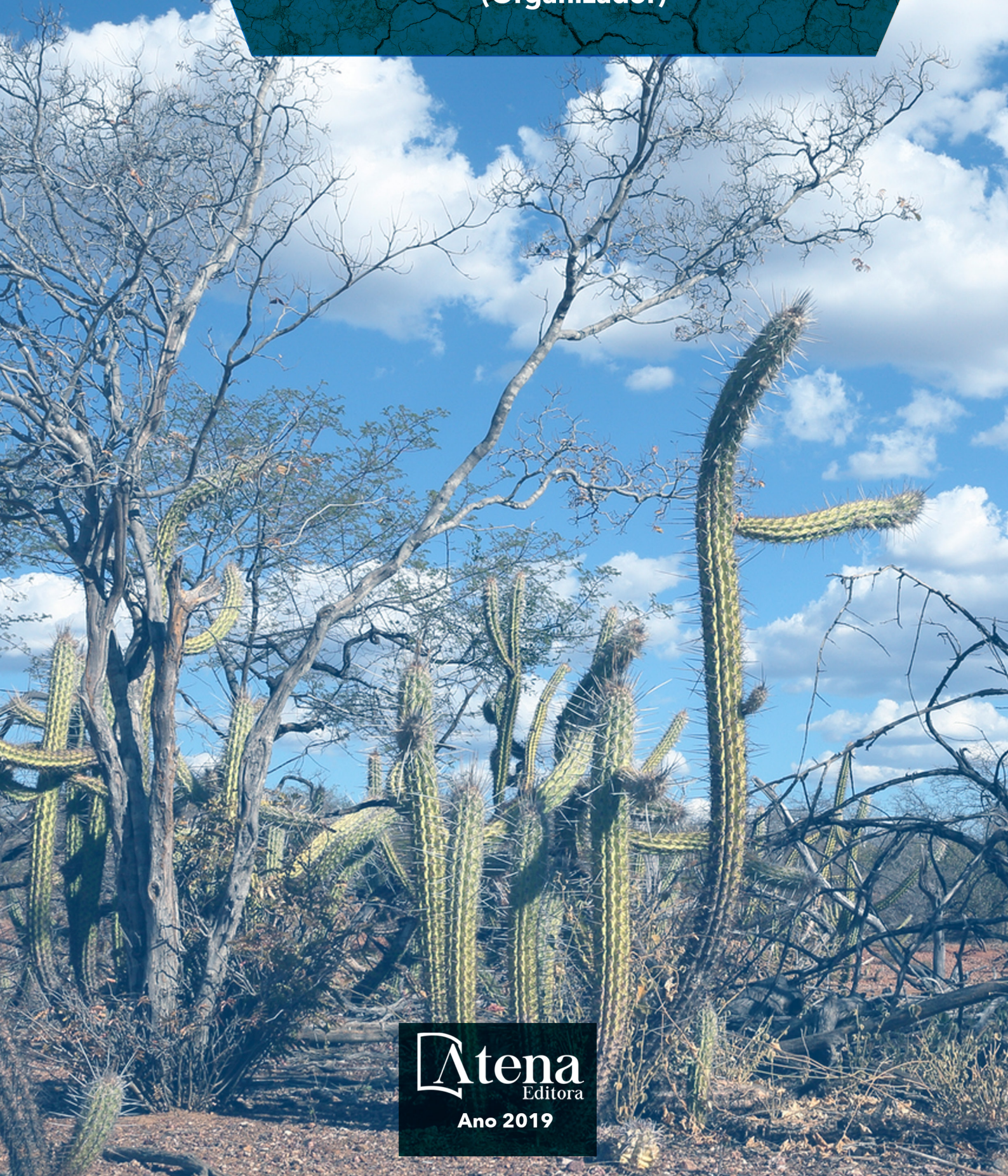


As Regiões Semiáridas e suas Especificidades 2

Alan Mario Zuffo
(Organizador)



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Alan Mario Zuffo
(Organizador)

As Regiões Semiáridas e suas Especificidades 2

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APRESENTAÇÃO

A obra “As Regiões Semiáridas e suas Especificidades” aborda uma série de livros de publicação da Atena Editora, em seu II volume, apresenta, em seus 23 capítulos, com conhecimentos tecnológicos das regiões semiáridas e suas especificidades. As Ciências estão globalizadas, englobam, atualmente, diversos campos em termos de pesquisas tecnológicas. O semiárido brasileiro tem características peculiares, alimentares, culturais, edafoclimáticas, étnicas, entre outros. Tais diversidades culminam no avanço tecnológico, nas áreas de Agronomia, Engenharia Florestal, Engenharia de Pesca, Medicina Veterinária, Zootecnia, Engenharia Agropecuária e Ciências de Alimentos que visam o aumento produtivo e melhorias no manejo e preservação dos recursos naturais, bem como conhecimentos nas áreas de políticas públicas, pedagógicas, entre outros. Esses campos de conhecimento são importantes no âmbito das pesquisas científicas atuais, gerando uma crescente demanda por profissionais atuantes no semiárido brasileiro e, também nas demais regiões brasileiras. Este volume dedicado à diversas áreas de conhecimento trazem artigos alinhados com a região semiárida brasileira e suas especificidades. As transformações tecnológicas dessa região são possíveis devido o aprimoramento constante, com base em novos conhecimentos científicos. Aos autores dos diversos capítulos, pela dedicação e esforços sem limites, que viabilizaram esta obra que retrata os recentes avanços científicos e tecnológicos, os agradecimentos do Organizador e da Atena Editora. Por fim, esperamos que este livro possa colaborar e instigar mais estudantes e pesquisadores na constante busca de novas tecnologias para o semiárido brasileiro, assim, garantir perspectivas de solução para o desenvolvimento local e regional para as futuras gerações de forma sustentável.

Alan Mario Zuffo

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CYTOGENETICS CHARACTERIZATION OF *TACINGA* BRITTON & ROSE (OPUNTIOIDEAE- CACTACEAE)

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ABSTRACT: The *Tacinga* genus includes eight species; seven are endemic to eastern Brazil and only occur in Northeastern of Venezuela. They are shrubs, sub-shrubs and lianas, with segmented or cylindrical cladodes and abundant glochids in their areoles. Their morphological characters have limited utility to distinguish species and to infer about the genetic relationships among them. The present work aimed to characterize six *Tacinga* species at chromosome level using CMA/DAPI fluorochromes. Their chromosome numbers ranged from $2n = 22$ (*T. braunii*,

T. funalis and *T. palmadora*) to $2n = 66$ (*T. wernerii*), with relatively symmetrical karyotypes, predominantly metacentric chromosomes, and average chromosome size of $2.68 \mu\text{m}$. The heterochromatic band pattern was characterized by the presence of at least one chromosome pair with conspicuous CMA⁺ terminal bands, usually linked and distended, with heteromorphisms on CMA⁺ interstitial bands observed in *T. funalis* and *T. inamoena*. The autopolyploidy and action of the transposable elements are mechanisms that could explain chromosomal evolution in *Tacinga*. On the other hand, the morphological diversity among disjunct populations of *T. inamoena* should be a result of its interspecific crosses. Considering this assumption, *T. inamoena* seems to be an excellent material to study polyploid complexes in which tetraploid species are often hybrids.

KEYWORDS: Chromosome banding, autopolyploidy, transposable elements and Opuntioideae.

1 | INTRODUCTION

Tacinga comprises eight species, which seven of them are endemic to eastern Brazil (ZAPPI et al., 2018), and only one, *T. lilae* Trujillo & Marisela Ponce, is endemic to Northeastern of Venezuela (MAJURE & PUENTE, 2014). They

are shrubs, sub-shrubs and lianas, with generally segmented or cylindrical cladodes, with abundant glochids in their areoles, globose or elongated fruits with glochids and few seeds (TAYLOR & ZAPPI, 2004). Representatives of this genus are common in the Brazilian semiarid physiognomy, with a prominent role in the ecology and sustainability of these ecosystems (NOBEL & BOBICH, 2002). They are important sources of food and water for several animals and contribute to soil formation on inselbergs that allowed the establishment of several other plants (TAYLOR & ZAPPI, 2004). Some species as *T. braunii* Esteves, *T. subcylindrica* M.Machado & N.P.Taylor and *T. weneri* (Eggl) N.P.Taylor & Stuppy are threatened with extinction (TAYLOR & ZAPPI, 2004; ZAPPI et al., 2018).

Due to the high degree of relationship among its most recently derived representatives, some species of this genus (*T. palmadora* vs *T. weneri*; *T. inamoena* vs *T. saxatilis*; and *T. funalis* vs *T. braunii*) are morphologically very similar and almost indistinct in their vegetative states (LAMBERT, 2009). Cytologically, *Tacinga* is poorly studied, with only chromosome counts for *T. funalis* and *T. palmadora*, with $2n = 22$ (Castro et al., 2013; 2016), *T. inamoena*, *T. saxatilis* with $2n = 44$, and *T. lilae* with $2n = 66$ (MAJURE et al., 2012; MAJURE & RIBBENS, 2012; MAJURE et al., 2014). This work aimed to do the chromosome characterization of Brazilian *Tacinga* species using CMA/DAPI banding for better understanding the karyotype evolution of this group.

2 | MATERIALS AND METHODS

Taxonomic sampling

The specimens of *Tacinga* (six species) were sampled throughout *Brazilian semiarid* region (Table 1) and cultivated in the Cactário Guimarães Duque of the Instituto Nacional do Semiárido (INSA). Vouchers were deposited in the herbarium EAN (acronyms according to Thiers, 2018) and scientific names according to Taylor & Zappi (2004).

Cytological procedures

Chromosome preparation

For chromosome analyses, root tips were pre-treated with 0.002 M 8-hydroxyquinoline at 4 °C for 20 hours, fixed in ethanol/glacial acetic acid 3:1 (v/v) for two hours at room temperature and stored at -20 °C for 24 hours in the same fixative. The root tips were digested in an enzyme mixture with 2% cellulase (Onozuka)/20% pectinase (Sigma) for 30 min at 37 °C. The meristem was dissected in acetic acid 45 % and squashed under cover slip. The cover slip was removed by freezing in liquid

nitrogen and the slides were air-dried and aged for three days.

Chromosome banding

The aged slides were stained with 0.1 mg mL⁻¹ chromomycine A3 (CMA) for 60 min and with 1 µg mL⁻¹ 4'-6-diamino-2-phenylindol (DAPI) for 30 min at room temperature. Slides were then mounted in McIlvaine (pH 7.0) buffer-glycerol (v/v, 1:1) containing 2.5 mM MgCl₂, and aged for at least three days for fluorochrome stabilization. The best slides were analyzed in a Zeiss® Axio Scope. A1 microscope a coplated with Axio Cam MRC5® camera. The Best metaphases were photographed using Axio vision® 4.8 software. All methaphases were homogeneously edited for brightness and contrast using Adobe Photoshop CS3® v. 10 (Adobe).

Karyotype measures

The chromosome measurements were performed using ImageJ® software (<http://wsr.imagej.net/distros/win/ij152-win-java8.zip>). Here, we followed the karyotype nomenclature of Guerra (1988) based on arms ratio (r), obtained by the long arm (q) compared with short arm (p); *i.e.*, $r = q/p$; and by the centromeric index (ci): $ci = p \times 100 / p + q$.

3 | RESULTS

In *Tacinga* the chromosome numbers ranged from $2n = 22$ (*T. braunii*, *T. funalis* and *T. palmadora*) to $2n = 66$ (*T. wernerii*), with relatively symmetrical karyotypes, predominantly metacentric chromosomes, and average chromosome size of 2.68 µm (Table 1). The heterochromatic banding pattern was characterized by the presence of at least one chromosome pair with conspicuous CMA⁺ terminal bands, which are often linked and distended (Fig. 1 a-h), and CMA⁺ interstitial heteromorphic bands observed in *T. funalis* (Fig. 1a) and *T. inamoena* (Fig. 1h).

Tacinga funalis has chromosome sizes ranging from 1.18 - 1.95 µm, only with metacentric chromosomes (22M), which three chromosomes presented CMA⁺ terminal bands and a single chromosome with CMA⁺ pericentromeric band. *Tacinga braunii* presented chromosomes ranging from 1.63 – 3.03 µm, a karyotype consisting mainly of metacentric chromosomes (20M + 2SM), with two CMA⁺ terminal bands. The chromosomes of *T. palmadora* ranged from 0.80 - 1.11 µm, with karyotypic formula consisting of 22 metacentric chromosomes, and presented a chromosome pair with conspicuous CMA⁺ terminal band and two interstitial ones. Chromosomes of *T. wernerii* ranged from 1.20 - 3.61 µm; its karyotypic formula consisted of 52M + 14SM, with two CMA⁺ terminal bands and two interstitial bands. *Tacinga subcylindrica* has chromosome

sizes ranging from 1.20 – 3,40 μm , consisting mainly of metacentric chromosomes (42M + 2SM), and two chromosome pair carrying CMA⁺ terminal bands, probably indicating nucleolus organizer regions (NORs) (Fig. 1e).

Although all analyzed populations of *T. inamoena* presented $2n = 44$, they differed in the karyotypic formula and patterns of heterochromatic bands (Figs. 1f-h): *cytotype* 1, collected in Algodão de Jandaira, Paraíba, presented chromosomes ranging from 0.54 - 1.35 μm , predominantly with metacentric chromosomes (40M + 4SM), and four CMA⁺ terminal bands (Fig. 2f); chromosomes of *cytotype* 2 from Morro do Chapéu, Bahia, ranged from 1.51 - 2.64 μm , with karyotypic formula of 32M + 12SM and only one chromosome pair with CMA⁺ terminal bands (Fig 1g); the *cytotype* 3, collected in both Brejo da Madre de Deus and Ourolândia, Pernambuco and Bahia states, respectively, presented chromosomes ranging from 1.92 – 3.34 μm , karyotypic formula of 44M, three chromosomes with CMA⁺ terminal bands and one single chromosome with a CMA⁺ interstitial band (Fig. 1h).

4 | DISCUSSION

Tacinga exhibits clear morphological affinities among its taxa, poor diversity in terms of number of species (8 ssp.), stability in chromosome numbers and presence of polyploid representatives ($2n = 4x = 44$ and $2n = 6x = 66$). From seven endemic species to the Brazilian semiarid, three were previously cytological analyzed, *T. funalis*, *T. palmadora* and *T. inamoena* (MAJURE et al., 2012; MAJURE & RIBBENS, 2012; MAJURE et al., 2014; CASTRO et al., 2016), where the present analysis confirmed the chromosome numbers to those species. In contrast, the patterns of heterochromatic bands for *T. palmadora* and *T. inamoena* observed in this study diverged from those reported by Castro et al. (2016). In addition, new chromosome counts were recorded in this study for *T. braunii* ($2n = 22$), *T. subcylindrica* ($2n = 44$) and *T. weneri* ($2n = 66$) (see Table 1). From a cytogenetic point of view, this group of plants may further be characterized by the presence of at least one chromosome pair with CMA⁺ terminal bands, often linked and distended. Interestingly, in both diploid and tetraploid individuals, was observed heteromorphisms on CMA⁺ bands (Figs 1a and 1h), revealing the dynamic nature of *Tacinga* genome under semiarid conditions. The heteromorphisms of GC-rich bands may be the result of transposable elements, described as being a type of repetitive DNA sequence, widely distributed in the plant genome, which seems to favor the rapid adaptation of the species due to changes in environmental conditions (HESLOP-HARRISON & SCHWARACHER, 2011; JOLY-LOPEZ & BUREAU, 2014).

The analysis of these data suggests that duplication of *Tacinga* genome occurred by autopolyploidization, probably associated with the action of transposable elements, and after several generations allowed the establishment and diversification of the polyploid lineages in the genus. The autopolyploidy seems to be more common in

plants than expected (PARISOD et al., 2010) and it has been reported in Cactaceae (HASSAN DAR & REHMAN, 2017, MAJURE et al., 2014), particularly in the Opuntiae tribe (MAJURE et al., 2012). Recent studies revealed that autopoliploids after a long time of genome duplication achieve greater genomic flexibility for tolerance and adaptation to the most diverse habitats in relation to their diploid relatives (HASSAN DAR & REHMAN, 2017; VAN DE PEER et al., 2017; ALIX et al., 2017; SOLTIS et al., 2014; PARISOD et al., 2010), especially when associated with genetic and epigenetic changes (SCHIFINO-WITTMANN, 2004).

T. inamoena seems to be an ecologically versatile and morphologically variable taxon, based on its heterozygosity in terminal and interstitial CMA⁺ bands and on the three distinct cytotypes observed. The accumulation of genetic variability should generate these distinct cytotypes or chromosome races that allowed its wide distribution and great phenotypic plasticity (MACHADO & TAYLOR, 2002). Other possible explanation is this variability came from interspecific crosses between *T. inamoena* and the other species of the group (TERAN & LOAYZA, 2008), due to their close interspecific phylogenetic relationship (MAJURE & PUENTE, 2014), generating natural hybrids. As an example, *Tacinga* × *quipa* (F.A.C.Weber) N.P.Taylor&Stuppy, a natural hybrid between *T. inamoena* and *T. palmadora* that sporadically occurs in sympatry with its parental species (TAYLOR et al., 2002; TAYLOR et al., 2004). In addition, the existence of putative hybrids between *T. inamoena* and other species of the genus has been frequently suggested in the literature (TAYLOR et al., 2002; LAMBERT, 2006; TERAN & LOAYZA, 2008), in which the tetraploid species of investigated polyploid complexes are more often putative hybrids. Although studies of species complexes are rare in Cactaceae, *T. inamoena* seems to be an excellent material for this type of investigation, considering its distinct morphological types found with sympatric distribution (TAYLOR et al., 2002; LAMBERT, 2009) and apparently with no intermediate morphotypes. Therefore, studies of meiotic behavior, reproductive biology and phytogeography should be conducted to better understand the evolution and diversification of *Tacinga*.

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Taxa	Voucher	Provenance*	2n	Karyotypic formula	Variation of chromosome size (µm)	Banding**	Previous counts***
						CMA+/DAPI-	
<i>Tacinga funalis</i> Britton & Rose	EMA 1764	Jacobina, BA	22	22M	1,18 – 1,95	3t+1i	<u>22</u> (C13; PW)
<i>T. braunii</i> Esteves	EMA 2002	Itinga, MG	22	20M + 2SM	1,63 – 3,03	2t	<u>22</u> (PW)
<i>T. palmadora</i> (Britton & Rose) N.P.Taylor & Stuppy	EMA 1589	Algodão de Jandaíra, PB	22	22M	0,80 – 1,11	2t+2i	<u>22</u> (C16; PW)
<i>T. wernerii</i> (Eggl) N. P.Taylor & Stuppy	EMA 1779	Morro do Chapéu, BA	66	52M + 14SM	1,20 – 3,61	2t+2i	<u>66</u> (PW)
<i>Tacinga subcylindrica</i> (M.Machado & N.P.Taylor)	EMA 2504	Jataúba, PE	44	42M + 2SM	1,20 – 3,40	4t	<u>44</u> (PW)
M.Machado & N.P.Taylor <i>T. inamoena</i> (K.Schum.) N.P.Taylor & Stuppy							
Cytotype 1	EMA 1602	Algodão de Jandaíra, PB	44	40M + 4SM	0,54 – 1,35	4t	<u>44</u> (M12; MR12; C13; PW)
Cytotype 2	EMA 1753	Morro do Chapéu, BA	44	32M + 12SM	1,49 – 2,67	2t	<u>44</u> (M12; MR12; C13; PW)
	EMA 1828	Morro do Chapéu, BA	44	12SM + 32M	1,53 – 2,60	2t	<u>44</u> (M12; MR12; C13; PW)
Cytotype 3	EMA 1645	Brejo da Madre de Deus, PE	44	44M	1,91 – 3,37	3t+1i	<u>44</u> (M12; MR12; C13; PW)
	EMA 1781	Ourolândia, BA	44	44M	1,94 – 3,31	3t+1i	<u>44</u> (M12; MR12; C13; PW)

Table 1: List of analyzed species of *Tacinga*, voucher, locality, cytogenetic data e previous counts.

*Abbreviation of the Brazilian States: PB = Paraíba; PE = Pernambuco; BA = Bahia; MG = Minas Gerais. ** i = interstitial; t = terminal. *** Underlined numbers refer to the chromosome number (2n) with previous records for those numbers in parentheses. Abbreviation of previous counts: Castro, et al., 2016; C13 = Castro et al., 2013; M12 = Majure et al., 2012; MR12 = Majure and Ribbens, 2012; PW = Present Work.

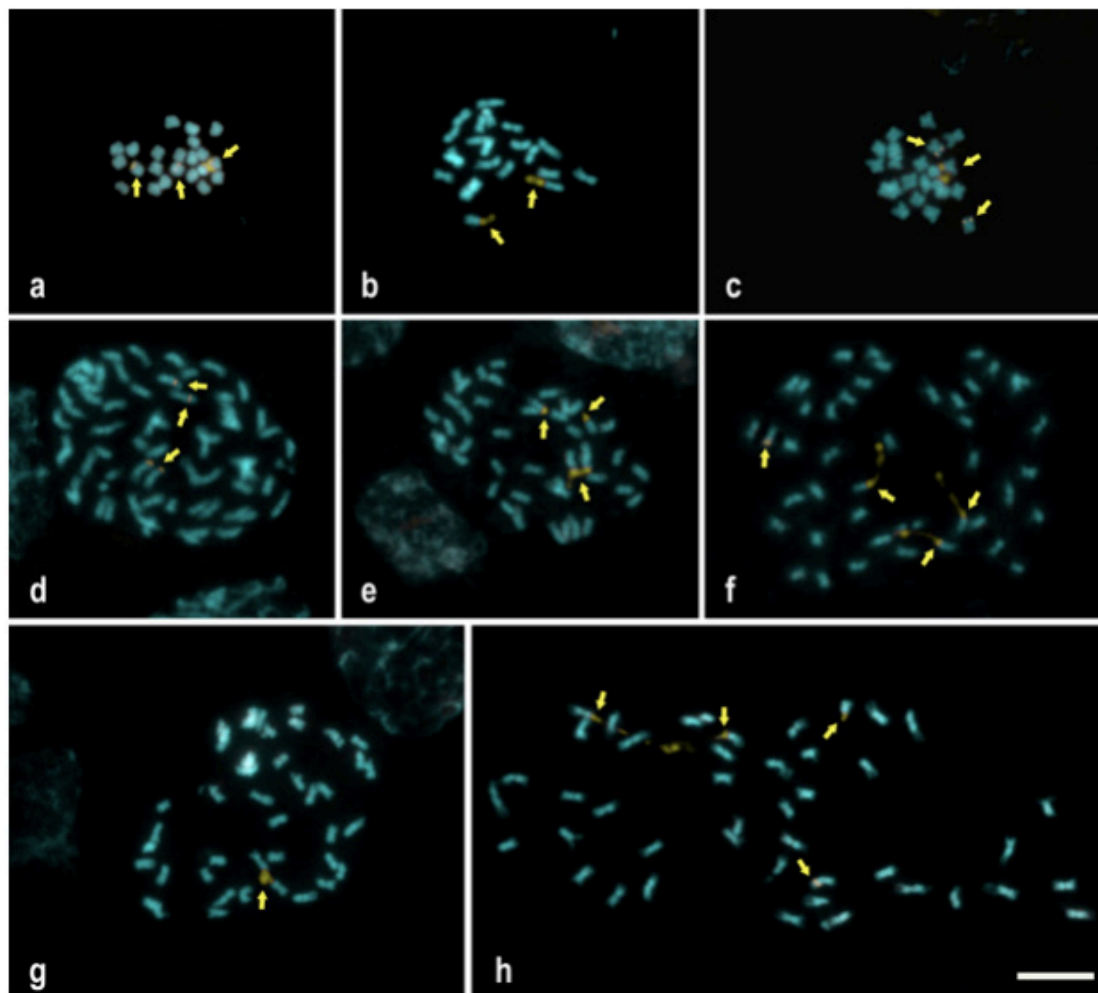


Figure 1: Chromosome metaphases stained with CMA/DAPI showing CMA bands (yellow) of some species of *Tacinga*. a. *T. funalis* with $2n = 22$ (22M); b. *T. braunii* with $2n = 22$ (2SM + 20M); c. *T. palmadora* ($2n = 22$, 22M). d. *T. wernerii* with $2n = 66$ (14SM + 52M); e. *T. subcylindrica* (44M); f-h. *Tacinga inamoena* with $2n = 44$: f. Cytotype 1, with 4SM + 40M collected in Algodão de Jandaira, PB; g. Cytotype 2, with 12SM + 32M collected in Morro do Chapéu, BA. h. Cytotype 3, with 44M collected in Brejo da Madre de Deus, PE. Arrows indicate CMA⁺ bands. Bar in “h” corresponds to 10 μm .

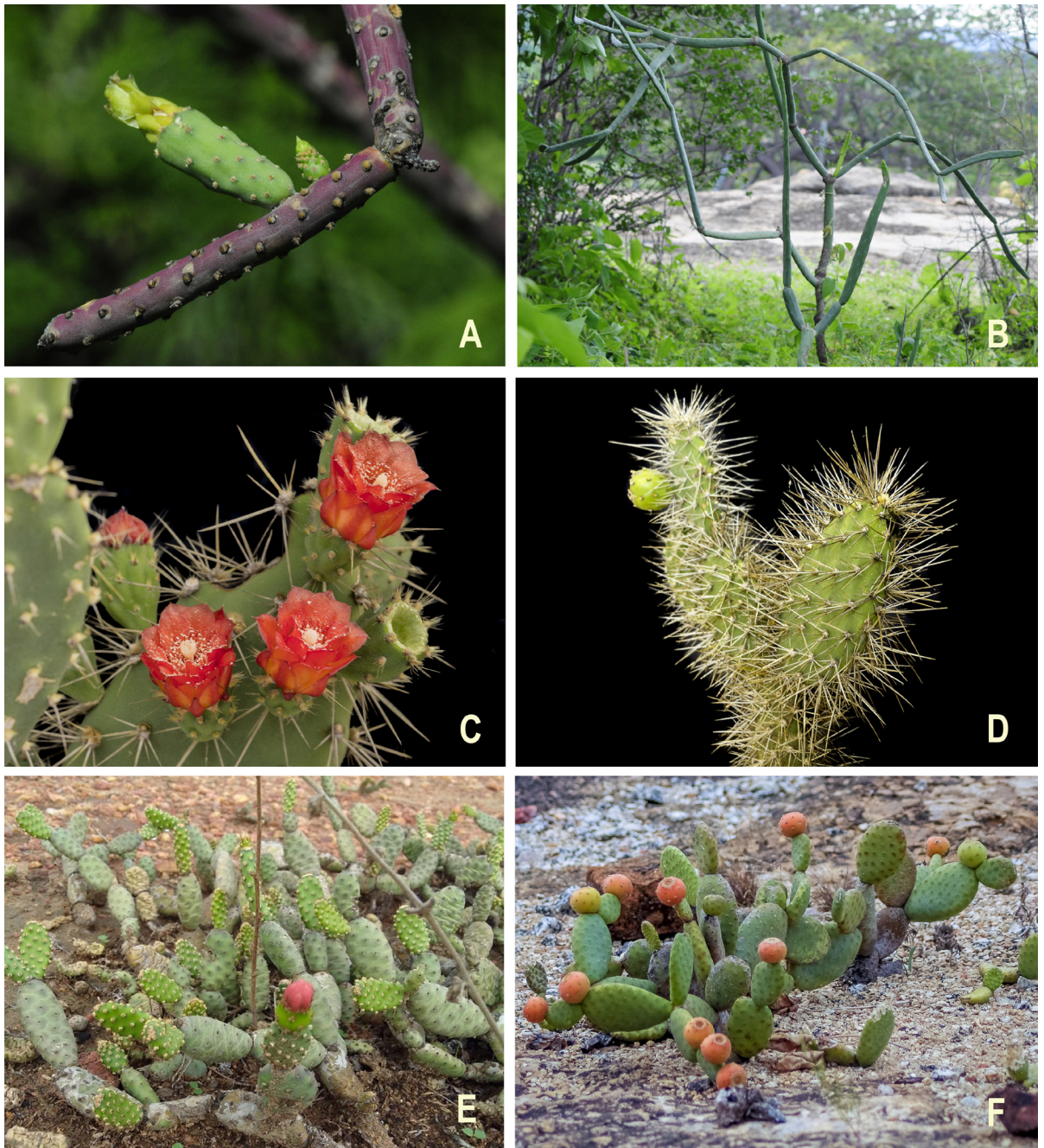


Figure 2. Selection of taxa analyzed: **A.** *Tacinga funalis* Britton & Rose (EMA 1764), **B.** *T. braunii* Esteves (EMA 2002), **C.** *T. palmadora* (Britton & Rose) N.P.Taylor & Stuppy (EMA 1589), **D.** *T. wernerii* (Eggl) N. P.Taylor & Stuppy (EMA 1779), **E.** *Tacinga subcylindrica* (Machado & N.P.Taylor) M.Machado & N.P.Taylor (EMA 2504), **F.** *T. inamoena* (K.Schum.) N.P.Taylor & Stuppy (EMA 1645). Photographs: E.M. Almeida, J. A. L. Neves and F. Lavorato.