836		22[a], 44[b]	11[c]	Diploid[d]; 2C: 0.88/0.90pg[e]	IPCN[a,c,d], Arumuganathan and Earle (1991)[e]
Coccinia (30)		12[a]				Darlington and Janaki Ammal (1945)[a]
	C. abyssinica Lamarck, 1753	12[a]	24[b]		-	Kumar and Subramaniam (1987)[a], Roy et al. (1991)[b]
	C. grandis	12[a]	24[b]	12[c]	Diploid[d], 2C (Flow cytometry): male- 0.943[e]/0.92[f] pg and female- 0.849[g]/ 0.73[h] pg; CSR: 1.33-4.71µm (male) and 1.35-2.26µm (female)[i]; 15m+4M+2sm+2m:sm+1m:st (Y) in male and 14m+6M+2sm+2m:st in female[j]; NORs-2[k]; chromosomal C bands[l]; centromeric, nucleolar CMA bands[m]; 45S (4)[n] rDNA signals, 2 signals adjacent to 5S[o]; GISH performed[p]; repetitive, organellar DNA hybridized[q]; centromere immunofluorescence[r]; heteromorphic sex chromosomes (largest Y)[s]; X-Y bivalent (meiosis)[t]	Bhowmick et al. (2012)[b,c,d,j,k,m,s,t], (2016) [b,d,f,h,j,k,n,s], Sousa et al. (2013)[b,d,e,g,i,k,l,n,o,r,s,t], Sousa et al. (2017)[b,d,k,n,o,p,q,r,s], Xie et al. (2019a)[b,n,o]
	C. hirtella Cogniaux, 1896		24[a]		Diploid[b]; 2C (flow cytometry): male-0.988pg[c]; 45S (4) and 45S+5S (2) adjacent rDNA signals[d], repetitive and organellar DNA hybridized[e]; centromere immunofluorescence performed[f]	Sousa et al. (2017)[a-f]
	C. sessilifolia Sonder, 1881		24[a]		Diploid[b]; 2C (Flow cytometry): male- 0.984pg, female- 0.998pg[c]; 45S (4) and 45S+5S (2) adjacent rDNA signals[d]; repetitive and organellar DNA[e]; centromere immunofluorescence performed[f]	Li et al. (2016)[a,b,d], Sousa et al. (2017)[a-f]
	C. trilobata		20[a]		Diploid[b]; 2C (flow cytometry): male- 1.263pg [c]; 45S (2) and 45S+5S (2) adjacent rDNA signals[d], repetitive, organellar DNA sequence hybridized[e]	Sousa et al. (2017)[a-e]
Ctenolepis Hooker, 1867	*C. garcinii* Burman, 1768		24[a]	12[b]	-	Kumar and Subramaniam (1987)[a], Beevy and Kuriachan (1996)[b]

Genera studied	Species studied	Chromosome no.			Ploidy, Genome size, Chromosome features	References
		x	2n	n		
Diplocyclos Endlicher, 1833	*D. palmatus*		24[a]		Diploid[b]; 45S (4) and 45S+5S (2) adjacent rDNA signals[c]	Li et al. (2016)[a-c]
Lagenaria Seringe, 1825	*L. leucantha* Rusby, 1896		22[a]	11[b]	-	IPCN[a,b], CCDB[a,b]
	L. leucantha var. *clavata* Makino, 1940		22[a]		-	CCDB[a]
	L. siceraria	11[a]	22[b]	11[c]	Diploid[d], 2C (flow cytometry): 0.734pg[e]; 2C (Feulgen densitometry):1.4pg[f]; CSR: 0.56–1.06µm[g]; metacentric and few sub-metacentric chromosomes[h]; 45S (2) and 45S+5S (2) adjacent rDNA signals[i]	Darlington and Janaki Ammal (1945)[a], Plant DNA C-Values Database[f], Beevy and Kuriachan (1996)[c], Achigan-Dako et al. (2008)[d,e], Waminal and Kim (2012)[b,d,g,h,i], Li et al. (2016)[b,d,i], Xie et al. (2019a)[b,i]
	L. siceraria var. *macrocarpa*		22[a]		-	CCDB[a]
	L. vulgaris Seringe, 1825		22[a]	11[b]	Diploid[c]; 2C (Feulgen densitometry): 1.40pg[d]	Bennet et al. (1982)[a,b,c]
Melothria		11[a], 12[b]				Darlington and Janaki Ammal (1945)[a,b]
	M. pendula Linnaeus, 1753		24[a]		Diploid[b]; 45S (2) and 45S+5S (2) adjacent rDNA signals[c]	Li et al. (2016)[a-c]
	M. perpusilla Blume, 1826		48[a]		-	Kumar and Subramaniam (1987)[a]
	M. scabra Naudin, 1866		24[a]		-	CCDB[a]
Peponium Engler, 1897	*P. betsiliense* Keraudren, 1960		24[a]		-	CCDB[a]
Solena	*S. amplexicaulis* Lamarck, 1785 (syn. *S. heterophylla*, *Melothria heterophylla*, *Zehneria umbellata*)		22[a], 24[b], 26[c], 36[d], 48[e]	11[f], 12[g], 24[h]	2-4 B[i]	Kumar and Subramaniam (1987)[a,b,c,e], Roy et al. (1991)[d,i], Beevy and Kuriachan (1996)[b,g,h], IPCN[b,d,e,f,g,h]
Zehneria	*Z. capillacea* Jeffrey, 1962 (syn. *Melothria capillacea*)		22[a]		-	CCDB[a]
	Z. indica Loureiro, 1790 (syn. *Melothria japonica*)	11[a]	22[b]	24[c]	Diploid[d]; 45S (2) and 45S+5S (2) adjacent rDNA signals[e]	Waminal and Kim (2015)[a,b,d,e]
	Z. marlothii Cogniaux, 1962		24[a]		Diploid[b]; 45S (2) and 45S+5S (2) adjacent rDNA signals[c]	Li et al. (2016)[a,b,c]
	Z. maysorensis Wight et Arnott, 1834		48[a]	24[b]	45S (2) and 5S (2) signals[c]	Beevy and Kuriachan (1996)[a,b], Xie et al. (2019a)[a,c]
	Z. mucronata Blume, 1856 (syn. *Melothria mucronata*)		22[a]	12[b]	-	Darlington et al. (1956)[a], CCDB[b]
	Z. scabra Sonder, 1862 (syn. *Melothria punctata*)		24[a], 48[b]		-	CCDB[a], Kumar and Subramaniam (1987)[b]
	Z. thwaitesii Schweinfurth, 1868		44[a]		-	CCDB[a]

genera included other than *Cucumis*; x: base number; 2n: zygotic number; n: gametic number; NOR: nucleolar organizing region; B: B chromosome; II: bivalents; IPCN: Index to Plant Chromosome Number Reports; CCDB: Chromosome Counts Database; superscripts correspond to references.

Table 7. Cytogenetic features of *Cucumis* (Benincaseae)#.

Species with subspecies/ varieties	Chromosome no.			Ploidy, Genome size, Chromosome features	References
	x	2n	n		
C. aculeatus Cogniaux, 1895		48[a]		Allotetraploid[b]; 24II[c]	IPCN[a-c], CCDB[a-c]
C. africanus Linnaeus, 1782	12[a]	24[b], 48[c]	12[d]	Diploid[e]; 2C (Feulgen microdensitometry): 1.782pg[f]; 4 satellited chromosomes[g]; 45S (4[b]/6[i]) rDNA signals, 2 co-localized 45S+5S signals[j]	IPCN[b,c], Yadava et al. (1984)[b,d], Ramachandran and Narayan (1985)[b,e,f], Yagi et al. (2015)[a,b,g,i,j], Zhang et al. (2016)[b,e,h,j]
C. angolensis Cogniaux, 1881.		24[a]			IPCN[a]
C. anguria Linnaeus, 1753		24[a]		Diploid[b]; majorly submetacentric and few nearly metacentric chromosomes[c]; 1 pair satellited[d]; 45S (2) and co-localized 45S+5S (2) rDNA signals[e], ScgCP enables chromosome identification[f]; GISH reveals cross species relationships[g]	Singh and Roy (1974)[a-d], Zhang et al. (2015)[a,g], (2016)[b,e], Li et al. (2018)[a,f]
C. anguria var. *anguria*	12[a]	24[b]	12[c]	Diploid[d]; 4 satellited chromosomes[e]; 45S (2) and co-localized 45S+5S (2) rDNA signals[f]	Yadava et al. (1984)[b,c], Yagi et al. (2015)[a,d,e,f]
C. anguria var. *longipes*		24[a]	12[b]	Diploid[c]; 2C (Feulgen microdensitometry): 1.587pg[d]	Yadava et al. (1984)[a,b], Ramachandran and Narayan (1985)[a,c,d]
C. anguria var. *longaculeatus*	12[a]	12.5		Diploid[c]; 4 satellited chromosomes[d]; 45S (2) and co-localized 45S+5S (2) rDNA signals[e]	Yagi et al. (2015)[a-e]
C. asper Cogniaux, 1901		24[a]		Diploid[b]; 45S (4) and 5S (2) signals detected[c]	IPCN[a], Zhang et al. (2016)[a,b,c]
C. callosus Rottler, 1803		14[a], 24[b]	12[c]	Diploid[d]; 2C (Feulgen microdensitometry):1.590pg[e]; 11m+1sm (haploid)[f]	Ramachandran and Narayan (1985)[a,d,e], Rajkumari et al. (2013)[b,c], (2015)[b,c,f]
*C. cinereus*Cogniaux, 1901 (syn. *Cucumella cinerea*)				2C (Feulgen microdensitometry): 0.5pg[a]	Bennet et al. (1982)[a]
C. diniae Raamsdonk et Visser, 1992		48[a]		-	IPCN[a]
C. dinteri Cogniaux, 1901		24[a]		Diploid[b]; 2C (Feulgen microdensitometry): 2.167pg[c]	IPCN[a], Ramachandran and Narayan (1985)[a-c]
C. dipsaceus Spach, 1838		24[a]	12[b]	Diploid[c]; 2C (Feulgen microdensitometry): 2.448pg[d]; 2m+8sm+2st (haploid)[e]; 45S (2) and co-localized 45S+5S (2) rDNA signals[f]	Yadava et al. (1984)[a,b], Ramachandran and Narayan (1985)[a,c,d], Rajkumari et al. (2015)[a,c,e], Zhang et al. (2016)[a,e,f]
*C. ficifolius*Richard, 1847		24[a], 48[b]	12[c]	Diploid[d]; 2C (Feulgen microdensitometry):1.373pg[e]; 45S (2) and co-localized 45S+5S (2) rDNA signals[f]	Yadava et al. (1984)[a,c], Ramachandran and Narayan (1985)[a,d,e], Zhang et al. (2016)[b,f]
C. figarei Naudin, 1859		48[a], 72[b]		Autoallopolyploid[c]; 2C (Feulgen microdensitometry): 3.886pg[e]; 36II[f]	IPCN[a-f], Ramachandran and Narayan (1985)[a,c,e]
C. heptadactylis Naudin, 1859		48[a]	23[b], 24[c], 52[d]	Autotetraploid[e]; 2C (Feulgen microdensitometry): 2.225pg[f]; 8 satellited chromosomes[g]; 45S (8) rDNA signals[h] of which 4 co-localized to 5S signals[i] or separate 5S (4) rDNA signals[j]; 10IV+4II[k]; irregular meiosis[l]	IPCN[a,e,k], Yadava et al. (1984)[a,b,d,e,l], Ramachandran and Narayan (1985)[a,e,f], Yagi et al. (2015)[a,e,g,h,i,j], Zhang et al. (2016)[a,e,h,j]
C. hookeri Naudin, 1870		24[a]	12[b]	Diploid[c]	Yadava et al. (1984)[a,b,c]
C. humifructus Stent, 1927		24[a]		Diploid[b]; 2C (Feulgen microdensitometry): 2.455 pg[c]	Ramachandran and Narayan (1985)[a,b,c]
C. hystrix Chakravarty, 1952	12[a]	24[b]		Diploid[c]; 2m+10sm (haploid)[d]; 45S (4) and co-localized 45S+5S (2) rDNA signals[e]; FISH with bulked oligo probe from cucumber chromosome C7[f], GISH reveals cross species relationships[g]	Rajkumari et al. (2015)[b,c,d], Han et al. (2015)[f], Zhang et al. (2015)[b,g], (2016)[a,b,c,e]
C. indicus Ghebretinsae et Thulin, 2007		20[a]		Diploid[b]; 4m+ 6sm (haploid)[c]	Rajkumari et al. (2015)[a-c]

Species with subspecies/ varieties	Chromosome no.			Ploidy, Genome size, Chromosome features	References
	x	2n	n		
C. javanicus Miquel, 1856 (syn. *Melothria assamica*)	12[a]	24[b], 48[c]		-	Kumar and Subramaniam (1987)[a], CCDB[b,c]
C. leiospermus Wight et Arnott, 1834 (syn. *Melothria leiosperma*)		24[a]		-	CCDB[a]
C. leptodermis Schweickerdt, 1933		24[a]	12[b]	-	Yadava et al. (1984)[a,b]
C. longipes Hooker, 1871		24[a]		-	IPCN[a]
C. meeusei Jeffrey, 1965		48[b]	22[c], 24[d]	Tetraploid[c]; 2C- 3.203pg (Feulgen microdensitometry)[f]; 45S (6) and co-localized 45S+5S (2) rDNA signals[g]	Yadava et al. (1984)[b,c,d], Ramachandran and Narayan (1985)[b,e,f], Zhang et al. (2016)[b,e,g]
C. melo Linnaeus, 1753	12[a]	20[b], 22[c], 24[d]	12[e]	Diploid[f]; 2C (Feulgen photometry): 0.94-1.04pg[g], 1.90pg[h]; 2C (Flow cytometry): 1.05pg[i]; 14m+10st (2SAT)[j]; 7m+5sm (haploid)[k]; 4 satellites[l] or 2 satellites[m]; CSR1.0-2.1µm[n]; CMA bands detected[e]; 45S (2) and co-localized 45S+5S (2) rDNA signals[g]; centromeric, telomeric, nulceolar and SSR probe hybridization reveals chromosomal relation[q]; ScgCP applied for comparative chromosome rearrangement studywith *C. sativus*[r]; FISH with bulked oligo probe from cucumber chromosome C7[s]; novel centromeric satellite DNA hybridized on chromosomes[t]; GISH reveals cross species relationships[u]; infraspecific positional differences in 45S (terminal and interstitial) -5S (terminal, subterminal and interstitial) rDNA signals[v]	CCDB[b,c], Plant DNA C-Values Database[h], Kumar and Subramaniam (1987)[a], Arumuganathan and Earle (1991)[g], Marie and Brown (1993)[i], Zhang (2005)[d,f,j,u], (2015)[d,r], Song and Kim (2008)[d,f,m], Han et al. (2009)[d,e,f,q], (2015)[s], Liu et al. (2010)[d,f,q], Hoshi et al. (2013)[d,f,l,n,o,p], Lou et al. (2014)[r], Rajkumari et al. (2013)[d,e], (2015)[d,f,k], Setiawan et al. (2018)[d,v], (2020)[d,t]
C. melo subsp. *melo*	12[a]	24[b]		Diploid; 45S (4) and 5S (2) rDNA signals[c]	Zhang et al. (2016)[a,c]
C. melo subsp. *agrestis* Naudin, 1859	12[a]	24[b]		Diploid; 45S (4) and 5S (2) rDNA signals[c]	Zhang et al. (2016)[a,c]
C. melo var. *agrestis*	12[a]	24[b]	12[c]	Diploid[d]; 2C (Feulgen microdensitometry): 2.483pg[e]; 10m+2sm (haploid)[f]; 1 pair satellited[g]	Singh and Roy (1974)[b,d,g], Yadava et al. (1984)[a-d], Ramachandran and Narayan (1985)[b,d,e], Beevy and Kuriachan (1996)[b,c], Rajkumari et al. (2015)[b,d,f]
C. melo var. *conomon* Thunberg, 1780		24[a]		Diploid[b]; 7m+3sm+2st (haploid)[c]	Zhang et al. (2005)[a], Rajkumari et al. (2015)[a,b,c]
C. melo var. *flexuosus* Linnaeus, 1763		24[a]		-	IPCN[a]
C. melo var. *inodorus*Jacquin, 1832		24[a]		Diploid[b]; 2C (flow cytometry): 0.64pg[c]	Karimzadeh et al. (2010)[a-c]
C. melo var. *melo*		24[a]	12[b]	Diploid[c]; 4m+8sm (haploid)[d]	Beevy and Kuriachan (1996)[a,b], Rajkumari et al. (2015)[b,c,d]
C. melo var. *momordica*Roxburgh, 1832		24[a]	12[b]	Diploid[c]; 2C (Feulgen microdensitometry): 2.291pg[d]; 6m+5sm+1st (haploid)[e]	Yadava et al. (1984)[a,b], Ramachandran and Narayan (1985)[a,c,d], Rajkumari et al. (2015)[a,c,e]
C. melo var. *muskmelon*		24[a]	12[b]		Yadava et al. (1984)[a,b]
C. melo var. *utilissimus* Roxburgh, 1832		24[a]	12[b]	Diploid[c]; 2C (Feulgen densitometry): 2.358 pg[d]	Yadava et al. (1984)[a,b]; Ramachandran and Narayan (1985)[a,c,d]
C. membranifolius Hooker, 1871		48[a]	24[b]	-	Yadava et al. (1984)[a,b]

Species with subspecies/ varieties	Chromosome no.			Ploidy, Genome size, Chromosome features	References
	x	2n	n		
C. metulifer Naudin, 1859 (syn. *C. metuliferus*)		24[a]	12[b]	Diploid[c]; 2C (Feulgen microdensitometry): 2.391pg[d]; metacentric, submetacentric, subtelocentric chromosomes[e]; CSR: 0.9–2.0 µmf; 4 satellites[g]; nucleolar and centromeric CMA-DAPI bands[h]; 45S (2) and co-localized 45S+5S (2) rDNA signals[i], satellite sequences[j] and telomeric DNA[k] hybridized on chromosomes; ScgCP applied for comparative chromosome rearrangement studywith *C. sativus*[l]; GISH reveals cross species relationships[m]	Yadava et al. (1984)[a,b,c], Ramachandran and Narayan (1985)[a,c,g], Ramachandran and Narayan (1990)[a,c,i], Hoshi et al. (2013)[a,c,e,f,g,h], Lou et al. (2014)[l], Yagi et al. (2014)[a,e,g,h,i,j,k], Li et al. (2016)[a,c,i], Zhang et al. (2015)[a,m], (2016)[a,c,i]
C. myriocarpus		24[a]	12[b]	Diploid[c]; 45S (2[d]/4[e]) and co-localized 45S+5S (2)[f] rDNA signals[d]	CCDB[a]; Zhang et al. (2016)[a,b,c,d,f], Yagi et al. (2015)[a-f]
C. myriocarpus subsp. *leptodermis* Schweickerdt, 1933	12[a]	24[b]		Diploid[c]; 4 satellited chromosomes[d]; 45S (3[e], 2[f]) and co-localized 45S+5S (2[g]) rDNA signals	Yagi et al. (2015)[a-g]
C. myriocarpus var. *myriocarpus*	12[a]	48[b]		Tetraploid[c]; 8 satellited chromosomes[d]; 45S (4) and co-localized 45S+5S (4) rDNA signals[e]	Yagi et al. (2015)[a-e]
C. prophetarum Linnaeus, 1755		24[a]	12[b]	Diploid[c], 2C (Feulgen Microdensitometry): 1.656 pg[d] 5m+7sm (haploid)[e]	Ramachandran and Narayan (1985)[a,c,d], Rajkumari et al. (2013)[a,b], (2015)[a,c,e]
C. prophetarum subsp. *zeyheri* Sonder, 1862		48[a]		-	IPCN[a]
C. pubescens Willdenow, 1805		24[a]	12[b]	-	IPCN[a]; Beevy and Kuriachan (1996)[b]
C. pustulatus Hooker, 1871		48[a], 72[b]	24[c]	Hexaploid[d], 45S (8) and co-localized 45S+5S (2) rDNA signals[e]; FISH with bulked oligo probe from cucumber chromosome C7[f]	Yadava et al. (1984)[a,c], Han et al. (2015)[f], Zhang et al. (2016)[b,d,e]
C. ritchiei Clarke, 1879		24[a]		Diploid[b], 8m+4sm (haploid)[c]	Rajkumari et al. (2015)[a,b,c]
C. sagittatus Peyritsch, 1860		24[a]	12[b]	Diploid[c], 2C (Feulgen microdensitometry):1.571pg[d]	Yadava et al. (1984)[a,b], Ramachandran and Narayan (1985)[a,c,d]
C. sativus Linnaeus, 1753	7[a]	14[b]	7[c]	Diploid[d], 2C (flow cytometry): 1.03pg[e] /1.77pg[f]; 12 metacentric and 2 sub-metacentric chromosomes[g]; CSR: 0.83-1.01µm[h], chromosomal C-bands[i]; centromeric 45S (10) and distal 5S (2) rDNA signals[j]; FISH with centromeric and telomeric[k] and SSR probe reveals chromosome evolution[l]; high resolution molecular cytogenetic map[m]; ScgCP applied for cross species chromosome rearrangement study[n]; FISH with bulked oligo probe from cucumber chromosome C7 in comparison with 5 *Cucumis* species[o]; GISH reveals cross species relationships[p]	Kumar and Subramaniam (1987)[a,b], Marie and Brown (1993)[f], Beevy and Kuriachan (1996)[b,c], Hoshi et al.(2008)[b,d,i], Barow and Meister (2003)[b,d,e], Han et al (2011)[b,d,k,l,m], Liu et al. (2010)[b,l], Waminal and Kim (2012)[b,d,g,h,j], Rajkumari et al. (2013)[b,c,f], Sun et al. (2013)[b,m], Lou et al. (2014)[b,n], Han et al. (2015)[o], Zhang et al. (2015)[b,p], Li et al. (2016)[b,d,j]
C. sativus var. Hokutosei	7[a]	14[b]		Diploid[c], 12 metacentric, 2 sub-metacentric chromosomes[d]; centromeric and telomeric signals[e]	Zhang et al. (2012)[a-e]
C. sativus var. *hardwickii* Royle, 1835	7[a]	14[b]		Diploid[c]; 2C (Feulgen Microdensitometry): 1.798pg[d]; 6m+1sm (haploid)[e]; centromeric 45S (6) and intercalary 5S (2) rDNA signals[f], centromeric, telomeric and SSR probe hybridization[g,h]; molecular cytogenetic map[i]	Ramachandran and Narayan (1985)[b,d], Zhao et al. (2011)[b,c,f,g], Yang et al. (2012)[b,h], Rajkumari et al. (2015)[b,c,e], Zhang et al. (2016)[a,b,c,f]
C. sativus var. Long green	7[a]	14[b]		Diploid[c], 12 metacentric, 2 sub-metacentric chromosomes[d]; centromeric and telomeric sequence signals[e]	Zhang et al. (2012)[a-e]
C. sativus var. *sativus* (CSS)	7[a]	14[b]		Diploid[c], centromeric 45S (10) and intercalary 5S (2) rDNA signals[d]; centromeric and distal repetitive sequence probes[e]; molecular cytogenetic map[f]	Zhao et al. (2011)[b-e], Yang et al. (2012)[b,f], Zhang et al. (2016)[a-d]

Species with subspecies/ varieties	Chromosome no.			Ploidy, Genome size, Chromosome features	References
	x	2n	n		
C. sativus cv. Winter Long		14[a]	7[b]	Diploid[e], C- banding[d], DAPI banding[e], 45S (6) and 5 S (2) rDNA signals[f], repetitive sequence based molecular karyotype in somatic and pachytene chromosomes[g]	Koo et al. (2002)[a-f], (2005)[a,b,g]
C. sativus var. *xishuangbannesis* Qi et Yuan Zhenzhen, 1983	7[a]	14[b]		Diploid[c], centromeric 45S (10) and intercalary 5S (2) rDNA signals[d]; centromeric and telomeric signals[e]	Zhao et al. (2011)[b,c,e], Zhang et al. (2016)[a-d]
C. setosus Cogniaux, 1881		24[a]	12[b]	Diploid[c]; 4m+5sm+3st (haploid)[d]	Rajkumari et al. (2013)[a-c], (2015)[a,c,d]
C. silentvalleyii Manilal et Sabu et Mathew, 1985		24[a]	12[b]	-	Rajkumari et al. (2013)[a,b]
C. trigonus Roxb.		24[a]	12[b]	-	Rajkumari et al. (2013)[a,b]
C. zambianus Widrl., J.H.Kirkbr., Ghebret. and K.R.Reitsma	12[a]	24[b]		Diploid[c]; 45S (2) and co-localized 45S+5S (2) signals[d]	Zhang et al. (2016)[a-d]
C. zeyheri Sond.		24[a], 48[b]		Diploid[c], Allotetraploid[d]; 2C (Feulgen densitometry): 1.682[c]/2.846 pg[f]; 4 satellites[g]; 45S (2) and co-localized 45S+5S (2) rDNA signals[h]; FISH with bulked oligo probe from cucumber schromosome C7[i]; 24II[j] , 12II[k], 11II+2I[l]	IPCN[a,b,d,j,k,l], Ramachandran and Narayan (1985)[a-f], Han et al. (2015)[i], Yagi et al. (2015)[a,c,g,h]
Cucumella cinerea (Cogn.) C.Jeffrey				2C (Feulgen Microdensitometry): 0.50pg[a]	Bennet et al. (1982)[a]
Mukia maderaspatana (L.) M.Roem. (syn. *Cucumis maderaspatanas* and *Melothria maderaspatana*)	12[a]	24[b]	11[c], 12[d]	-	CCDB[b,c], Rajkumari et al. (2015)[b,d]
Oreosyce africana Hook.f. (syn. *Cucumis subsericeus*)	12[a]	48[b]		Tetraploid[c]; co-localized 45S and 5S rDNA signals (2)[d]; FISH with bulked oligo probe from cucumber chromosome C7[e]	Han et al. (2015)[e], Zhang et al. (2016)[a-d]

#x: base number; 2n: zygotic number; n: gametic number; NOR: nucleolar organizing region; SAT: satellite chromosome; ScgCP: Single-copy gene-based chromosome painting (Lou et al. 2014); I: univalent, II: bivalent, IV: tetravalent; CCDB: Chromosome Counts Database; superscripts correspond to reference.

Table 8. Cytogenetic information in Cucurbiteae #.

Genera studied	Species studied	Chromosome no.			Ploidy, Genome size, Chromosome features	References
		x	2n	n		
Cayaponia Silva Manso, 1836	*C. laciniosa* Linnaeus, 1753		24[a]		-	Kumar and Subramaniam (1987)[a]
Cucurbita		10[a], 12[b]			-	Darlington and Janaki Ammal (1945)[a,b]
	C. andreana Naudin, 1896		40[a]			CCDB[a]
	C. argyrosperma Huber, 1867 (syn. *C. mixta* Pangalo, 1930)		40[a]		2C (flow cytometry): 0.748 pg[b]	Sisko et al. (2003)[a,b]
	C. cylindrata Bailey, 1943		40[a]	20[b]	-	CCDB[a,b]
	C. digitata Gray, 1853	10[a], 12[b]	40[c]	20[d]	-	Darlington and Janaki Ammal (1945)[a,b], CCDB[c,d]
	C. ecuadorensis Cutler et Whitaker, 1969				2C: 0.72pg[a]	Plant DNA C Value database[a]
	C. ficifolia Bouché, 1837 (syn. *C. melanosperma* Gasparrini, 1847)		40[a]		2C (flow cytometry): 0.933pg[b]	Plant DNA C- Values Database[a,b]
	C. foetidissima Kunth, 1817	10[a], 12[b]	40[c], 42[d]		2C (flow cytometry): 0.686pg[e]	Darlington and Janaki Ammal (1945)[a,b], Plant DNA C- Values Database[c,e], CCDB[c,d]
	C. indica (unresolved)		40[a]		-	IPCN[a]
	C. lundelliana Bailey, 1943			20[a]	2C (flow cytometry): 0.72pg[b]	CCDB[a], Plant DNA C Value database[b]
	C. maxima Duchesne, 1786	20[a]	24[b], 40[c], 44[d], 48[e]	20[f]		Kumar and Subramaniam (1987)[a,c,d,e], Beevy and Kuriachan (1996)[f], CCDB[c,f]
	C. moschata Duchesne, 1786	10[a], 12[b]	24[c], 40[d], 44[e],48[f]		Diploid[g]; 2C (Feulgen microdensitometry): 0.90pg[h]; 2C (flow cytometry): 0.708[i]/ 0.97[i]pg; 36 metacentric and 4 sub-metacentric chromosomes[k]; CSR: 1.05-1.78μm[l], 45S (10) and 5S (4) rDNA signals[m]	CCDB[f], Plant DNA C-Values Database[h,i], Kumar and Subramaniam (1987)[a-f], Barrow and Meister (2003)[j], Xu et al. (2007)[d,m], Waminal et al. (2011)[g,d,k,l,m]
	C. okeechobeensis ssp. *martinezii* Bailey, 1943		40[a]		2C (flow cytometry): 0.74pg[b]	Plant DNA C- Values Database[a,b]
	C. palmata Watson, 1876	10[a], 12[b]	40[c], 42[d]	20[e]	-	Kumar and Subramaniam (1987)[a,b], CCDB[c,d,e]
	C. pedatifolia Bailey, 1943		40[a]		-	CCDB[a]
	C. pepo Linnaeus, 1753	10[a], 12[b]	22[c], 24[d], 28[e], 40[f], 42[g], 44[h], 46[i], 80[j]	20[k]	2C (flow cytometry): 0.74pg[l]; 0.864[m]; 1.109 pg-1.064 pg[n]; 1.18pg[o]; 45S (10) and 5S (4) rDNA signals[p]	Kumar and Subramaniam (1987)[a-j], CCDB[f,k], Marie and Brown (1993)[l], Barow and Meister (2003)[o], Rayburn (2008)[n], Plant DNA C- Values Database[m], Xie et al. (2019b)[f, p]
Sicana Naudin, 1862	*S. odorifera* Vellozo, 1831		40[a]	20[b]	-	IPCN[a,b]

x: base number; 2n: zygotic number; n: gametic number; CCDB: Chromosome Counts Database; superscripts correspond to references.

terstitial telomeric repeats which were suggested to be the result of chromosome fusion from ancestral genome. The co-localization of 45S and 5S rDNA loci in *A. tenerum* and *Thladiantha dubia* Bunge, 1833, have been thought to imply regional synteny and shared ancestral traits (Xie et al. 2019b). In the tribe Cucurbiteae, detailed karyotype analysis is known only in *Cucurbita moschata* Duchesne, 1786 and *C. pepo* Linnaeus, 1753, showing conserved 45S and 5S rDNA signals (non-co-localized) in independent analyses (Table 8).

Karyotypes and chromosome sizes are reported in ten species of Momordiceae (Table 3). Interspecific differences have been observed and found to correlate with phylogenetic

relationship within *Momordica* (Ghosh et al. 2021). Infraspecific delimitation of Indian *M. charantia* varieties was based on fluorochrome banding pattern and genome size divergence (Table 3), corresponding to infraspecific distinction reported in the Japanese bitter gourd cultivars (Kido et al. 2016). FISH in three *Momordica* species revealed 45S and 5S rDNA sites to be localised on different chromosomes (Table 3). In context of the genome sequence of bitter gourds (Matsumura et al. 2020), further scopes for cytogenetic and genomic investigation remain open.

Karyotype and chromosome size is reported in eight 8 species of Sicyoeae (Table 5). Fluorochrome banding pattern has facilitated comparative analysis in *Luffa* species occurring in India (Tables 1, 5) (Bhowmick and Jha 2015a, 2021). The cultivated ridged gourd (*L. acutangula* Linnaeus, 1753) showed three CMA^+ satellite bearing pairs (Fig. 1A–C, J) as in the wild *L. echinata* Roxburgh, 1814 (Fig. 1G–I, L), while the sponge gourd (*L. aegyptiaca* Miller, 1768 has two satellited pairs (Fig. 1D–F, K). *Luffa acutangula* and *L. echinata* also showed up distal DAPI bands (Fig. 1J, L), absent in *L. aegyptiaca* (Fig. 1K). *Trichosanthes* species (2n = 22) have inter-specific differences (Fig. 2) as well as infraspecific distinction (*T. cucumerina* Linnaeus, 1753) in fluorochrome banding pattern (Tables 1, 5, Fig. 2A–H). The male and female plants of *T. dioica* show similar chromosome number, morphology and genome size but show differences in fluorochrome banding pattern (Fig. 2I–P, Table 5). The 11^{th}, 12^{th} and 13^{th} pairs (CMA^+) are marker chromosomes in *Luffa* (Fig. 1, Table 1) while the 10^{th} and 11^{th} pairs are conserved CMA^+ satellited pairs in *Trichosanthes* (Fig. 2, Table 1). Eight species of Sicyoeae have been subjected to FISH (Table 5). The polyploid and diploid species have differences in the number of rDNA loci, showing separate localization of the 45S and 5S rDNA signals except *Sicyos angulatus* Linnaeus, 1753 and *Trichosanthes kirilowii* Maximowicz, 1859 (Table 5).

Benincaseae generally reveal two distal 45S rDNA loci of which at least one locus is either adjacent to 5S rDNA locus (Table 6) or co-localized in the same chromosome as in most of the *Cucumis* species (Table 7). Exceptionally, a wild species of *Benincasa* (*B. fistulosa* Stocks, 1851) has non-adjacent 45S and 5S signals (Li et al. 2016). GC rich satellites were observed in the 12^{th} pair of chromosomes showing CMA^+ bands in cultivated Indian ashgourd (*B. hispida*) (Fig. 3 A–C, J, Tables 1, 6). *Lagenaria siceraria* Molina, 1782 and *Cucumis melo* Linnaeus, 1753 are the other two genera having similarity in rDNA hybridization profile, agreeing with phylogenetic affinity (Li et al. 2016).

Citrullus colocynthis Linnaeus, 1753 and *C. lanatus* Thunberg, 1794 may share a common ancestor both having two 45S rDNA loci and one 5S locus. Loss of one 45S rDNA locus has given way to *C. rehmii* De Winter, 1990 while gain of one 5S rDNA locus has been proposed to lead to *C. ecirrhosus* Cogniaux, 1888 and *C. lanatus* var. *citroides* Bailey, 1930 (presently *C. amarus* Schrader, 1836) (Reddy et al. 2013; Li et al. 2016). GISH using *C. lanatus* var. *citroides* genome has revealed divergence from *C. lanatus* var. *lanatus* (Reddy et al. 2013).

The genus *Cucumis* is the largest in Benincaseae with 65 species of which 39 have been studied (Table 7). Among the *Cucumis* species with x = 12, co-localization rDNA loci (45S and 5S rDNA) have been documented in 14 species, including *C. melo* (Table 7). However, the number of 45S sites is generally four, which may be six or eight in some

cases (Table 7). rDNA hybridization data strongly corroborated with the 'fusion' theory for derivation of x = 7 (*C. sativus*) from x = 12 (*C. melo*) (Waminal and Kim 2012) which is substantiated by genomic studies (Li et al. 2011). There are ten pericentromeric/ centromeric 45S and two distal 5S rDNA sites in *C. sativus* while six 45S rDNA sites were reported in *C. sativus* var. *hardwickii* Royle, 1835 (Koo et al. 2005; Zhang et al. 2012). Comparative chromosome painting (Lou et al. 2014) and GISH (Zhang et al. 2015) proved high colinearity between cucumber and melon. Based on chloroplast and nuclear DNA (ITS) phylogeny, *C. melo* (melon) has been found to be sister to a clade comprising *C. sativus* and related genera (*Dicaelospermum* Clarke, 1879 and *Mukia* Arnott, 1840) (Renner et al. 2007). rDNA site co-localization was found to coincide with geographical origin of 12 *Cucumis* species (Zhang et al. 2016). The chromosomal affinity between *C. metuliferus* Schrader, 1838, *C. anguira* Linnaeus, 1753, *C. zeyheri* Sonder, 1862, *C. myriocarpus* Naudin, 1859 and polyploid *C. heptadactylis* Naudin, 1859 (dioecious) (Yagi et al. 2015) can be substantiated by their phylogenetic proximity based on chloroplast and nuclear DNA (ITS) sequences (Renner et al. 2007). rDNA distribution of *C. metuliferus* was also the reason to consider proximity with *Citrullus naudinianus* Sonder, 1862, (previously *Acanthosicyos naudinianus* Sonder, 1862) (Reddy et al. 2013). Infraspecific differences were documented in *Cucumis melo* on the basis of 45S- 5S rDNA signals (linked or separated) which also possessed unique centromeric satellites (Setiawan et al. 2018, 2020). Moreover, chromosome painting method elucidated chromosomal rearrangement in some *Cucumis* species (Lou et al. 2014; Li et al. 2018).

The dramatic evolution of Y chromosome was validated in karyotypes (Fig. 3 D–I, K–L) of *Coccinia grandis* (Table 6). The 45S rDNA sites enabled confirmation of NORs in the 8th and 12th pair containing distal GC rich CMA^{+} signals in *C. grandis* (Fig. 3 D–I, K–L, Tables 1, 6). 45S and 5S rDNA hybridization pattern was similar in three other *Coccini*a species and *Diplocyclos palmatus* Linnaeus, 1753 (Table 6). The three closely related dioecious species of *Coccinia* accumulated Y chromosome repeats and displayed sex chromosome turnover (Sousa et al. 2017). Strong centromeric CMA bands (Fig. 3 D–I, K–L, Table 1) were observed in *C. grandis* except Y chromosome (Fig. 3 I, L), presenting a possibility that *CgCent* (CL1) is a feature of centromeres of dioecious *Coccinia* species (Sousa et al. 2017). In addition, non-nucleolar CMA^{+} heterochromatin might be associated with sexual differentiation of autosomes in dioecious *C. grandis* (Fig. 3) which is also a marker in *Trichosanthes dioica* (Fig. 2, Table 1), opening good scope for further study.

Distinct 45S rDNA sites are higher in number than 5S rDNA sites in Cucurbitaceae (Fig. 4) (Waminal and Kim 2012). The distal 45S rDNA loci are conserved genomic landmarks (Fig. 4) while 5S rDNA loci are relatively diverse (Fig. 4). Based on the literature reports, some NORs (Type I) included chromosomes showing non-colocalized 45S and 5S rDNA sites in seven species of Benincaseae, one species each from Cucurbiteae and Momordiceae and two species of Sicyoeae. The rearrangement of 45S rDNA site in *Cucumis sativus*, probes for chromosome number reduction which may be a consequence of diploidization. The second type (Type II) shows colocalised 45S and 5S rDNA loci, either adjacent or distant, but always on the same chromosome and found in one species each of Benincaseae, Sicyoeae and Actinostemmateae. The third type (Type III) was characterized by chromosomes with non-colocalized and

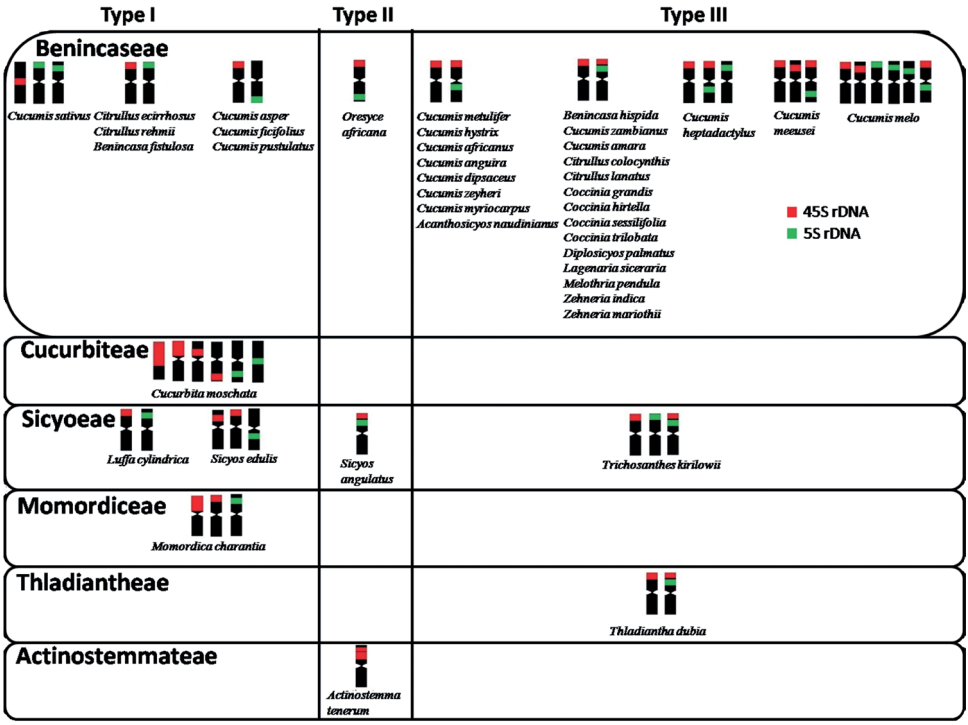


Figure 4. Types of chromosomes bearing the NORs as per available reports of rDNA hybridization in Cucurbitaceae. Type I: Chromosomes with only non-colocalised 45S and 5S rDNA sites, Type II: Chromosomes with colocalised 45S and 5S rDNA sites, Type III: Chromosomes with both non- colocalised and colocalised 45S and 5S rDNA sites. See text for explanation.

colocalised 45S and 5S rDNA loci, as in 14 species of Benincaseae and one species each of Sicyoeae and Thladiantheae. The rDNA sites of majority of *Cucumis* species were of non-adjacent type. Hence, type III NORs in majority of Benincaseae genera advocates conservation of the marker chromosomes having distal NOR (45S rDNA). *Gynostemma pentaphyllum* and some polyploid *Cucumis* reveal rDNA loci reduction after polyploidization (Zhang et al. 2016; Pellerin et al. 2018).

Correlation between parameters

Chromsome numbers in Cucurbitaceae range from x = 5 to x = 16. The most prevalent number x = 12 (Fig. 5) is considered ancestral (Xie et al. 2019b), followed by x = 11, 13, 14 and 10 (Fig. 5). The present regression analyses for 41 taxa (including 16 Indian taxa) (Table 9) revealed significant linear correlation between 2n and HCL, between ploidy and genome size and between ploidy and HCL (Fig. 6). Therefore, an increase in ploidy/ 2n number is linked with increase in HCL. There was no significant correlation between 2C genome size and chromosome numbers. Cytogenetic parameters may not reflect residual evidence of CCT in Cucurbitraceae at present, as reasoned by Alix et al. (2017).

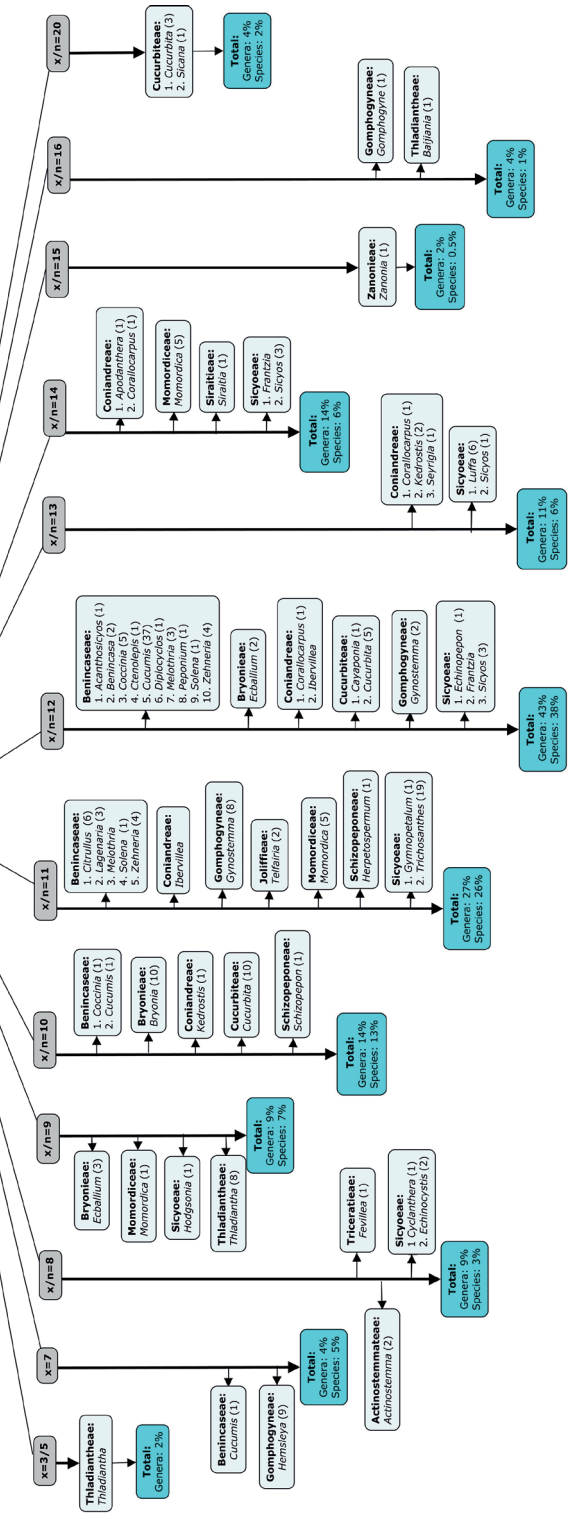


Figure 5. The types of different base numbers (x, based on published reports) or possible base numbers (x/n, based on reported haploid counts) in Cucurbitaceae. The numbers in brackets beside names of genera signify the number of species whose chromosome counts are reported. The % of genera and species with a particular chromosome number, is indicated at the end arrow (out of a total of 44 genera and 188 species with chromosome counts).

Table 9. Data on fundamental cytogenetic parameters utilized for statistical analysis.

Species	2n Chromosome no.	Ploidy	2C genome size (pg)	MCL (µm)	HCL (µm)	References
Gynostemma pentaphyllum	66	6	3.62			Zhang et al. (2013), Pellerin et al. (2018)
Zanonia indica	30	2		1.47	22.12	Lekhak et al. (2018)
Momordica balsamina	22	2		1.30	14.3#	Bharathi et al. (2011)
Momordica charantia var. *charantia*	22	2	0.72	1.97	21.77	Ghosh et al. (2018)
Momordica charantia var. *muricata*	22	2	1.16	2.19	24.19	Ghosh et al. (2018)
Momordica cochinchinensis	28	2	2.64	2.27	31.86	Ghosh et al. (2021)
Momordica cymbalaria	18	2	3.74	3.75	33.79	Ghosh et al. (2021)
Momordica dioica	56	4	3.36	2.75	77.1	Ghosh et al. (2021)
Momordica sahyadrica	28	2		1.34	18.76	Bharathi et al. (2011)
Momordica subangulata	56	4	3.06	2.15	60.3	Ghosh et al. (2021)
Luffa acutangula	26	2		2.20	28.63	this study
Luffa cylindrica	26	2	1.56	2.98	38.77	Bhowmick and Jha (2015a), this study
Luffa echinata	26	2		3.17	41.26	this study
Trichosanthes cucumerina	22	2		3.47	37.855	Bhowmick and Jha (2019), this study
Trichosanthes cucumerina subsp. *cucumerina* Anguina	22	2		3.43	37.74	Bhowmick and Jha (2019), this study
Trichosanthes dioica Male	22	2	2.27	3.71	40.82	Bhowmick and Jha (2015a), this study
Trichosanthes dioica Female	22	2	2.32	3.71	40.82	Bhowmick and Jha (2015a), this study
Benincasa hispida	24	2	1.97	3.17	38.08	Bhowmick and Jha (2015a), this study
Citrullus lanatus	22	2		1.33#	14.67	Waminal et al. (2011)
Coccinia grandis male	24	2	0.92	1.80	20.32	Bhowmick et al. (2012, 2016), this study
Coccinia grandis female	24	2	0.73	1.86	19.85	Bhowmick et al. (2012, 2016), this study
Coccinia hirtella	24	2	0.988			Sousa et al. (2017)
Coccinia sessilifolia Male	24	2	0.984			Sousa et al. (2017)
Coccinia sessilifolia Female	24	2	0.998			Sousa et al. (2017)
Coccinia trilobata	20	2	1.263			Sousa et al. (2017)
Lagenaria siceraria	22	2	0.734	1.79	20.06	Achigan-Dako et al. (2008)
Cucumis africanus	24	2		2.08	25.045	Yagi et al. (2015)
Cucumis anguria var. *anguria*	24	2		2.13	25.6	Yagi et al. (2015)
Cucumis anguria var. *longaculeatus*	24	2		2.10	25.195	Yagi et al. (2015)
Cucumis heptadactylus	48	4		2.09	50.225	Yagi et al. (2015)
Cucumis melo	24	2	1.05	1.50	17.8#	Marie and Brown (1993), Hoshi et al. (2013)
Cucumis melo var. *inodorus*	24	2	0.64			Karimzadeh et al. (2010)
Cucumis myriocarpus var. *leptodermis*	24	2		1.93	23.19	Yagi et al. (2015)
Cucumis myriocarpus var. *myriocarpus*	48	4		2.25	53.985	Yagi et al. (2015)
Cucumis zeyheri	24	2		2.30	27.56	Yagi et al. (2015)
Cucumis sativus	14	2	1.03, 1.77##	2.07#	14.50	Barow and Meister (2003), Marie and Brown (1993), Waminal and Kim (2012)
Cucurbita argyrosperma	40		0.748			Roy et al. (1991), Sisko et al. (2003)
Cucurbita ecuadorensis	40		0.933			Sisko et al. (2003)
Cucurbita foetidissima	40		0.686			Sisko et al. (2003)
Cucurbita moschata	40	2	0.708, 0.97##	1.26#	25.19	Sisko et al. (2003), Barrow and Meister (2003), Waminal et al. (2011)
Cucurbita okeechobeensis ssp. *martinezii*	40		0.74			Sisko et al. (2003)

calculated from chromosome measurements reported in publications, ## different entries for same taxa were taken from different reports

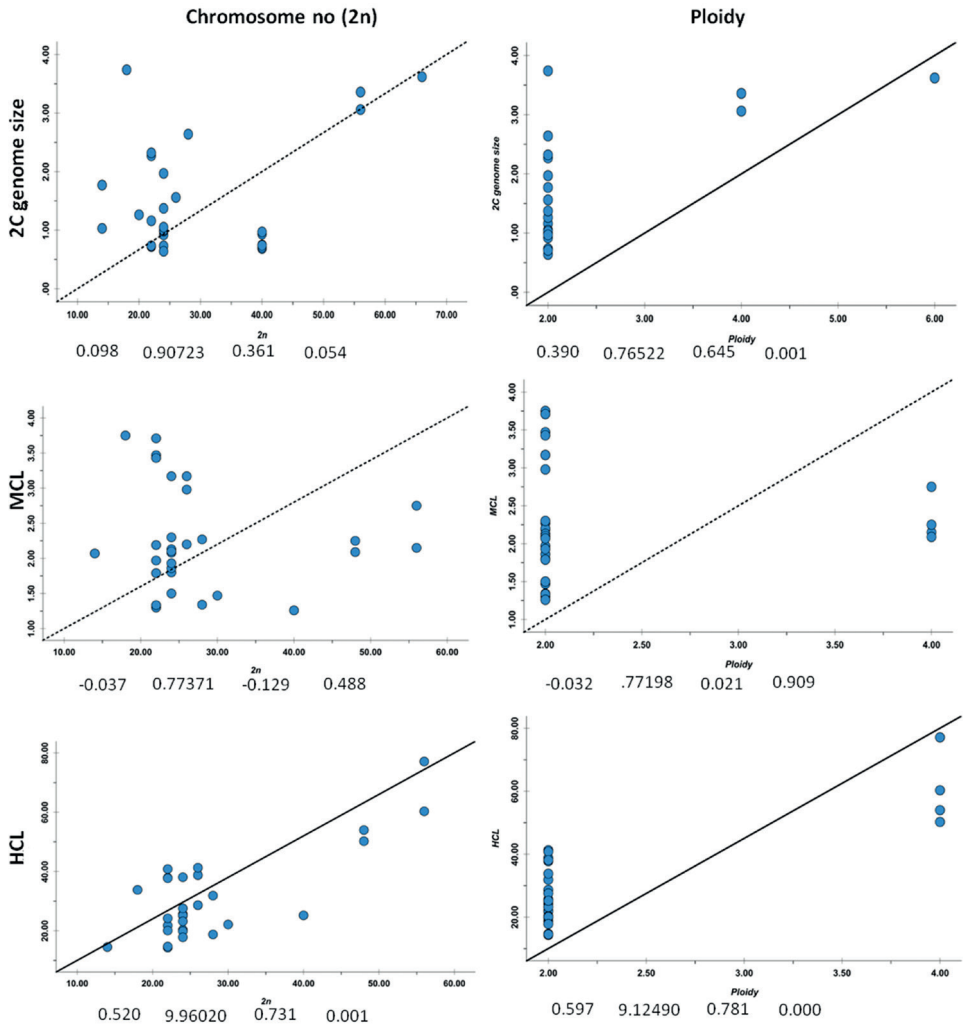


Figure 6. Scatter plots of 2n chromosome number and ploidy level (predictor variables) versus 2C genome size, MCL (mean chromosome length) and HCL (total length of haploid chromosome set) in Cucurbitaceae taxa. Symbols below plots depict regression analysis parameters; square: adjusted R square, circle: standard error of the estimate, triangle: Pearson Correlation, star: 2-tailed significance of Pearson Correlation. Regular lines indicate significant linear regression and dotted lines indicate not significant linear regress

Future directions

Chromosome number and genome size information in the basal clades (understudied tribes) should be given attention to infer ancient base numbers. The parameters of fundamental and molecular cytogenetics are inevitable for genomic interpretation (Weiss-Schneeweiss and Schneeweiss 2013; Deakin et al. 2019) and hence relevant to spot genetic resources and relationships with wild relatives. The current review is not exhaustive but supersedes the scopes of general web resources and brings an offline resource exclusive for Cucurbitaceae.

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Karyotype description and comparative chromosomal mapping of rDNA and U2 snDNA sequences in *Eigenmannia limbata* and *E. microstoma* (Teleostei, Gymnotiformes, Sternopygidae)

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Abstract

The genus *Eigenmannia* Jordan et Evermann,1896 includes electric fishes endemic to the Neotropical region with extensive karyotype variability and occurrence of different sex chromosome systems, however, cytogenetic studies within this group are restricted to few species. Here, we describe the karyotypes of *Eigenmannia limbata* (Schreiner et Miranda Ribeiro, 1903) and *E. microstoma* (Reinhardt, 1852) and the chromosomal locations of 5S and 18S rDNAs (ribosomal RNA genes) and U2 snDNA (small nuclear RNA gene). Among them, 18S rDNA sites were situated in only one chromosomal pair in both species, and co-localized with 5S rDNA in *E. microstoma*. On the other hand, 5S rDNA and U2 snRNA sites were observed on several chromosomes, with variation in the number of sites between species under study. These two repetitive DNAs were observed co-localized in one chromosomal pair in *E. limbata* and in four pairs in *E. microstoma*. Our study shows a new case of association of these two types of repetitive DNA in the genome of Gymnotiformes.

Keywords

Electric fish, fish cytogenetics, freshwater fishes, karyotype evolution, repetitive DNA

Introduction

The order Gymnotiformes is an endemic freshwater group inhabiting the Neotropical region and consisting of species capable of emitting low voltage continuous electric discharges (Alves-Gomes 2001; Lavoué et al. 2012). Among them, *Eigenmannia* is the most species-rich genus of the family Sternopygidae (Gymnotiformes), with 27 recognized species (Fricke et al. 2021). On the other hand, *Eigenmannia* is not a monophyletic assembly, and it is considered taxonomically ambiguous due to little morphological variation between species, which makes it difficult to define species-specific diagnostic characters (Alves-Gomes 1998; Albert 2001; Peixoto and Ohara 2019).

In recent years, cytogenetic studies in *Eigenmannia* were mainly limited to some species and / or karyomorphs (i.e., different karyotype forms), revealing a variable karyotype macrostructure, with diploid chromosome numbers ranging from (2n) = 28 to 46 chromosomes (Arai 2011; Silva et al. 2015b). In addition, different sex chromosome systems have been described, identifying standard systems such as XX/XY, ZZ/ZW in *E. virescens* (Almeida-Toledo et al. 2001; Henning et al. 2011; Fernandes et al. 2020), derived ZZ/Z0 system in *E.* prope *trilineata* (Araya-Jaime et al. 2017b), and multiple sex chromosome system $X_1X_1X_2X_2/X_1X_2Y$ in *Eigenmannia* sp2 (Almeida-Toledo et al. 2000; Sene et al. 2014; Araya-Jaime et al. 2015), as well as species/karyomorphs without heteromorphic sex chromosomes (de Almeida Toledo et al. 1984; Almeida-Toledo et al. 2000, 2001; Silva et al. 2009; Henning et al. 2011).

The physical mapping of repetitive sequences in gymnotiform species has provided important data on the structure and organization of the genome that has allowed us to understand the processes of karyotypic evolution that these species have experienced, recognizing Robertsonian rearrangements as the most frequent mechanisms of chromosomal variability in Gymnotiformes (Milhomem et al. 2008; Giora and Fialho 2009; Nagamachi et al. 2010; da Silva et al. 2014; Utsunomia et al. 2014, 2018; Suárez et al. 2017; Rodrigues et al. 2021). The mapping of ribosomal DNA genes (18S rDNA and 5S rDNA) has been widely used in molecular cytogenetics of Gymnotiformes, where the evidence provided by several studies has made it possible to establish two distribution patterns of these sequences: i) 18S rDNA loci located on a single chromosome pair and ii) 5S rDNA sites located in multiple chromosomal pairs, which may be associated with transposable elements or U2 snDNA (small nuclear RNA gene) sequences (Scacchetti et al. 2011, 2012; Utsunomia et al. 2014; da Silva et al. 2016; Araya-Jaime et al. 2017b; Sochorová et al. 2018; Rodrigues et al. 2021).

Recently, the mapping of genes belonging to the U snDNA family increased the knowledge about the dynamics of tandemly repeated multigene families in vertebrates. This multigene family harbors genes coding for nine types of non-coding RNAs; namely U1, U2, U4, U4 atac, U5, U6, U6 atac, U11 and U12; which constitute a portion of the RNA-protein complex of the spliceosome (Valadkhan 2005; Matera and Wang 2014). In fish cytogenetics, the use of these repetitive markers is relatively recent, with data being reported for several groups, including Characiformes (Silva et al. 2015a; Santos et al. 2017; Serrano et al. 2017), Batrachoidiformes (Ubeda-Manzanaro et al.

2010), Cyprinodontiformes (Araya-Jaime et al. 2017a), Gadiformes (García-Souto et al. 2015), Perciformes (Xu et al. 2017), Cypriniformes (Sember et al. 2018), among others. In gymnotiform fish genomes, the cytogenetic reports of these sequences are restricted to U2 snDNA, recognizing two general chromosomal patterns: i) grouped in a single pair of chromosomes or ii) scattered throughout the genome and, in some cases, associated with 5S rDNA (Utsunomia et al. 2014; Araya-Jaime et al. 2017b). In this way, the U snDNA sequences represent a good repetitive marker to provide information on the evolutionary relations between closely related species, infer the homology between certain chromosomes present in different lineages, and trace the origin and evolution of specific chromosomes, in the context of the great karyotype diversity found among Gymnotiformes.

With the aim of expanding our knowledge about the chromosomal structure and the dynamics of repetitive DNA sequences in the *Eigenmannia* genome, we present for the first time the karyotype and chromosomal location of three repetitive DNA classes (18S and 5S rDNA and U2 snDNA) in *E. microstoma* and *E. limbata* from the Sao Francisco and the Amazon River basin, respectively. Our results show a new case of physical association between the 5S rDNA and U2 snDNA in Gymnotiformes.

Material and methods

Twelve individuals of *Eigenmannia limbata* and eight of *E. microstoma*, from the Amazon basin and the San Francisco River basin, respectively, were analyzed in this study (Fig. 1). After dissection, the specimens were fixed and preserved in 70% ethanol. Finally, these specimens were deposited in the fish collection of the Laboratório de Biologia e Genética de Peixes, UNESP, Botucatu-SP. The animals were collected in accordance with Brazilian environmental protection legislation (Collection Permission MMA/IBAMA/SISBIO-number 3245) and the procedures for fish sampling, maintenance and analysis were performed in compliance with the Brazilian College of Animal Experimentation (**COBEA**) and approved (protocol 504) by the Bioscience Institute/ Unesp Ethics Committee on the use of Animals (**CEUA**).

Mitotic chromosomes were obtained by direct preparation from the cephalic kidney according to Foresti et al. (1993), and slides for conventional analysis were stained with 5% Giemsa solution in a phosphate buffer at pH 6.8. The constitutive heterochromatin (CH) was detected following Sumner (1972). Images were captured with a digital camera (Olympus DP90) in the Olympus BX6 epifluorescence photomicroscope and acquired using cellSens Dimension (Olympus, Sapporo-Japan). Image treatment, optimization of brightness and contrast was performed using the Adobe Photoshop CS6 program. The arm ratio (Levan et al. 1964) was used to classify the chromosomes as metacentric (m), submetacentric (sm), subtelocentric (st), and acrocentric (a). For counting the total number of chromosome arms or fundamental number (NF), chromosomes m, sm, st were considered bi-armed, while acrocentric chromosomes (or indistinguishable st/a) were classified as mono-armed chromosomes.

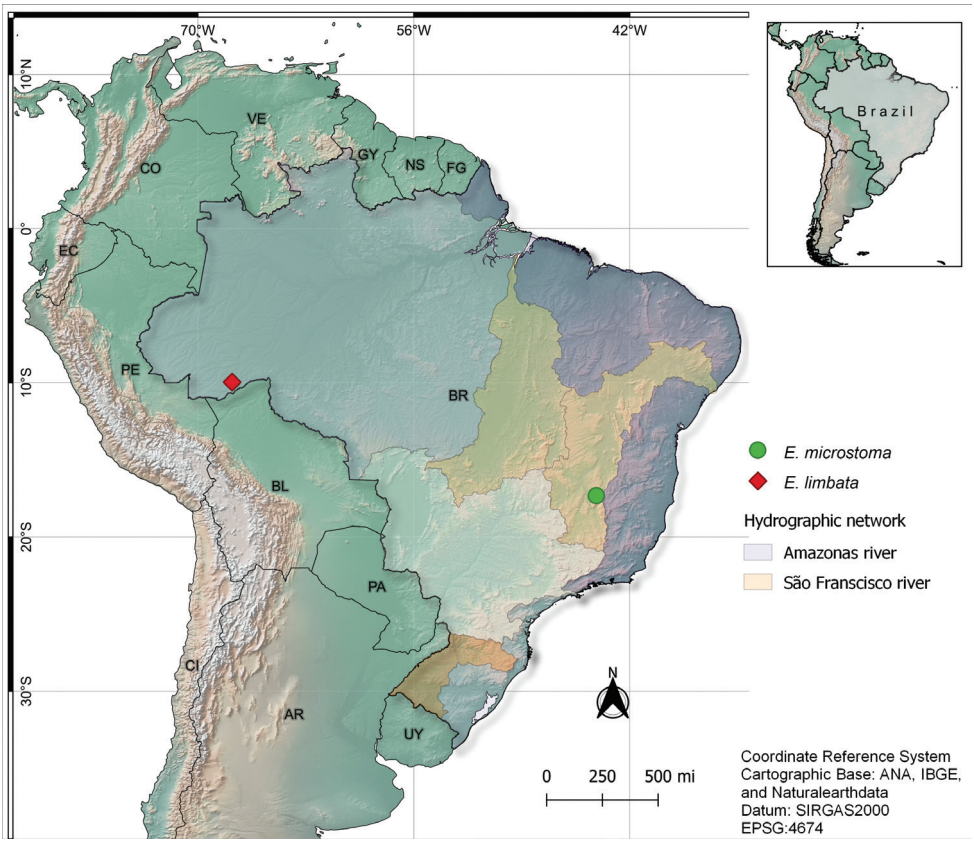

Figure 1. Location of *Eigenmannia* species in the Amazon and São Francisco basins.

Fluorescence *in situ* hybridization (FISH) procedure was performed according to Pinkel et al. (1986). The 18S, 5S rDNA and U2 snRNA gene probes were obtained from the genomic DNA of *E. microstoma* which was extracted using Wizard Genomic DNA Purification Kit (PROMEGA, Madison, Wisconsin, USA). The rDNA probes were amplified by polymerase chain reaction (PCR), using the primers 18SF (5' CCGCTTTGGTGACTCTTGAT 3') and 18SR (5' CCGAGGACCTCACTAAACCA 3') (White et al. 1990), 5SF (5' TACGCCCGA TCTCGTCCGATC 3') and 5SR (5' CAGGCTGGTATGGCCGTAACG 3') (Pendas et al. 1994) and U2F (5' ATCGCTTCTCGGCCTTATG 3') and U2R (5' TCCCGGCGGTACTGCAATA 3') (Bueno et al. 2013). PCR products were verified in 1% agarose gel. 18S rDNA probe (600 pb long fragment) were labeled with biotin-14-dATP (Dig Nick Translation mix, Roche, Applied Science, Penzberg, Germany), while the U2 snRNA gene probe (150 bp) was labeled by PCR with biotin-16-dUTP (Roche). Hybridization signals were detected using FITC–avidin (conjugated fluorescein isothiocyanate–avidin; Sigma-Aldrich, St Louis, MO, USA). 5S rDNA probe (300 pb) was labeled with digoxigenin-11-dUTP (Biotin Nick Translation mix, Roche) and the hybridization signals were detected using anti-

digoxigenin-rhodamine (Roche). The chromosomes were counterstained with 0.2 µg/mL of 4', 6-diamidino-2-phenylindole (DAPI) in the Vectashield mounting medium (Vector, Burlingame, CA).

Results

The diploid chromosome number (2n) of the *E. microstoma* was 38 chromosomes, with a karyotype composed of 8m + 10sm + 20a chromosomes (NF = 56), while *E. limbata* had 2n = 38 and karyotype composed of 8m + 4sm + 26a chromosomes (NF = 50). Morphologically differentiated sex chromosomes were not found in either species (Table 1).

Table 1. Cytogenetic features and collection sites of *Eigenmannia* species.

Species (N)	2n	Karyotype formula	Sample localities	Hydrographic basin	Coordinates (DDM)
E. limbata (♂7, ♀5)	38	8m+4sm+26a	Rio Branco-AC	Amazonas	9°57'27.10"S, 67°46'55.40"W
E. microstoma (♂5, ♀3)	38	8m+10sm+20a	Francisco Dumont-MG	São Francisco	17°18'57.80"S, 44°10'23.00"W

C-banding technique revealed significant differences in the patterns of CH distribution between the analyzed species. Both species displayed pericentromeric regions of CH in all chromosomes and *E. microstoma* possessed additional interstitial blocks on several chromosomes (Fig. 2).

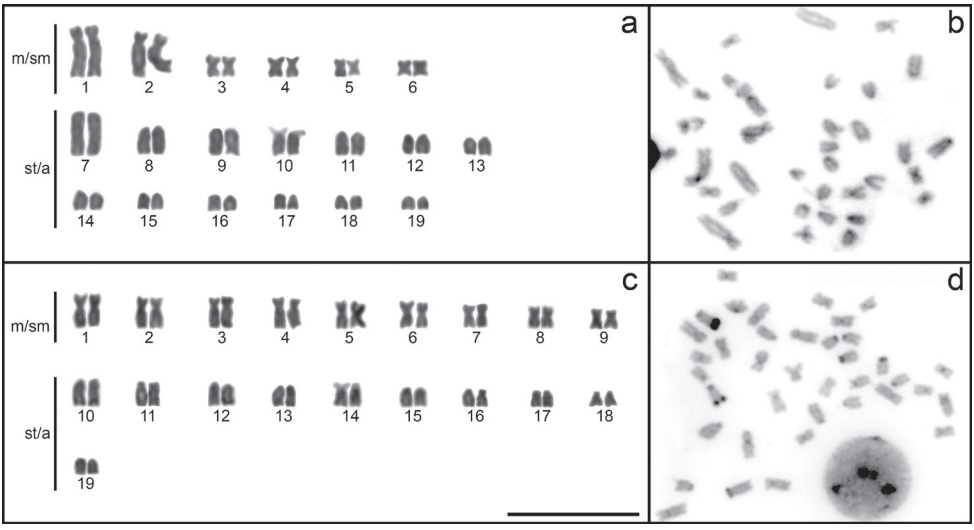

Figure 2. Chromosomes stained with Giemsa and C-banded **a, b** karyotype and C-banded metaphase of *E. limbata* **c, d** karyotype and C-banded metaphase of *E. microstoma*. Scale bar: 10 µm.

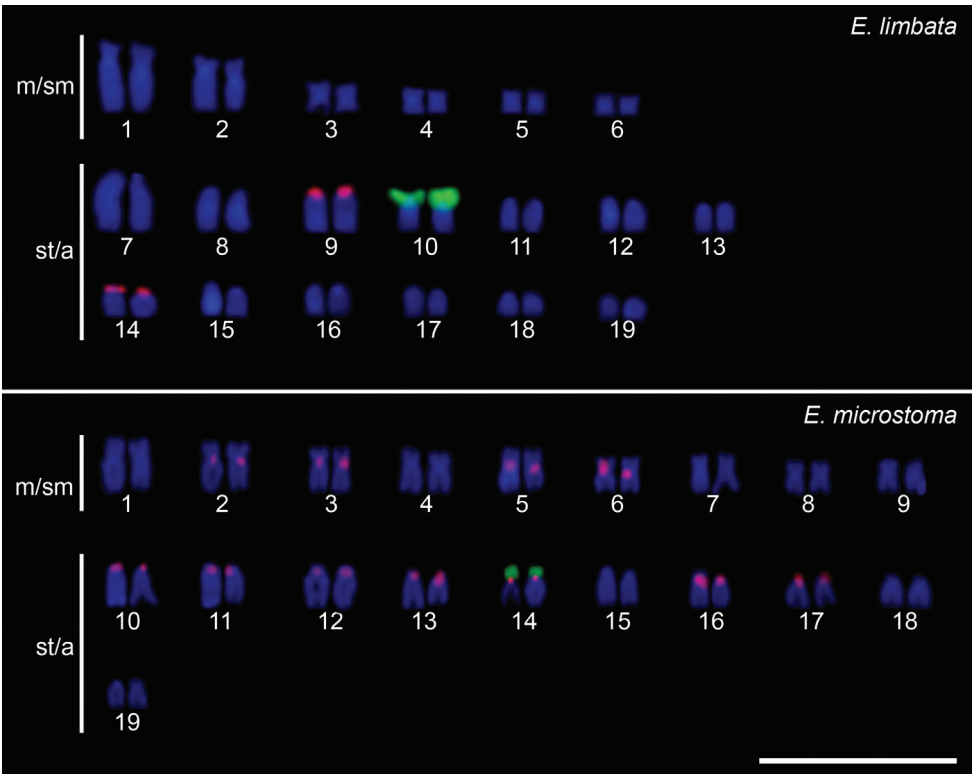

Figure 3. Karyotypes of *Eigenmannia* species after FISH with 5S (red) and 18S (green) ribosomal DNA probes and counterstained with DAPI. Scale bar: 10 μm.

The 18S rDNA site was located by FISH in a single chromosomal pair in both species, namely pair No. 10 in *E. limbata* and pair No. 14 in *E. microstoma* (Fig. 3). The 5S rDNA sites showed a considerable variation in the number and locations in analyzed species. These sites were detected in two chromosomal pairs in *E. limbata* and in 11 chromosomal pairs in *E. microstoma* (Fig. 3).

The distribution of the U2 snDNA sites was variable in terms of the number, chromosomal location, and number of co-localized sites with 5S rDNA between species. U2 snDNA sites were placed on three chromosomal pairs (11, 12 and 14) in *E. limbata* and in the chromosome pairs Nos 10, 12, 16 and 17 in *E. microstoma* (Fig. 4). These sites were co-localized with the 5S rDNA sites in the pair No. 14 in *E. limbata* and in all pairs in *E. microstoma* (Fig. 4). The location of all repetitive DNAs mapped by FISH is summarized in the ideogram presented in Fig. 5.

Discussion

The species *E. limbata* and *E. microstoma* were analyzed cytogenetically for the first time, showing the same 2n (38 chromosomes), but different NF and karyotypic

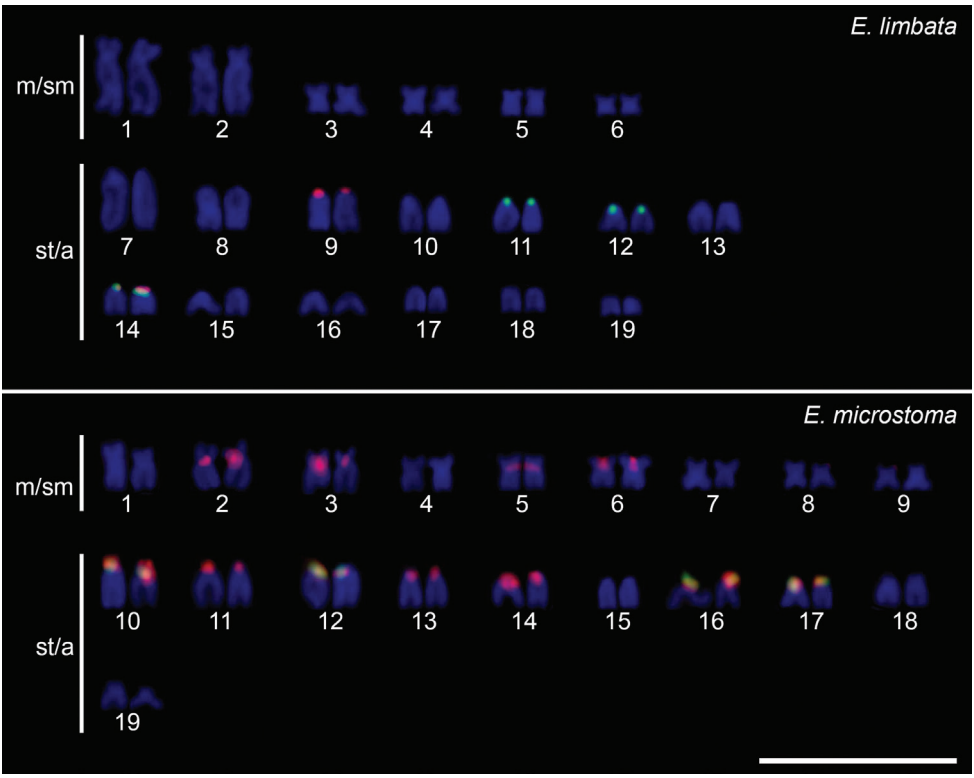

Figure 4. Karyotypes of *Eigenmannia* species after FISH with 5S rDNA (red) and U2 snDNA (green) probes and counterstained with DAPI. Note that, the two repetitive DNAs are located adjacently on the same pair (14) in *E. limbata* and they are located adjacently on four chromosome pairs (10, 12, 16 and 17) in *E. microstoma*. Scale bar: 10 μm.

structure (Table 1). Previous cytogenetic studies in *Eigenmannia* have consistently reported this same 2n (38 chromosomes), but there are wide variations in terms of the reported karyotypic formula, NF and sex chromosome system (Almeida Toledo et al. 1984; Moysés et al. 2010; Henning et al. 2011; de Sene et al. 2014; Araya-Jaime et al. 2017b; Fernandes et al. 2020). These differences in karyotypic structure and NF can be explained by the occurrence of Robertsonian rearrangements, which may be participating as an important postzygotic reproductive isolation mechanism in *Eigenmannia.* This circumstance could be related to their low population sizes and low mobility, which would facilitate the fixation of chromosomal polymorphisms (Moysés et al. 2005, 2010; Giora and Fialho 2009; Silva et al. 2009, 2015b).

A single chromosome pair carrying the NOR has been reported for most of the species of the Sternopygidae family, although the chromosomal location of the NOR varies between species and populations; therefore, a simple NOR phenotype can be an ancestral feature in the genome of Sternopygidae (de Almeida-Toledo et al. 2001; dos Santos Silva et al. 2008; de Sene et al. 2014; Araya-Jaime et al. 2017b; Fernandes et al. 2020; Rodrigues et al. 2021). However, within Gymnotidae, the

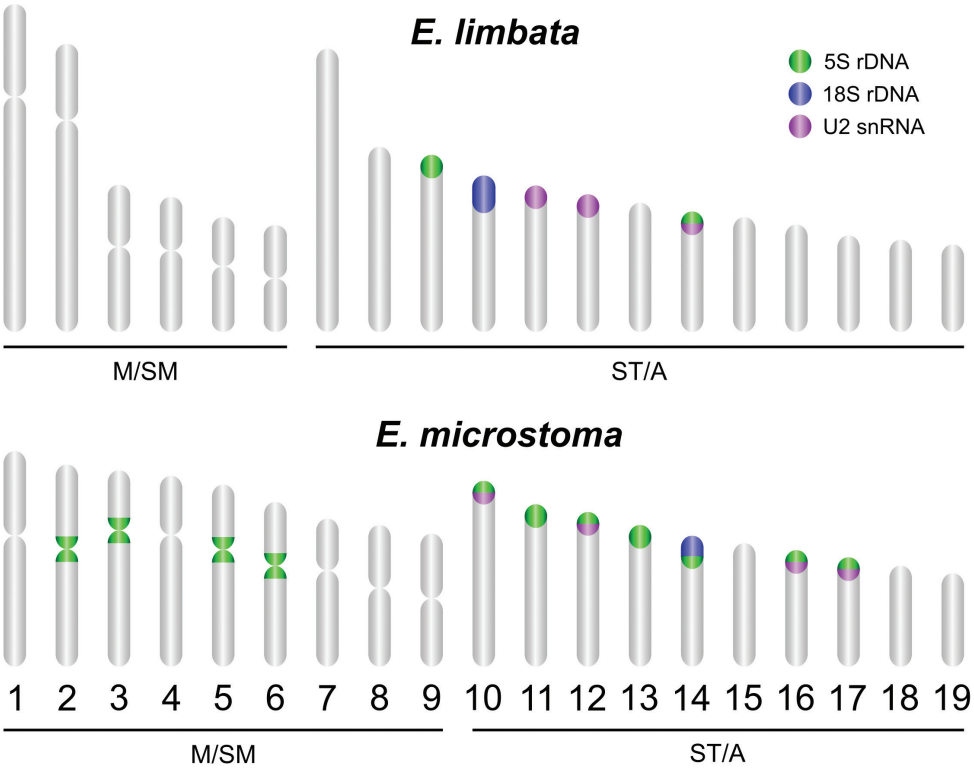

Figure 5. Idiogram of *Eigenmannia* species showing the location of repetitive DNAs.

case of *Gymonotus coatesi* is reported as the only representative of this family with multiple 18S rDNA sites (Machado et al. 2017). Furthermore, a considerable variability has been observed in the size of the NOR region within Sternopygidae (dos Santos Silva et al. 2008; de Sene et al. 2014; Silva et al. 2015b; Fernandes et al. 2017; Rodrigues et al. 2021). Accordingly, we observed this NOR heteromorphism between *E. limbata* and *E. microstoma*, in which the NOR region of *E. limbata* is considerably larger than that of *E. microstoma* (Fig. 3). This could be a consequence of tandem duplication of ribosomal genes which could form through several mechanisms including unequal exchange of sister chromatids or unequal crossing over during meiosis (Charlesworth et al. 1994; Eickbush and Eickbush 2007; Bianciardi et al. 2012).

On the other hand, multiple 5S rDNA sites observed in *E. limbata* and *E. microstoma* (Fig. 3) appear to be a widely recognized feature within Gymnotiformes, with evidence in representatives of *Gymnotus* (da Silva et al. 2011; Scacchetti et al. 2011, 2012; Utsunomia et al. 2014; da Silva et al. 2016, 2019), *Eigenmannia* (de Sene et al. 2014; Araya-Jaime et al. 2017b; Fernandes et al. 2020), *Sternopygus* (Fernandes et al. 2017) and *Archolaemus* (Rodrigues et al. 2021). Ribosomal DNA sites are considered as hot spots for chromosomal rearrangements due to their organization

into long stretches of conserved tandemly repeated sequences and their high transcription activity, which means they are susceptible to chromosomal breakage and/or non-allelic homologous recombination, increasing thus the probability of occurrence of chromosomal rearrangements, such as fusions, fissions and inversions (Rosa et al. 2012; Barros et al. 2017; Potapova and Gerton 2019; Warmerdam and Wolthuis 2019; Deon et al. 2020). Furthermore, the rDNA dynamics has been also correlated with the insertion of transposable elements, or other repetitive DNAs, into non-transcribed spacers (NTS) of 5S rDNA units, as has been observed in the genomes of *G. inaequilabiatus* (Scacchetti et al. 2012), *G. paraguensis* (da Silva et al. 2011) and *G. mamiraua* (da Silva et al. 2016). Thus, both mentioned mechanisms could explain the chromosomal dynamics of these sequences in gymnotiform genomes (de Sene et al. 2014; da Silva et al. 2016; Araya-Jaime et al. 2017b; Fernandes et al. 2017). In our case, given that 5S rDNA probe was prepared from the genomic DNA of *E. microstomata* in which we then revealed 22 signals, and that only four signals were evidenced in *E. limbata*, a possible explanation may be that the 300 bp long 5S rDNA fragment contains inserts of other repeats in its NTS region which might have promoted spreading of 5S rDNA clusters and/or generated additional non-5S rDNA signals in *E. microstomata*. In that case, only four signals in *E. limbata* might mean that the signal pattern is much less affected by the action and/or additional accumulation of the associated repeat(s). Although a single consistent PCR amplification product was obtained to be a template for the FISH probe preparation, thereby evidencing a lack of detectable amounts of 5S rDNA sequence variants or truncated copies, we cannot directly evaluate the possible presence and contribution of other repeats as we did not sequence the 5S rDNA fragment and consequently weren't looking for admixed repetitive sequences. We may, however, conclude that the chromosomal behavior of the 5S rDNA sites observed in this work is congruent with the patterns previously reported for *Eigenmannia*, such as the number of variable sites and their association with 18S rDNA and U2 snDNA clusters (de Sene et al. 2014; Araya-Jaime et al. 2017b).

The results presented here, for *E. limbata* and *E. microstoma*, represent the first case, within *Eigenmannia*, of multiple sites for U2 snDNA (Fig. 4), highlighting in *E. microstoma* the presence of three chromosomal pairs carrying U2 snDNA sites, where one of them (pair No 14) is co-localized with 5S rDNA, while in *E. limbata*, the four chromosomal pairs carrying U2 snRNA genes are co-localized with 5S rDNA. The previous report by Araya-Jaime et al. (2017b) described the karyotype of *E.* aff *trilineata* with a single U2 snDNA site being co-localized with 5S rDNA. These results reinforce the dynamic nature of these sequences and show that the 5S rDNA / U2 snRNA association would be a characteristic feature of the *Eigenmannia* genome. In other Gymnotiformes, six *Gymnotus* species are reported with a single U2 snRNA carrier pair, while only in *G. pantal* and *Archolaemus janae*, multiple sites for U2 snRNA have been reported (Utsunomia et al. 2014; Rodrigues et al. 2021). None of the species mentioned above exhibits co-localization between U2 snDNA with other sequences been reported.

Conclusion

In the present work, the cytogenetic analysis carried out in the species *E. limbata* and *E. microstoma* reinforced the chromosomal variability reported for the genus, evidencing the occurrence of notable differences between the karyotypes of the species / karyomorphs studied up to here, even though the 2n mostly observed is 2n = 38 chromosomes. The chromosomal location of the 5S and 18S rDNA clusters observed in the species studied here followed the same pattern observed in Gymnotiformes with a single NOR-bearing pair and multiple sites for 5S rDNA. On the other hand, the dynamic nature of the U2 snRNA sites stands out, together with the co-localization with 5S rDNA genes, as a characteristic feature of the *Eigenmannia* genome. Finally, the results presented here reinforce the postulate that cytogenetic features (conventional and molecular) could be considered as important markers for taxonomic diagnosis and for the description and characterization of the existing biodiversity in Gymnotiformes.

Author's contribution

Conceptualization: CAJ, CO, FF; experimental design: CAJ, LRRS, FF; collected samples: CAJ, DMZAS, LRRS, CNN; cytogenetics analyses: CAJ, DMZAS, LRRS, CNN, FF; contributed with reagents/materials/analysis tools: DMZAS, CO, FF. All authors wrote, read, and approved the manuscript.

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First cytogenetic data on Afrotropical lutefishes (Citharinidae) in the light of karyotype evolution in Characiformes

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Abstract

The Afrotropical lutefish family Citharinidae (Citharinoidei, Characiformes) comprises three genera with eight species in total. Although Citharinidae have been studied in terms of taxonomy and systematics, no cytogenetic information was available for any representative of the family. Furthermore, only one species out of 116 in Citharinoidei (*Distichodus affinis* Günther, 1873) has been studied cytogenetically. Here, we report the karyotypes of *Citharinus citharus* (Geoffroy St. Hilaire, 1809) from West Africa and *Citharinus latus* Müller et Troschel, 1844 from Northeast Africa. The former has the diploid chromosome number 2n = 40 and the fundamental number FN = 80, while the latter has 2n = 44 and FN = 88. Hence, these karyotypes consist exclusively of bi-armed chromosomes. Such karyotypes were previously found in *D. affinis* and in many lineages of Neotropical species of another suborder of Characiformes, Characoidei. In contrast, the karyotypes dominated by uni-armed elements are typical for a number of phylogenetically basal lineages of Afrotropical and Neotropical Characoidei. We discuss the importance of our data on Citharinidae for the understanding of the karyotype evolution within the order Characiformes.

Keywords

Africa, Characoidei, chromosomes, Citharinoidei, *Citharinus*, karyotype evolution

Introduction

Characins, the order Characiformes, are classified into two suborders: Citharinoidei and Characoidei. The former includes two Afrotropical families: Citharinidae with eight species in three genera and Distichodontidae with 108 species in 16 genera, while the latter suborder (Characoidei) contains more than 2,000 species in two Afrotropical (Alestidae and Hepsetidae) and 20 Neotropical families (Nelson et al. 2016; Betancur et al. 2018; Froese and Pauly 2022). The suborders are undoubtedly monophyletic, while the monophyly of the order as a whole has been put into question by some studies of its molecular phylogeny (reviewed by Arcila et al. 2017; Betancur et al. 2018). The time of the divergence of the characin suborders is estimated between 115 and 145 Mya (Lavoué 2019, but also see Arroyave et al. 2013).

There is no cytogenetic information about any citharinid species, whereas the karyotype of the only distichodontid species, *Distichodus affinis* Günther, 1873, was analyzed by Rab et al. (1998). In contrast, the extensive literature on the cytogenetics of Characoidei, both Afrotropical (Post 1965; Hinegardner and Rosen 1972; Krysanov and Golubtsov 2014; Carvalho et al. 2017; Mohamed et al. 2019) and Neotropical (reviewed by Oliveira et al. 1988, 2007, 2009; Galetti et al. 1994; Fenocchio et al. 2003; Nirchio et al. 2014; Pazian et al. 2018, Sassi et al. 2020), demonstrates the substantial variety of the karyotype structure and different modes of its evolution in hundreds of cytogenetically studied species.

Here, we present the first data on the karyotypes of two species of the genus *Citharinus* Cuvier, 1816. We then cytogenetically compare these species with the nearest studied relative, *D. affinis*, and other characins. Finally, we discuss the importance of these data for the understanding of the karyotype evolution within the order Characiformes.

Materials and methods

Seven individuals of an undetermined sex (UD) of *Citharinus citharus* (Geoffroy St. Hilaire, 1809), standard length (SL) of 61–91 mm, and three individuals (a female, a male and a UD individual) of *C. latus* Müller et Troschel, 1844, SL = 63–84 mm, were karyotyped. For each individual, at least 10 complete metaphases were analyzed to establish the diploid chromosome number and the karyotype structure. The total numbers of complete metaphase plates studied for each species were 101 and 42, respectively. *Citharinus citharus* were purchased from the Nigerian aquarium fish dealers through the mediation of the company Aqua Logo Engineering (https://www.aqualogo-engineering.ru) in October of 2021, while *C. latus* individuals were collected in southwestern Ethiopia by the Joint Ethiopian-Russian Biological Expedition (JERBE) from the Alvero River just downstream of the Abobo Dam (7°52'23"N, 34°29'48"E) in November of 2017. This river belongs to the Sobat River drainage discharging into

the White Nile in South Sudan. Nigerian fish were kept in the Moscow laboratory in a 100-l aquarium with permamently aerated and filtered water for one to ten days before treatment. Ethiopian fish were caught with a cast net and delivered in 80-l plastic containers into the field laboratory, where they were kept in permamently aerated water for several hours before treatment.

Before preparation, fish were treated intraperitoneally with 0.025% colchicine (0.01 ml / 1 g of their weight) for 1–2 hours (for *C. citharus*, in laboratory conditions) or 0.1% colchicine for 3–4 hours (for *C. latus*, in field conditions). Then, fish were euthanized with an overdose of tricaine methanesulfonate (MS-222), identified, measured with an accuracy of ±1 mm, dissected for gonad examination and tissue sampling, and preserved in 10% formaldehyde. Species identification was done based on the morphological characters (mostly, the number of scales in the lateral line for *C. citharus* and the relative size of adipose fin for *C. latus*, according to Gosse 1990; Golubtsov et al. 1995). Vouchers are deposited at the Severtsov Institute of Ecology and Evolution (Moscow), under provisional labels of JERBE.

Chromosome preparations were obtained from *C. citharus* following Bertollo et al. (2015) and from *C. latus* following Kligerman and Bloom (1977), with some modifications for both protocols. Briefly, the kidneys of *C. citharus* were suspended in 10 ml of a 0.075M KCl hypotonic solution and incubated for 20 min at room temperature; then 1 ml of the freshly prepared 3:1 methanol : acetic acid fixative was added and the cell suspension was centrifuged for 5 minutes at 1000 rpm. Afterwards, the supernatant was discarded, 5 ml of the fixative were added, and the cell suspension was kept at 4 °C for 15–20 min. These procedures were repeated two more times. After the third centrifugation and the elimination of the supernatant, 0.5–1.0 ml of the fixative was added and the final cell suspension was left for storage at -20 °C. To prepare chromosome spreads, several small drops of the cell suspension were released onto various sections of a slide, previously maintained in distilled water at 4 °C, then the slides were transferred to a hot plate (45 °C) for drying. As for *C. latus*, the kidney tissue was incubated with a 0.075M KCl hypotonic solution for 20 min and fixed in three changes of the 3:1 methanol : acetic acid fixative. To prepare slides, the fixed tissue was incubated with the 50% glacial acetic acid, suspended, and dropped onto hot slides (45 °C). The chromosome spreads of both species were stained conventionally with 4% Giemsa solution in a phosphate buffer solution at pH 6.8 for 8 min.

The chromosome spreads were analysed using an Axioplan 2 Imaging microscope (Carl Zeiss, Germany) equipped with a CV-M4^{+}CL camera (JAI, Japan) and the Ikaros software (MetaSystems, Germany). Final images were processed using the Photoshop software (Adobe, USA). Karyotypes were established according to the centromere position following the nomenclature by Levan et al. (1964). Chromosomes were classified as metacentric (m) or submetacentric (sm), grouped according to their morphology and ordered by the decrease of their size. To determine the fundamental number (FN), metacentrics and submetacentrics were considered bi-armed.

Results and discussion

The karyotype of *C. citharus* has 2n = 40 and consists of 26 metacentrics (m) and 14 submetacentrics (sm), the fundamental number FN = 80 (Fig. 1, above). The karyotype of *C. latus* has 2n = 44 and consists of 30 m and 14 sm, FN = 88 (Fig. 1, below). No distinguishable sex chromosomes were observed in complements of the two *Citharinus* species, similar to the report by Rab et al. (1998) for *Distichodus affinis*.

Citharinus citharus has nine chromosome pairs (nos. 1–3 and 14–19) noticeably larger than others, while *C. latus* has seven large chromosome pairs (nos. 1–4 and 16–18). This difference could be explained by two fusions of four pairs of smaller chromosomes (if the karyotype of *C. latus* is considered ancestral) or fissions of two pairs of larger chromosomes (if the karyotype of *C. citharus* is considered ancestral). However, another possible scenario would be an independent origin of karyotypes of the two *Citharinus* species. Namely, *D. affinis* exhibits 2n = 48 (Rab et al. 1998), while 2n = 50–54 is typical for the phylogenetically basal groups of Characoidei (Arai 2011; Machado et al. 2011; Cioffi et al. 2012; Krysanov and Golubtsov 2014; Arcila et al. 2017; Carvalho et al. 2017). Therefore, the two *Citharinus* karyotypes could evolve via the different numbers of chromosome fusions from an ancestral karyotype with the diploid chromosome number higher than those displayed by the two *Citharinus* species. Obviously, these scenarios are speculative due to the lack of cytogenetic data for the most of genera and species of Citharinoidei. However, our results, together with

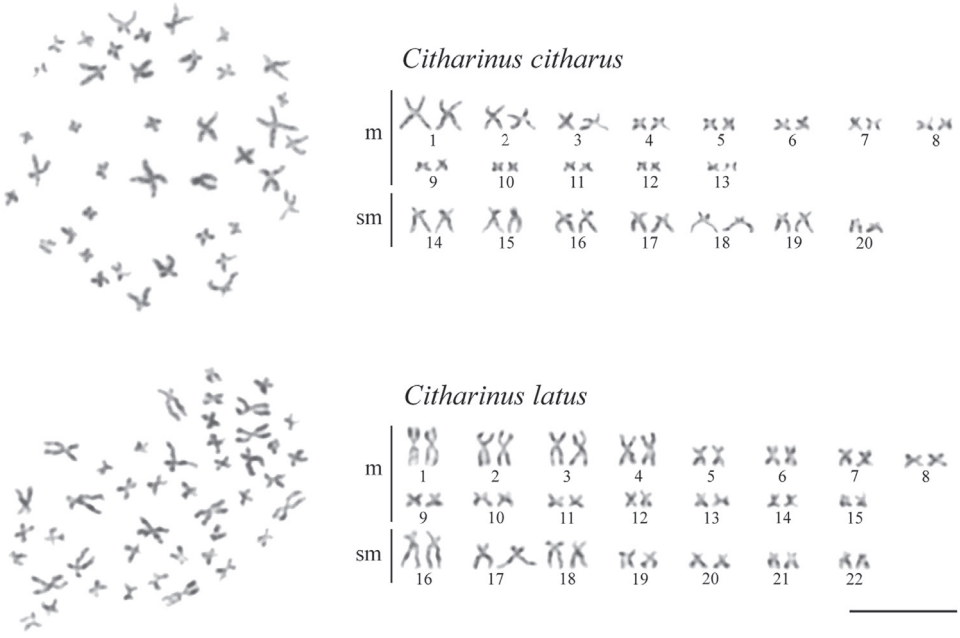

Figure 1. Metaphase chromosome plates (left) and karyotypes (right) of *Citharinus citharus* and *C. latus* after conventional Giemsa staining. Scale bar: 10 μm.

the basal location of the family Citharinidae in the phylogeny of all Citharinoidei and a distant location in this phylogeny of *D. affinis* (Arroyave et al. 2013; Betancur et al. 2018), suggest a substantial role of chromosome fusions/fissions in the evolution of Citharinoidei karyotypes.

Of note, all the three Citharinoidei species with studied karyotypes – both *Citharinus* species presented here and *D. affinis* studied by Rab et al. (1998) – have exclusively bi-armed chromosomes. However, due to the fragmentary cytogenetic data for Citharinoidei, we cannot reliably conclude whether this karyotype structure is typical for the suborder.

In comparison, the suborder Characoidei that is better studied cytogenetically demonstrates a wide variation in karyotype structures even in its basal groups. Specifically, karyotypes with exclusively bi-armed chromosomes are found in the family Crenuchidae (Oliveira et al. 2007; Arai 2011; Machado et al. 2011) which is a basal group of all Characoidei (Arcila et al. 2017). Of note, bi-armed karyotypes are also characteristic of some other families of the suborder, namely Anostomidae, Chilodontidae, Curimatidae and Prochilodontidae; however, they are nested far from the root of the Characoidei phylogeny (Arai 2011; Arcila et al. 2017). In contrast to Crenuchidae, two groups of other Characoidei families stem out rather close to the basal nodes but the karyotype structures of most of their members that were cytogenetically studied are enriched with or even dominated by uni-armed chromosomes. The first group consists of Neotropical Ctenoluciidae and Lebiasinidae, while the second group comprises Afrotropical Hepsetidae and Alestidae, as well as Neotropical Erythrinidae (Arai 2011; Cioffi et al. 2012; Krysanov and Golubtsov 2014; Arcila et al. 2017; Carvalho et al. 2017; Mohamed et al. 2019). The reported exceptions in these groups are genera *Hoplerythrinus* Gill, 1896 and *Hoplias* Gill, 1903 (Erythrinidae) whose karyotypes are almost completely dominated by bi-armed chromosomes (Arai 2011; Cioffi et al. 2012).

Importantly, our new data on the two Citharinoidei karyotypes suggest a revision of the current hypothesis about the ancestral chromosome number of the order Characiformes. Namely, based almost exclusively on the cytogenetic data from the other suborder of Characiformes, Characoidei, the chromosome number 2n = 54 was suggested to be ancestral for the whole order (Oliveira et al. 1998, 2007; Cioffi et al. 2012; Carvalho et al. 2017). However, cytogenetic data on the family Citharinidae and, in general, the suborder Citharinoidei is also important for the understanding of the karyotype evolution in Characiformes, because the family Citharinidae is a basal group for Citharinoidei, while Citharinoidei is a basal group for all Characiformes (Arcila et al. 2017). In this context, our data on Citharinidae suggest the possibility of a lower ancestral chromosome number for the order Characiformes and indicates the need of futher cytogenetic studies in the phylogenetically basal groups of Citharinoidei and Characoidei to clarify the evolution of the chromosome number and the karyotype structure in Characiformes.

On the other hand, some authors recently proposed a new hypothetical molecular phylogeny of the ray-finned fishes where the suborder Citharinoidei is separated into

an order Citharinifomes and considered as a sister group to Characiformes + Siluriformes (Dornburg and Near 2021; Melo et al. 2021). Consequently, according to this new hypothesis, the karyotypes of Citharinoidei/Citharinifomes and Characoidei/Characiformes could evolve more independently. However, even in that case our new data, together with any future cytogenetic studies of Citharinoidei/Citharinifomes, will help to reconstruct the evolutionary history of karyotypes in a part of the superorder Ostariophysi.

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