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Original article

Chromosome number, genome size and heterochromatin evolution in diploid species of *Ipomoea* and related genera (Convolvulaceae: Convolvuloideae)

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ABSTRACT

Convolvuloideae is a group of plants formed by genera with n = 15 and high stability of chromosome numbers. Despite being stable, polyploidy is the main mechanism of karyotype evolution for the group and seems to be related to speciation events. The present work aimed to comparatively analyze the karyotypes of diploid species from Convolvuloideae, with emphasis on *Ipomoea*, using fluorochrome banding and genome size. New counts were recorded for five species belonging to *Camonea* and *Ipomoea,* all with $2n$ = 30. The basic number x = 15 has been suggested for Convolvuloidae. The first genome size records are presented here for the genera *Camonea*, *Distimake* and *Stictocardia*, as well as for six species of *Ipomoea*. Genome size ranged from 1C = 0.78 pg in *I. bahiensis* to 1.38 pg in *Distimake dissectus*. Two types of heterochromatin bands were identified in Convolvuloideae, CMA^+ bands were the predominant type, while $DAPI^+$ bands were less frequent, with four banding variation described. Small genome sizes and stable chromosome numbers possibly represent evolutionarily strategies associated with adaptation and speciation in the clade, while the implications of heterochromatin variation remain unknown.

Keywords: basic chromosome number, chromosome banding, fluorochromes, karyotype evolution, polyploidy.

Introduction

The karyotypic changes that occur throughout a monophyletic group of plants often result in a wide diversity of chromosome numbers, which draw attention as evidence of the evolutionary process (Chase *et al.* 2023). These data, together with phylogenetic or ecological approaches, allows to distinguish different evolutionary strategies in plants, test hypotheses about the directions of karyological changes, as well as about the relationship between chromosome number variation and speciation/adaptation (Carta *et al.*

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2018). However, for different groups of plants, karyotypes do not always vary in chromosome number, and entire clades with different genera and species show impressive numerical stability (Guerra 2008).

On the other hand, more detailed karyological analysis in plants with chromosome number stability have revealed structural chromosome reorganization among species, including synteny and collinearity breakage, centromeric repositioning, inversions and translocations involving many pairs of non-homologous chromosomes (Báez *et al.* 2019; Martins *et al*. 2021). Some of these reorganizations appear to be related to ancient whole genome duplication events, as in the genus *Ipomoea*, with occurrence of interchromosomal rearrangements between pseudo-chromosome 1, 5 and 12 in *I. aquatica* and pseudo-chromosome 5, 14 and 15 in *I. triloba*, without however modifying the total number of chromosomes (Hao *et al.* 2021).

The clade Convolvuloideae is another example of a group consistently formed by genera with $n = 15$ and high stability of chromosome numbers. Despite being monophyletic, the intergeneric relationships in the clade are still poorly understood, with the tribe Merremieae (already dissolved) emerging as paraphyletic while the monophyly of Ipomoeeae is strongly supported by molecular and palynological data (Simões *et al.* 2022). Convolvuloideae is little known in karyological terms, with several genera lacking any information about chromosome count in the literature, and several others with only one species recorded. Furthermore, only the genera *Calystegia* and *Ipomoea* have genome size data available in the literature.

The most kariologically known genus in Convolvuloideae is *Ipomoea*, which also stands out for having the largest number of species, with about 800 species currently recognized (Wood *et al.* 2020). The genus has a higher occurrence in tropical and subtropical regions (Wood 2017), with 430 species recorded in the Americas (Wood & Scotland 2017), being cited as the most economically important for the food industry and with ornamental use (Simões *et al.* 2022). Despite being partially stable, with $2n = 30$ in most species (72% of records), polyploidy is the main mechanism of karyotype evolution recorded for the group and seems to be related to speciation events, as in *I. argillicola*, *I. biflora*, *I. cairica*, *I. cordatotriloba*, *I. gracilis*, *I. littoralis*, *I. lonchophylla*, *I. racemigera*, *I. repens*, *I. saintronanensis*, *I. tabascana*, *I. tiliacea*, *I. trifida* with $2n = 60$, or to domestication as in *Ipomoea* × *leucantha* Jacq. with $2n = 90$ and *I. batatas* with $2n = 45, 60, 84, 90, 120$. Furthermore, there are records of B chromosomes in *I. carnea*, suggesting that the group presents some ancestral karyological event related to structural rearrangements involving A chromosomes. B chromosomes are supernumerary dispensable chromosomes, while A chromosomes are referred to all the other chromosomes in the genome (Pokorná & Reifová 2021). In terms of genome size *Ipomoea* is also the most studied genus from the family Convolvulaceae, ranging from 1C = 0.63 pg in *I. quamoclit* (Veselý *et al.* 2012) to 1C = 2.30 pg in *I. batatas* (Ozias-Akins & Jarret 1994).

Polyploidy (or whole genome duplication) consists of the accumulation of multiple copies of one genome in the same nucleus, above the diploid level. It can be defined as allopolyploidy when associated with hybridization between two or more species, or autopolyploidy when it occurs within a single species. The main contribution of polyploidy to the evolution of angiosperms is the generation of genetic variability, which can serve as raw material for the origin of evolutionary novelties (Jiao *et al.* 2011; Carta *et al.* 2018). An ancestral polyploidy event has been mapped into the phylogeny of angiosperms as well as at the origin of seed plants, indicating that all angiosperms are paleopolyploid (Jiao *et al.* 2011). Recently, a hexaploidization event was confirmed at the origin of Convolvulaceae about 40–46 million years ago (Zhang *et al.* 2022). It is possible to state that polyploidy partly explains the rapid diversification and dominance of angiosperms in terrestrial environments. Several plant lineages show the same pattern of rapid diversification when related to allopolyploidy, as in *Nicotiana* section *Suaveolentes* (Chase *et al.* 2023), as well as autopolyploidy in the complex *Epidendrum nocturnum* (Cordeiro *et al.* 2022), and may also be related to the origin of new genera, such as *Aniseia* in the clade Convolvuloideae (Rice *et al.* 2015).

In numerically stable groups, such as Convolvuloideae, more detailed analyzes, such as comparative genomics and oligo-FISH, can reveal a wide diversity of structural alterations and karyotype differences in a group of related species (Montenegro *et al.* 2022). However, these analyzes are expensive and require whole-genome sequencing and chromosome-level assembly of some reference species, which is not always available. An efficient, fast and accessible way to assess chromosome variation in stable groups is through the use of different cytogenetic approaches, which may indicate differences in chromosome structure. Thus, the combined use of different cytogenetic information, including chromosome number, banding patterns and genome size, in a phylogenetic context, is extremely important for understanding the factors related to diversification, speciation and evolution of species in a clade (Souza *et al.* 2012; Acosta *et al.* 2016; Moraes *et al.* 2017).

The present work aimed to comparatively analyze the karyotypes of diploid representatives of different genera in Convolvuloideae, with emphasis on *Ipomoea*, using banding technique with the fluorochromes CMA and DAPI and the quantification of the genome size by flow cytometry. In addition, the reconstruction of the ancestral basic chromosome number of Convolvuloideae was carried out through the analysis of the chromosome number variation recorded for the clade in the phylogenetic context proposed by Simões *et al.* (2022), aiming to answer the following questions: (1) What are the types and patterns of heterochromatin bands in species of Convolvuloideae?

(2) How does genome size vary among species in Convolvuloidae? Is this variation informative about the evolutionary genomes dynamics in Convolvuloidae? (3) What is the most likely basic chromosome number for the clade Convolvuloidae? (4) Does the analyzed karyological dataset support any model of chromosome evolution for the clade Convolvuloideae?

Material and methods

Botanical collection and documentation

Nineteen species belonging to the clade Convolvuloideae, collected in the field and maintained in cultivation at the experimental garden of the Laboratory of Plant Cytogenetics of the Federal University of Paraíba (UFPB) were analyzed. All plant material studied was herborized and the specimens were deposited at the Herbarium Prof. Jayme Coelho de Morais from UFPB and Professor Vasconcelos Sobrinho from the Federal Rural University of Pernambuco (Tab. 1). With regard to species identification, relevant literature was used, in addition to comparisons with previously identified materials.

Chromosome number variation

Initially, a review of all chromosome number records of Convolvuloideae species available in the literature was performed (Table S1). For this purpose, three databases were consulted: The Chromosome Counts Database – CCDB (Rice *et al.* 2015), Index to Plant Chromosome Numbers - IPCN

Table 1. Species of Convolvuloideae analyzed with CMA/DAPI banding and flow cytometry. Species, vouchers, collection sites, chromosome numbers (2*n*), genome size (1C) in picograms and heterochromatin banding patterns are presented.

Taxon	Voucher	Provenance	2n*	1C*	Heterochromatin bands	
					Terminal	Proximal
Camonea umbellata (L.) A.R.Simões & Staples	CSDornelas 01	Areia, PB (6°58'30''S/35°41'28''W)	**30**	**0.97**	8 CMA$^+$	-
Distimake dissectus ARSimões & Staples	GWStaples 1463	Florida, USA (29°33'42''N/81°44'00''W)	30	**1.38**	3 CMA$^+$	2 DAPI$^+$
D. macrocalyx (Ruiz & Pav.) AR Simões & Staples	GWStaples 1727	Areia, PB (6°58'30''S/35°41'28''W)	30		4 CMA$^+$	-
Ipomoea acanthocarpa Hochst. ex Choisy	JAlencar 60	Ilha do Ferro, AL (9°44'17''S/37°31'38''W)	30		4 CMA$^+$	-
I. asarifolia (Desr.) Roem. & Schult	CSDornelas 06	Areia, PB (6°58'30''S/35°41'28''W)	30	**1.0**	6 CMA$^+$	2 CMA$^+$
I. bahiensis Willd.	LPFelix 17640	Areia, PB (6°58'30''S/35°41'28''W)	**30**	**0.78**	5 CMA$^+$/16 DAPI$^+$	4 CMA$^+$
I. bonsai D. Santos & Alencar	SLC 90	Cratéus, CE (5°09'50''S/40°39'38''W)	30		6 CMA$^+$	-
I. brasiliana Meisn.	CSDornelas 03	Areia, PB (6°58'30''S/35°41'28''W)		**1.23**		
I. cárnea Jacq.	CSDornelas 07	Areia, PB (6°58'30''S/35°41'28''W)		**1.24**		
I. crinicalyx Meisn.	JLourenço 156	Guarapari, ES (20°40'25''S/40°30'00''W)	**30**		4 CMA$^+$	1 CMA$^+$
I. indica (Burm.) Merr.	GWStaples 1711	Bezerros, Pernambuco (8°14'01''S/35°45'17''W)	30	**0.84**	7 CMA$^+$	-
I. mucuroides			**30**			
I. nil (L.) Roth.	CSDornelas 05	Areia, PB (6°58'30''S/35°41'28''W)		0.87		
I. quamoclit L.	JLourenço 30	Serra de Itabaiana, SE (10°44'58''S/37°20'25''W)	30	0.86	6 CMA$^+$	-
I. queirozii J.R.I.Wood & L.V.Vasconc.	LPFelix 18198	Juazeiro, BA (9°25'06''S/40°29'03''W)		**1.18**		
Ipomoea sp.	LPFelix 17576	Juazeiro, BA (9°25'06''S/40°29'03''W)	30	**1.04**	3 CMA$^+$	1 CMA$^+$
Operculina hamiltonii (G.Don) DFAustin & Staples	DSantos 29	Araçagi, PB (6°51'03''S/35°22'53''W)	30		2 CMA$^+$	-
O. turpethum (L.) Silva Manso	MChristenhusz SN	Australia	30		6 CMA$^+$	2 CMA$^+$
Stictocardia tillifolia (Desr.) Hallier fil	GWStaples 1564	Thailand	30	**1.08**	6 CMA$^+$	-

*unpublished chromosome numbers and genome size in bold

(Goldblatt & Johnson 1979) and IAPT/IOPB Chromosome data, in addition to the records available in specialized literature.

Basic chromosome number

The chromosome number records, as well as the counts performed during the execution of this work, were used to reconstruct the basic chromosome number for each clade, as well as for the genera, using ChromEvol v.2 (http://www.zoology.ubc.ca/prog/chromEvol.html) (Mayrose *et al.* 2010; Glick & Mayrose 2014). The maximum likelihood approach was used to test the number and directions of changes in chromosome numbers along the branches in the phylogeny proposed by Simões *et al.* (2022). This estimate made it possible to infer which model of chromosome evolution best explains chromosome number variation in Convolvuloideae. The best model was selected using the Akaike Information Criterion (AIC). The haploid chromosome numbers were used to reconstruct the basic ancestral chromosome number, through phylogeny, using the ChromEvol program. Haploid chromosome numbers were plotted as categorical data under maximum likelihood, applying ancestral character reconstruction analysis.

Some chromosome numbers recorded for some genera in Convolvuloideae are very discrepant in the individual context in each genus. During the literature review, it was observed that many records in the original publications, especially those records between the 10's and the 80's, lacked photographic documentation of metaphases, sometimes being mentioned in chromosome number lists, or referring to hybrid analyses. Thus, for the reconstruction of the basic number, the chromosomal numbers confirmed for each genus were used, or those whose original publications presented documentation of the metaphases. The chromosome numbers of hybrids when mentioned in publications were also excluded.

Cytogenetic analysis

The analysis of mitotic metaphases were performed from root tips of plants growing at the experimental garden of the Plant Cytogenetics Laboratory of UFPB - Federal University of Paraíba, Campus II. The root tips were pre-treated with 8-hydroxyquinoline (8-HQ) 0.002 M for 24 hours at 10 °C, fixed in absolute ethanol/glacial acetic acid (v/v) 3:1 for 2 hours at room temperature and stored in freezer at – 20°C.

For preparing the slides, the root tips were digested in a solution containing 2% cellulase (Onozuka) and 20% pectinase (Sigma) (w/v) at 37 °C for 120 min. The material was crushed in 45% acetic acid and frozen in liquid nitrogen to remove the coverslip. Slides were stained with DAPI glycerol (2µg/mL) (1:1, v/v) to select the best slides. Subsequently, slides were carnoy bleached for 30 min. and kept in absolute ethanol at room temperature for two hours (Guerra & Souza 2002).

CMA/DAPI staining

Double staining with the fluorochromes chromomycin A_3 (CMA) and 4'-6-diamidinino-2-phenylindole (DAPI) was performed as described by Barros e Silva and Guerra (2010). The slides were stained with 10µL of CMA (0.2 mg mL^{-1}) for 1h, and subsequently with 10µL of DAPI (1 µg mL^{-1}) for 30 min. Slides mounted in glycerol/Mcllvaine buffer. Then, the slides were aged for three days in a dark chamber to stabilize the fluorochromes. The best metaphases were photographed in a Zeiss epifluorescence photomicroscope equipped with an AxioCam MRC5 video camera with the aid of Axiovision 4.8 software. Images were processed for brightness and contrast using Photoshop CS3 software.

Genome size

From young and completely expanded leaves for each species, a suspension of nuclei was prepared with WPB buffer (Tris-MgCl2) as described by Loureiro *et al.* (2007). Genome size was estimated by flow cytometry with a BD Accuri™ cytometer. The DNA content was calculated based on three different measurements for each individual analyzed according to the proportionality of the fluorescence intensity obtained. Histograms were generated by the BD Accuri™ C6 Plus software v1.0.1. by using the fluorescence pulse histogram area for analysis. The G1 peak of the diploid species of *Pisum sativum* L. (1C = 4.54 pg DNA content) and *Zea mays* L. (1C = 2.73 pg) was used as an internal standard. Seeds of *P. sativum* and *Z. mays* were obtained from the Institute of Experimental Botany, Olomouc, Czech Republic.

Results

Chromosome number variation

Chromosome numbers, vouchers, collection sites, chromosome numbers, genome size and heterochromatic banding patterns are organized in Table 1. The chromosome number of all analyzed species was $2n = 30$ (Figs. 1, 2), registered for five genera in Convolvuloideae (Table 1). New chromosome counts were recorded for five species belonging to two genera, as follows: $2n = 30$ for *Camonea umbellata* (Fig. 1A), *Ipomoea bahiensis* (Fig. 2A), *I. crinicalyx* (Fig. 2C), *I. mucuroides* (Fig. 2E) and *I. quamoclit* (Fig. 2F). A count of $2n = 30$ was also recorded for an undetermined species, *Ipomoea* sp. (Fig. 2G).

Previous records of $2n = 30$ were confirmed for *Distimake dissectus* (Fig. 1C), *D. macrocalyx*, (Fig. 1D), *I. acanthocarpa* (Fig. 1E), *I. asarifolia* (Fig. 1F), *I. bonsai* (Fig. 2B), *I. indica* (Fig. 2D), *Operculina hamiltonii* (Fig. 2H), *O. turpethum* (Fig. 2I) and *Stictocardia tiliifolia* (Fig. 2J).

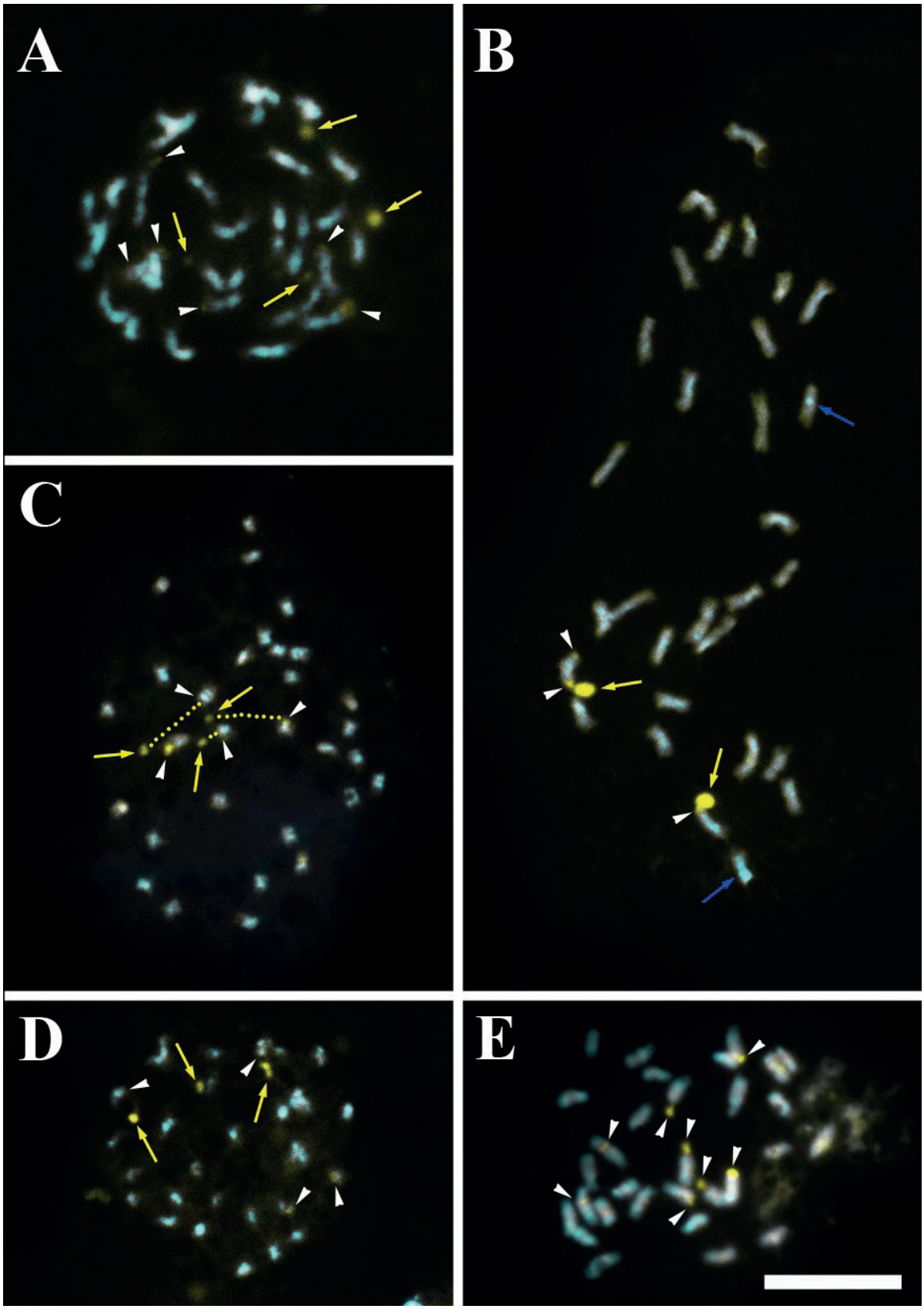

Figure 1. CMA/DAPI banding in diploid species of the clade Convolvuloideae with 2*n* = 30. **A.** *Camonea umbellata*; **B.** *Distimake dissectus*; **C.** *D. macrocalyx*; **D.** *Ipomoea acanthocarpa*; **E.** *I. asarifolia*. White arrowheadss point to CMA^+ bands. Yellow arrows point to satellites. Blue arrows in **B** point to $DAPI^+$ bands. Dotted lines in **C** represent distention of NORs. Bar in **E** is equivalent to 10μm.

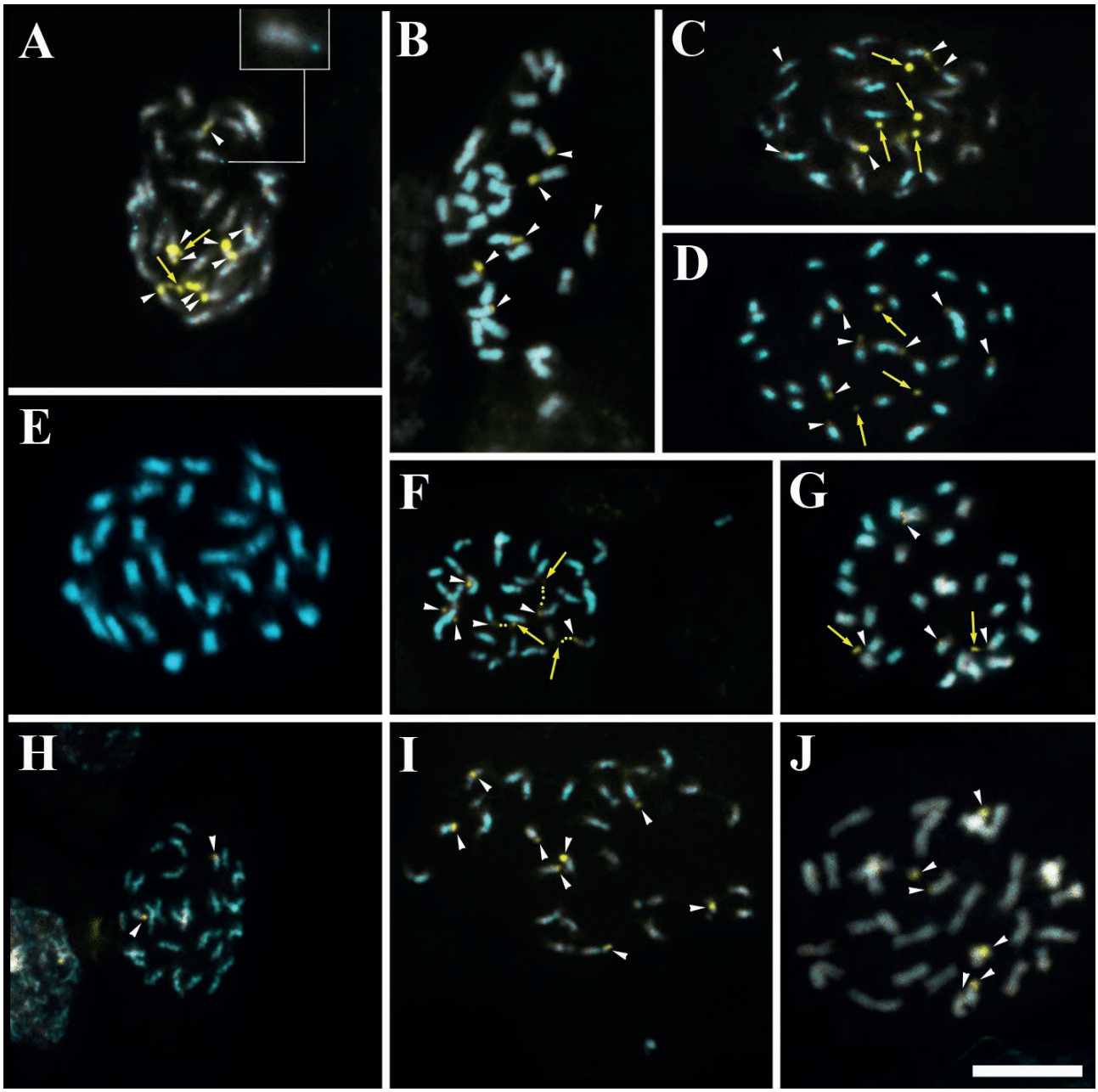

Figure 2. CMA/DAPI banding in diploid species of the clade Convolvuloideae with 2*n* = 30. **A.** *Ipomoea bahiensis*; **B.** *I. bonsai*; **C.** *I. crinicalyx*; **D.** *I. indica*; **E.** *I. mucuroides* (stained with DAPI only); **F.** *I. quamoclit*; **G.** *Ipomoea* sp.; **H.** *Operculina hamiltonii*; **I.** *O. turpethum*; **J.** *Stictocardia tillifolia*. White arrowheadss point to CMA$^+$ bands. Yellow arrows point to satellites. Insert in **A** highlights dotlike DAPI$^+$ terminal bands. Dotted lines in **F** represent distention of NORs. Bar in **J** is equivalent to 10μm.

Reconstruction of the basic chromosome number

The hypothesis of chromosome evolution presented here was based on the phylogenetic tree proposed by Simões *et al.* (2022) (Fig. 3). The best model from ChromEvol, according to AIC, was "Constant rate" (Table 2), which considers three parameters: rate of gain of a chromosome, rate of loss of a chromosome, rate of genome duplication. The basic chromosome number $x = 15$ is suggested for the clade Convolvuloidae. The expectation value (Expectation of events: f =1.38) indicates that polyploidy is the more frequent form of chromosome number change. Our analysis suggests that the clade Convolvuloideae presents a pattern of chromosome evolution based on chromosome number stability, with polyploidy restricted to speciation events within terminal lineages.

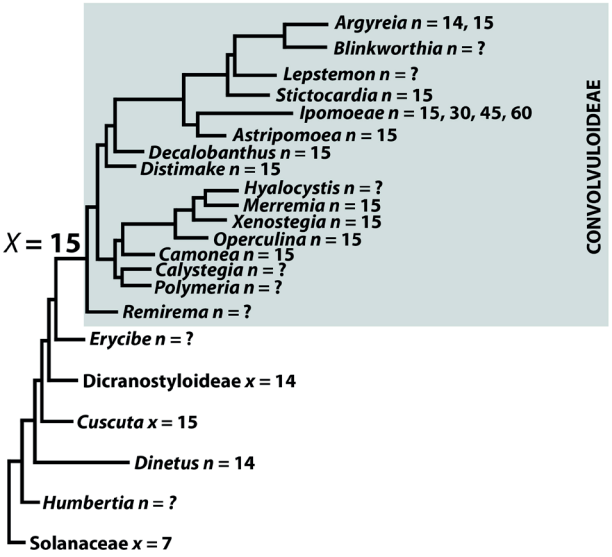

Figure 3. More likely basic chromosome number for Convolvuloideae by maximum likelihood estimated in ChromEvol, based on the phylogeny proposed by Simões *et al.* (2022). Records of haploid chromosome numbers are presented for each clade (including genera).

Table 2. Summary of the eight ChromEvol models for the phylogenetic tree proposed by Simões *et al.* (2022). The Log-likelihood and the AIC values are presented. The best model, Constant Rate, is indicated by the * and this model was re-run under Optimize Model option with 1,000 simulations.

Model	ML	
	Likelihood	AIC
Constant rate	-66.08	138.2*
Constant rate demi-polyploidy	-68.11	142.2
Constant rate demi-polyploidy est	-65.69	139.4
Constant rate no duplication	-73.39	150.8
Linear rate	-65.11	140.2
Linear rate demi-polyploidy	-65.63	141.3
Linear rate demi-polyploidy est	-65.12	142.2
Linear rate no duplication	-67.08	142.2

Genome size

The 1C value was quantified for 12 species and four genera (Table 1). Genome size ranged from 1C = 0.78 pg in *Ipomoea bahiensis* to 1C = 1.38 pg in *Distimake dissectus*, both unpublished. New records of genome size are also presented for the genera *Camonea* and *Stictocardia*: *C. umbellata* with 1C = 0.97 pg and *S. tiliifolia* with 1C = 1.08 pg. Unpublished genome size data were also recorded for *I. asarifolia* with 1C = 1.0 pg, *I. brasiliana* with 1C = 1.23, *I. carnea* with 1C = 1.24 pg, *I. indica* with 1C = 0.84 pg and *I. queirozii* with 1C = 1.18 pg. In addition, genome size was also recorded for the undetermined species *Ipomoea* sp. with 1C = 1.04 pg. The genome size for *I. quamoclit* was 1C = 0.86 pg and for *I. nill* was 1C = 0.87 pg.

Chromosome banding

Two types of heterochromatin were identified among the analyzed species, forming CMA$^+$ and DAPI$^+$ bands, with variation in number and chromosome region (Table 1). All species analyzed exhibited CMA$^+$ bands (Figs. 1 and 2). Only two species, *Distimake dissectus* (Fig. 1B, blue arrows) and *Ipomoea bahiensis* (Fig. 2A, insert), exhibited DAPI$^+$ bands. Some species have chromosomes with terminal and/or pericentromeric regions more strongly stained with DAPI than with CMA, without, however, forming conspicuous bands.

Heterochromatic bands were observed on the following chromosome positions:

(1) CMA$^+$ bands only on terminal chromosome regions: two bands in *Operculina hamiltonii* (Fig. 3H); four bands in *Distimake macrocalyx* (Fig. 1C), *I. acanthocarpa* (Fig. 1D); six bands in *I. bonsai* (Fig. 2B), *I. quamoclit* (Fig. 2F), and *Stictocardia tiliifolia* (Fig. 2J); seven bands in *I. indica* (Fig. 2D); eight bands in *Camonea umbellata* (Fig. 1A);

(2) CMA$^+$ bands on terminal and pericentromeric regions: three terminal and one pericentromeric bands in *Ipomea* sp. (Fig. 2G), four terminal and one pericentromeric band in *I. crinicalyx* (Fig. 2C), six terminal bands and two pericentromeric bands in *I. asarifolia* (Fig. 1E) and *O. turpethum* (Fig. 2I);

(3) CMA$^+$ bands on terminal regions and DAPI$^+$ bands on pericentromeric regions: One species, *D. dissectus*, with three terminal CMA$^+$ bands and two pericentromeric DAPI$^+$ bands (Fig. 1B);

(4) CMA$^+$ and DAPI$^+$ bands on terminal regions, and CMA$^+$ bands on pericentromeric regions: five terminal CMA$^+$ bands, 16 terminal DAPI$^+$ bands, and four pericentromeric CMA$^+$ bands in *I. bahiensis* (Fig. 2A).

Some terminal CMA$^+$ bands, probably corresponding to NORs, form small satellites (Figs. 1 and 2, yellow arrows), more or less detached portions from the chromosomes, in the following species: two in *Distimake dissectus* (Fig. 1B), *I. bahiensis* (Fig. 2A) and *Ipomoea* sp. (Fig. 2G), three in *D. macrocalyx* (Fig. 1C), *I. acanthocarpa* (Fig. 1D), *I. indica* (Fig. 2D) and *I. quamoclit* (Fig. 2F), four in *Camonea umbellata* (Fig. 1A) and *I. crinicalyx* (Fig. 2C).

Discussion

Chromosome number variation

For Convolvuloideae, the most frequent number in the literature is consistently $2n = 30$, occurring in all genera. With a reasonable number of cytologically known species, *Ipomoea* conserves the same chromosome number in most species, occasionally breaking stability with polyploidy and the occurrence of B chromosomes (Yeh & Tsai 1995). Some species have diploid and polyploid cytotypes, such as *Ipomoea cairica*, *I. cordatotrilobai* (both ruderal species), *I. gracilis* and

I. trifida (Darlington & Wylie 1956; Jones 1970; Yen *et al.* 1992; Ozias-Akins & Jarret 1994; Yeh & Tsai 1995; Chiarini 2000), suggesting that these polyploid individuals have autopolyploid origin. The potential advantage of polyploids in natural, managed and disturbed environments under changing climates and new stresses is well known (Heslop-Harrison *et al.* 2023). On the other hand, some species possibly have a polyploid origin, such as *I. batatas*, *I. biflora*, *I. lonchophylla*, *I. racemigera* and *I. saintronanensis* that do not have records of diploid contraparts (Sampathkumar 1968; Yen *et al.* 1992; Ozias-Akins & Jarret 1994).

In different genera, including *Ipomoea*, few records of discrepant chromosome numbers, such as $2n = 28$ or 32-38, are suggestive of dysploidy in Convolvuloideae. Species of *Ipomoea* with $2n = 28$, for example, *I. coptica*, *I. hederifolia*, *I. lobata* (Sampathkumar 1979), *I. rubriflora* (Chiarini 2000), also have records of $2n = 30$ (Ward 1984; Yen *et al.* 1992; Rice *et al.* 2015). However, most are old counts without documentation of metaphases, which makes it difficult to estimate the reliability of that data. Despite the occurrence of disploid numbers in the literature, certain counts may be due to mistaken botanical identification or technical difficulties in counting the chromosomes. There are eight chromosome counts for the genus *Argyreia*, five species with $2n = 30$ and three with $2n = 14$ (Rice *et al.* 2015). Only two species, *A. nervosa* (Burm.) Bojer and *A. wallichii* Choisy, have their taxonomic status confirmed, both with $2n = 30$ and at least one of the species with $2n = 14$ was transferred for *Stictocardia*.

Basic chromosome number

Our reconstruction of the basic chromosome number for Convolvuloideae point to a possible scenario of very consistent chromosome evolution along the diversification of the main lineages in the clade. However, the analysis of the evolutionary process itself, beyond the probability values for a given ancestral chromosome number, must be better constructed from the available evidence in a broader context. Unfortunately, the scarcity of chromosome number data for the family Convolvulaceae as a whole is very high, and the present discussion is limited to just establishing the best hypothesis in line with the currently available data.

Convolvulaceae and Solanaceae are sister families in Solanales, and show similar patterns of chromosome variation, with records of intergeneric dysploid series. The most striking difference between these sister families is the occurrence, in Solanaceae, of lineages of more recent diversification and allopolyploid origin (Bombarely *et al.* 2016; Zhang *et al.* 2022; Chase *et al.* 2023). In Convolvulaceae there is no consistent evidence of genera with polyploid origin but *Aniseia* with $2n = 60$ (Rice *et al.* 2015), a genus with 25 species and only one chromosome count.

Our results propose an ancestor with $x = 7$ for Convolvulaceae, related to the ancestor with x = 7 proposed for Solanaceae (Bombarely *et al.* 2016). After the divergence, a polyploidy event possibly originated the ancestor with $x = 14$ of the main lineages in Convolvulaceae, at the origin of the clade with the genus *Dinetus*, sister group of the large clade that includes the two main lineages of the family designated as Dicranostyloideae and Convolvuloideae (Stefanović *et al.* 2002; Simões *et al.* 2022) (Fig. 3). Recent evidence confirmed two independent polyploidization events in order Solanales, one in Solanaceae and one in Convolvulaceae that occurred 43–49 and 40–46 million years ago, respectively (Zhang *et al.* 2022). The genus *Cuscuta*, despite its uncertain position in the family, has been extensively studied in terms of chromosome numbers, whose basic number $x = 15$ was suggested as the most likely by Ibiapino *et al.* (2022).

Changes in genomes via dysploidy are suggested here also for the origin of Convolvuloideae, starting from $x_1 = 14$ to $x_2 = 15$ at the origin of the clade. It is still speculative to state that dysplody played a predominant role in the origin of Convolvuloideae, as well as to determine that $x = 15$ is the basic number of that clade. Despite these limitations, it has already been demonstrated that there is a diversity of scenarios, represented by lineages at genus level, evidencing different patterns of chromosome variation that appear to be non-random in Convolvulaceae.

Genome size

Convolvuloideae is a little known in terms of genome size, with only two genera with 1C value estimates available in the literature: *Calystegia* (two species: Bai *et al.* 2012) and *Ipomoea* (26 species: Ozias-Akins & Jarret 1994; Bennett *et al.* 1998; Veselý *et al.* 2012; Bou Dagher-Kharrat *et al.* 2013). We expanded these records by including *Camonea*, *Distimake* and *Stictocardia* in our analyses, as well as presenting unpublished genome size data for eight species of *Ipomoea*. Based on these records, including those previously available, Convolvuloideae shows a 3.6-fold variation from 1C = 0.63 pg in *I. quamoclit* (Veselý *et al.* 2012) to 1C = 2.30 pg in polyploids of *I. batatas* (Ozias-Akins & Jarret 1994).

Chromosome banding

Despite the stability of chromosome numbers in Convolvuloideae, especially in the genus *Ipomoea* whose sampling was more representative in the present work, the high variation in number and position of heterochromatin bands is suggestive of structural alterations, at least related to the repetitive component of the genomes. All species analyzed here were diploid and showed CMA^+ terminal bands, sometimes associated with NORs forming satellites, as commonly observed in other groups of plants (Castro *et al.* 2016; Ibiapino *et al.* 2020). Several diploid species have a single pair of CMA^+ bands related to NORs, with a corresponding and regular increase in the number of bands in neopolyploids, as observed in Cactaceae (Castro *et al.* 2016; 2020). After ancestral polyploidy events, especially when a lineage enters a new adaptive zone, genomes can experience complex adjustments that result in variation

of repetitive DNA sequences (Stebbins 1950; Dodsworth *et al.* 2015; Wendel 2015; Escudero & Wendel 2020). The variation observed in the number of CMA^+ terminal bands of the species analyzed here (which vary from 1 to 8) seems to corroborate this hypothesis.

We have shown that the combined analyses of chromosomes numbers, genome size and heterochromatin banding in a phylogenetic context are important to understand the biology and evolution of plant species. It has become increasingly evident that the basic number of the clade Convolvuloideae is consistently $x = 15$, and that plant groups exhibit different strategies of karyotype evolution, some based on maintenance of chromosome numbers, while other groups exhibit series of chromosome number changes. In addition, we demonstrated that plant groups with stable numbers can exhibited other levels of variation, as genome size and heterochromatin band patterns. The high heterochromatin band diversity observed in the clade Convolvuloideae is compatible with the polyploid origin of Convolvulaceae (Zhang *et al.* 2022), despite numerical stability of the clade. Our results suggested that dynamic changes in heterochromatin organization also play a role in shaping karyotypes of Convolvuloideae, and the heterochromatic banding variation may be a promising tool for taxonomic investigations of the clade Convolvuloideae.

Supplementary material

The following online material is available for this article:
Table S1. Species of the family Convolvulaceae with records of haploid (n) and/or diploid (2n) chromosome numbers, organized according to Stefanović *et al.* (2002). Chromosome numbers in bold correspond to taxonomically unresolved species.

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