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Genome size evolution and chromosome numbers of species of the cryptanthoid complex (Bromelioideae, Bromeliaceae) in a phylogenetic framework

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We describe the chromosome numbers and genome sizes of species of the cryptanthoid complex of Bromeliaceae in a phylogenetic framework and their relationship with habitat preferences. The 2C DNA contents varied 2.13-fold among species, ranging from 0.76 to 1.66 pg. A significant difference in DNA content was found among *Cryptanthus*, *Hoplocryptanthus* and *Rokautskyia*. Moreover, species from campos rupestres and the Atlantic Forest had lower and higher genome size values, respectively. The smaller genome sizes of *Hoplocryptanthus* spp. from campos rupestres may be related with the large genome constraint. The species show a highly conserved ploidy (with $2n = 32$ and 34), although the genome sizes varied considerably. The observed variation in chromosome numbers seems to be influenced by dysploidy, but additional investigations are needed. Our study demonstrates that the genome size variation in the cryptanthoid complex species is not strictly related to the phylogenetic relationships and has probably been influenced by different evolutionary processes.

KEYWORDS: ancestral character states – Atlantic Forest – campos rupestres – dysploidy – flow cytometry – genome constraint.

INTRODUCTION

Cryptanthus Otto & A.Dietr. is a genus of Bromeliaceae endemic to Brazil and is part of the cryptanthoid complex (subfamily Bromelioideae) (Leme *et al.*, 2017). Initially, *Cryptanthus* was divided into two subgenera: *Cryptanthus* subgenus *Cryptanthus*, comprising andromonoecious plants, and *Cryptanthus* subgenus *Hoplocryptanthus* Mez, comprising monoecious species (Mez, 1896;Ramírez-Morillo, 1996, 1998). In a

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phylogenetic study of the sister genus *Orthophytum* Beer (Louzada *et al.*, 2014), the few sampled *Cryptanthus* spp. were not recovered as a monophyletic group, although the segregation of the species of the genus in different clusters did not receive statistical support. In a more comprehensive phylogenetic analysis based on DNA data for *Cryptanthus*, Cruz *et al.* (2017) clarified the infrageneric relationships, suggesting that subgenus *Hoplocryptanthus* is paraphyletic, and Leme *et al.* (2017), based on morphological features and molecular data, proposed the recognition of subgenus *Cryptanthus* as *Cryptanthus s.s.* and divided subgenus *Hoplocryptanthus* into three new genera, *Hoplocryptanthus* (Mez) Leme, S.Heller & Zizka, *Rokautskyia* Leme, S.Heller & Zizka and *Forzzaea* Leme, S.Heller & Zizka. With *Lapanthus* Louzada & Versieux, *Orthophytum* and *Sincoraea* Ule, Leme *et al.* (2017) referred to these genera as the cryptanthoid complex.

Species of the cryptanthoid complex have, in general, a narrow geographical distribution in several different habitats. Most of the species are endemic to the Atlantic Forest domain, but some species also occur in xeric environments, e.g. *Cryptanthus bahianus* L.B.Smith., that occurs in Caatinga, and several other species that occur on campos rupestres (rocky outcrops) (Leme *et al.*, 2017). Given habitat loss due to anthropogenic activities and the narrow endemism, some of these species are already considered threatened (Martinelli *et al.*, 2008; Versieux *et al.*, 2008).

Cryptanthus has autapomorphic features such as the tendency to andromonoecy (Ramírez-Morillo, 1996, 1998; Ramírez-Morillo & Brown, 2001). Moreover, *Cryptanthus* and *Hoplocryptanthus* exhibit unusual chromosome numbers ($2n$ = 32, 34 and 36), with x = 17 considered as the base number, different from the base number proposed for the remaining lineages of the family, x = 25 (Gitaí *et al.*, 2014). Based on karyological analyses and genome size estimates, it is accepted that dysploidy better explains this basic chromosome number, considering that *Cryptanthus* spp. ($2n$ = 32 and 34) do not have significantly different genome sizes than other bromeliads (Gitaí *et al.*, 2014). Another remarkable but not unique cytological feature is the presence of B chromosomes in *C. bahianus* and *C. praetextus* É.Morren & Baker, which have also been observed in other bromeliads, such as *Bromelia karatas* L. and *Hohenbergia* aff. *utriculosa* Ule (Cotias-de-Oliveira *et al.*, 2000; Gitaí *et al.*, 2014).

The restricted occurrence of many species, habitat degradation and unique karyological features indicate the potential to address a wide range of questions on the evolutionary patterns of species of the cryptanthoid complex. Despite this, these genera (especially *Cryptanthus* and *Hoplocryptanthus*) have been considerably overlooked in studies on the evolution of groups of bromeliad, with just a few previous studies focusing on phylogenetic relationships and basic cytogenetic characters (Ramírez-Morillo, 1998; Ramírez-Morillo & Brown, 2001; Gitaí *et al.*, 2014; Cruz *et al.*, 2017). Therefore, genome size estimation is an important approach that can provide additional information on the karyotype evolution of the group (Pellicer *et al.*, 2018).

Variation in genome size of angiosperms is *c.* 2440-fold, which is mainly related to polyploidy (or whole-genome duplications) and deviation in copy numbers of repetitive DNA clusters (Dodsworth, Leitch & Leitch, 2015; Pellicer *et al.*, 2018). Most of the genomic DNA of land plants is composed of non-coding repetitive sequences (e.g. satellite DNA and transposable elements; Dodsworth *et al.*, 2015) and, whenever recent polyploidy events are absent, changes in such DNA portions can largely contribute to either downsizing or upsizing of genomes, making these changes one of the main evolutionary forces to drive genome size variation among related species (Cavalier-Smith, 2005; Heslop-Harrison, 2012; Pellicer *et al.*, 2018). Furthermore, alterations at the chromosome level usually cause sudden and marked changes in genome size, whereas molecular mechanisms are more gradual, producing only slight modifications (Dušková *et al.*, 2010).

Differences in genome sizes, and thus the actual amount of nuclear DNA, may have an impact on biological aspects of the plants, e.g. limiting those with larger genomes from develop specific traits or capabilities and influencing their presence or the absence in certain types of niche (Knight, Molinari & Petrov, 2005; Francis, Davies & Barlow, 2008; Suda *et al.*, 2015). Genome size variation can also be related to the rates of molecular evolution or even speciation in plant species (Pellicer *et al.*, 2018). The biological and the evolutionary meaning of the genome size variation are better understood when also considered the phylogenetic relationships, which are fundamental data to explain the genome size evolution outweighing any correlation with ecogeographic variables (Loureiro *et al.*, 2010). Therefore, when searching for the causes of variation in genome size, adaptive and non-adaptive (e.g. hybridization) components need to be considered (Dušková *et al.*, 2010).

In Bromeliaceae, there have been several investigations to estimate genome sizes (e.g. Ramírez-Morillo & Brown, 2001; Zonneveld, Leitch & Bennett, 2004; Leitch *et al.*, 2010; Moura, Forzza & Cristiano, 2018, Müller, Zotz & Albach, 2019). Some of those studies included *Cryptanthus* spp. (Favoreto *et al.*, 2012; Gitaí *et al.*, 2014), although the available data do not sufficiently cover the taxonomic diversity of this group.

Table 1. Studied material with voucher numbers, genome size, CV values and chromosome number of some species. Abbreviations: AF, Atlantic Forest; CA, Caatinga; CR, campos rupestres; HB, Herbarium Bradeanum; IBt, Instituto de Botânica; LC, Living collection; RE, restinga; RG, Refúgio dos Gravatás, living collection, in Teresópolis, Rio de Janeiro; SP, Herbarium of Instituto de Botânica. [K]Kew Gardens database (Ramírez-Morillo & Brown, 2001)

Species	Voucher	2C value (pg)	CV value	Habitat type	2*n*
Cryptanthus acaulis Lindl. Beer	*Leme, 3359* (RG)	1.38	2.45	AF	34
Cryptanthus alagoanus Leme & J.A.Siqueira	*Leme, 5380* (RG)	0.78	3.11	AF	-
Cryptanthus arelii H.Luther	*Leme, 2830* (RG)	0.98	2.78	CR	-
Cryptanthus bahianus L.B.Sm.	*SEL 87–382*	0.76K	-	CA	34 + 1-3B
Cryptanthus beuckeri É.Morren	*Leme, 7341* (RG)	1.46	2.54	AF	-
Cryptanthus bromelioides Otto & A.Dietr.	*Leme, 2229* (RG)	1.52	2.87	AF	-
Cryptanthus burle-marxii Leme	*Leme, 6260* (RG)	1.32	2.90	AF	34
Cryptanthus capitellatus Leme & L.Kollmann	*Leme, 7988* (RG)	1.36	2.78	AF	-
Cryptanthus colnagoi Rauh & Leme	*Leme, 5144* (RG)	1.34	2.89	AF	-
Cryptanthus coriaceus Leme	*Leme et al. 1114* (HB)	0.84	2.31	AF	-
Cryptanthus correia-araujoi Leme	*Leme et al. 2704* (HB)	1.66	2.78	AF	34
Cryptanthus delicatus Leme	*Leme, 2236* (RG)	1.46	3.25	AF	-
Cryptanthus dianae Leme	*Leme, 3872* (RG)	1.36	3.12	AF	32 + 1-3 B
Cryptanthus giganteus Leme & A.P.Fontana	*Leme, 6913* (RG)	1.38	2.65	AF	-
Cryptanthus lutherianus I.Ramirez	*Leme, 6956* (RG)	0.90	3.25	AF	34
Cryptanthus lyman-smithii Leme	*Leme, 4344* (RG)	1.38	3.78	AF	-
Cryptanthus marginatus L.B.Sm.	*Leme, 0290* (RG)	1.40	2.74	AF	32
Cryptanthus maritimus L.B.Sm.	*Leme et al. 1582* (HB)	1.48	2.78	AF	-
Cryptanthus pickelii L.B.Sm.	*Leme, 7516* (RG)	1.28	2.64	AF	-
Cryptanthus reisii Leme	*Leme, 5015* (RG)	0.88	2.12	AF	-
Cryptanthus sinuosus L.B.Sm.	*Leme, 2868* (RG)	1.04	2.67	AF	-
Cryptanthus teretifolius Leme	*Leme et al. 3073* (HB)	1.40	2.89	AF	-
Cryptanthus ubairensis I.Ramirez	*Leme, 7788* (RG)	1.00	7.11	AF	-
Cryptanthus venecianus Leme & L.Kollmann	*Leme, 7743* (RG)	1.32	2.78	AF	-
Cryptanthus viridipetalus Leme	*Leme, 8016* (RG)	1.34	2.45	AF	-
Cryptanthus zonatus (Vis.) Beer	*Leme, 6559* (RG)	1.04	2.67	AF	34
Forzzaea leopolodo-horstii Leme, S.Heller & Zizka	*Leme, 8414* (RG)	1.14	2.00	CR	-
Hoplocryptanthus caracensis Leme, S.Heller & Zizka	*Leme, 1853* (RG)	0.78	1.99	CR	-
Hoplocryptanthus ferrarius Leme, S.Heller & Zizka	*Leme, 6540* (RG)	0.82	2.78	CR	34
Hoplocryptanthus glaziovii Leme, S.Heller & Zizka	*Leme, 1856* (RG)	0.80	2.34	CR	-
Hoplocryptanthus regius Leme, S.Heller & Zizka	*Leme et al. 6372* (HB)	0.78	2.84	CR	-
Hoplocryptanthus schwackeanus Leme, S.Heller & Zizka	*Leme, 7205* (RG)	0.82	2.68	CR	-
Hoplocryptanthus tiradentesensis Leme, S.Heller & Zizka	*Leme, 7266* (RG)	0.80	2.56	CR	-
Rokautskyia latifolia Leme, S.Heller & Zizka	*Leme, 5220* (RG)	1.10	2.22	AF	-
Rokautskyia microglazioui Leme, S.Heller & Zizka	*Leme, 0152* (RG)	0.94	2.89	AF	-
Rokautskyia odoratissima Leme, S.Heller & Zizka	*Leme, 5207* (RG)	1.10	2.89	AF	-
Rokautskyia pseudoglasioui Leme, S.Heller & Zizka	*Leme et al. 1560* (HB)	1.00	2.56	AF	32
Rokautskyia pseudoscaposa Leme, S.Heller & Zizka	*Leme, 5211* (RG)	1.04	2.78	AF	-

Table 1. Continued

Species	Voucher	2C value (pg)	CV value	Habitat type	2*n*
Rokautskyia sanctaluciae Leme, S.Heller & Zizka	*Leme, 6699* (RG)	1.00	2.24	AF	-
Rokautskyia scaposa Leme, S.Heller & Zizka	*Leme, 5214* (RG)	1.04	2.67	AF	-
Rokautskyia whitmanii Leme, S.Heller & Zizka	*Leme, 5209* (RG)	1.06	2.32	AF	-
Sincoraea amoena Leme, S.Heller & Zizka	*Louzada, 7106 IBt* (LC)	1.04	2.78	CR	-
Sincoraea heleniceae Leme, S.Heller & Zizka	*Louzada et al. 2544* (SP)	1.08	2.56	CR	-
Sincoraea ophiuroides Leme, S.Heller & Zizka	*Louzada, 88 IBt* (LC)	1.02	2.56	CR	-
Sincoraea ulei Leme, S.Heller & Zizka.	*Louzada, 91 IBt* (LC)	1.50	2.78	CR	-

In the present study, we analysed the chromosome numbers and estimated genome sizes of species in the cryptanthoid complex, interpreting the results in a phylogenetic framework to address the following questions. (1) What is the variation of the genome size (DNA 2C-values) within and among the genera? (2) How does the observed genome size variation correlate with the molecular phylogeny of the groups and chromosome counts? (3) How does the observed genome size evolution correlate with species habitat preferences?

MATERIAL AND METHODS

Plant material and FCM analysis

Forty-five accessions were analysed (Table 1), including 26 *Cryptanthus* spp., six *Hoplocryptanthus* spp., one *Forzzaea* sp., eight *Rokautskyia* spp. and four *Sincoraea* spp. Fresh leaves (*c*. 20–30 mg) from each accession were chopped on ice with an internal reference standard (*Solanum lycopersicum* L. 'Stupické', 2C = 1.96 pg) in 1 mL of buffer (LB01 or $MgSO_4$, depending on the accession) to release the nuclei, according to Doležel, Binarova & Lucretti (1989). The chopped tissues were captured through two layers of cheesecloth using a plastic pipette, filtered through a 50 µm nylon filter and collected into a polystyrene tube. The suspension of nuclei was stained with 25 µL of 1% propidium iodide (w/v) and 5 µL of 20 mg L^{-1} RNase was added to each sample. The samples were stored at 4 °C in the dark and analysed after 1–2 h. At least 10 000 nuclei from three different runs were analysed for each sample using a FACSCalibur cytometer (Becton-Dickinson). Each output flow cytometry (FCM) histogram from Cell Quest software was analysed using WinMDI 2.8 software (Fig. 1).

Phylogenetic analysis and ancestral character state reconstruction

The phylogenetic tree used in the reconstruction of ancestral character states was based on the AFLP data obtained by Cruz *et al.* (2017). For the tree reconstruction, we considered *Hoplocryptanthus* as the earliest-diverging group, according to Leme *et al.* (2017). The maximum likelihood tree with all previously analysed accessions was adjusted by trimming the species without any information on genome size in Mesquite v.3.5 (Maddison & Maddison, 2018).

For the reconstruction of ancestral character states of genome size, the program R v.3.2.1 (http://cran.r-project.org) was used. The phylogenetic signal of genome size was tested with the λ-value, as described by Pagel (1999), using the 'phylosig' optimization of the Phytools package (Revell, 2012). Ancestral genome sizes were calculated using the function 'ace' with the maximum likelihood optimization with the aid of the package Ape (Paradis, Claude & Strimmer, 2004).

Cytogenetic analysis

Ten *Cryptanthus* spp. were collected in Ipojuca (Pernambuco, Brazil, latitude 08° 31' 48" S/longitude 35° 01' 05" W) or from seeds from the scientific living collection of E.M.C. Leme (Refúgio dos Gravatás, Teresópolis, Rio de Janeiro, Brazil) (Table 1).

For slide preparation, root tips were collected and then pre-treated at 18 °C in 2 mM 8-hydroxyquinoline for 4.5 h. The roots were fixated in ethanol:acetic acid (3:1 v/v) at room temperature (*c*. 25 °C) for 24 h and stored at –20 °C. In the preparation of the conventional staining slides (*C. marginatus* L.B.Sm.), fixed root tips were washed in distilled water and hydrolysed in 5M HCl at room temperature for 20 min. The slides were prepared by squashing the meristematic tissue in 45% acetic acid. In addition, the slides were stained in a 2% Giemsa solution for 15 min and assembled with Entellan (Merck). For the other species, fixed root tips were washed in distilled water and digested in an enzymatic solution containing 2% cellulase (Onozuka R-10, Serva) and 20% pectinase (v/v) (Sigma-Aldrich) for 3 h at 37 °C. Slide preparation followed Carvalho & Saraiva (1993) with the modifications introduced by Vasconcelos *et al.* (2010). In addition, the slides

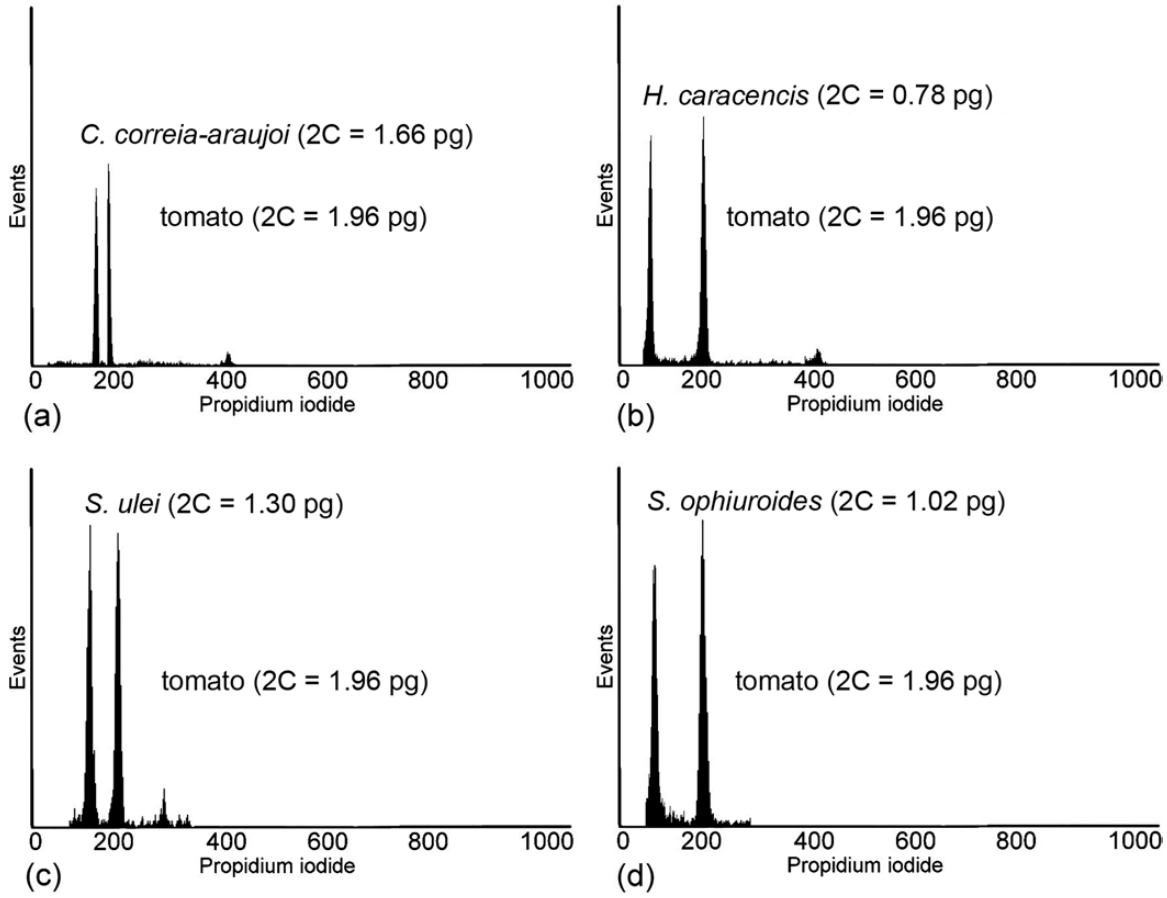

Figure 1. Fluorescence histograms of simultaneous analysis of propidium iodide stained nuclei isolated from fresh tissue of internal standard (*Solanum lycopersicum*) with A and B, species *Cryptanthus correia-araujoi* and *Hoplocryptanthus caracensis* and C and D, *Sincoraea ulei* and *S. ophiuroides*, showing two peaks corresponding to G0/G1 of each species.

were stained in a solution of 2 μg mL^{-1} DAPI (4', 6-diamidino-2-phenylindole)/glycerol (1:1, v/v).

The cells were analysed using a Leica DMLB epifluorescence microscope, and images were captured through a Leica DFC 340FX camera and the software Leica CW 4000. The images were optimized for better brightness and contrast with Adobe Photoshop CS6 (Adobe Systems Incorporated). The relative chromosome size was measured using the MicroMeasure v.3.3 program, with ten metaphases being analysed per species. B chromosomes were classified by the absence of homologues, considering morphology and chromosome measurements.

RESULTS

Here, we compiled genome sizes for 45 out of 152 species (29.6%) of the cryptanthoid complex, of which 40 are reported for the first time (Table 1). The analysis revealed a pronounced variation in nuclear DNA content among species (2.13-fold), ranging from 2C = 0.76 pg (*C. bahianus*) to 2C = 1.66 pg (*C. correia-araujoi* Leme) (Table 1). A continuous variation of the DNA 2C-values was arranged according to the preferred habitat, which was much clearer for *Hoplocryptanthus*, in which the species from campos rupestres had lower DNA contents (2C = 0.70–0.84 pg) than those from the Atlantic Forest (2C = 0.98–1.12 pg). In *Hoplocryptanthus*, we observed smaller genome sizes (2C = 0.80 pg on average) than in other genera including *Rokautskyia* (2C = 1.02 pg on average). On the other hand, most *Cryptanthus* spp. (which occur in the Atlantic Forest except for *C. arelii* H.Luther and *C. bahianus*) had larger genome sizes (2C = 1.52–1.66 pg), although without any clear distribution pattern of DNA content along the clusters (Fig. 2).

The coefficient of variation (CV) of the G0/G1 peak ranged from 1.99 to 7.11 (Table 1). In the vast majority of the estimations (84.6%), the CV value was < 3.0%, and only 1.92% of CV values were > 4.0%. In all cases,

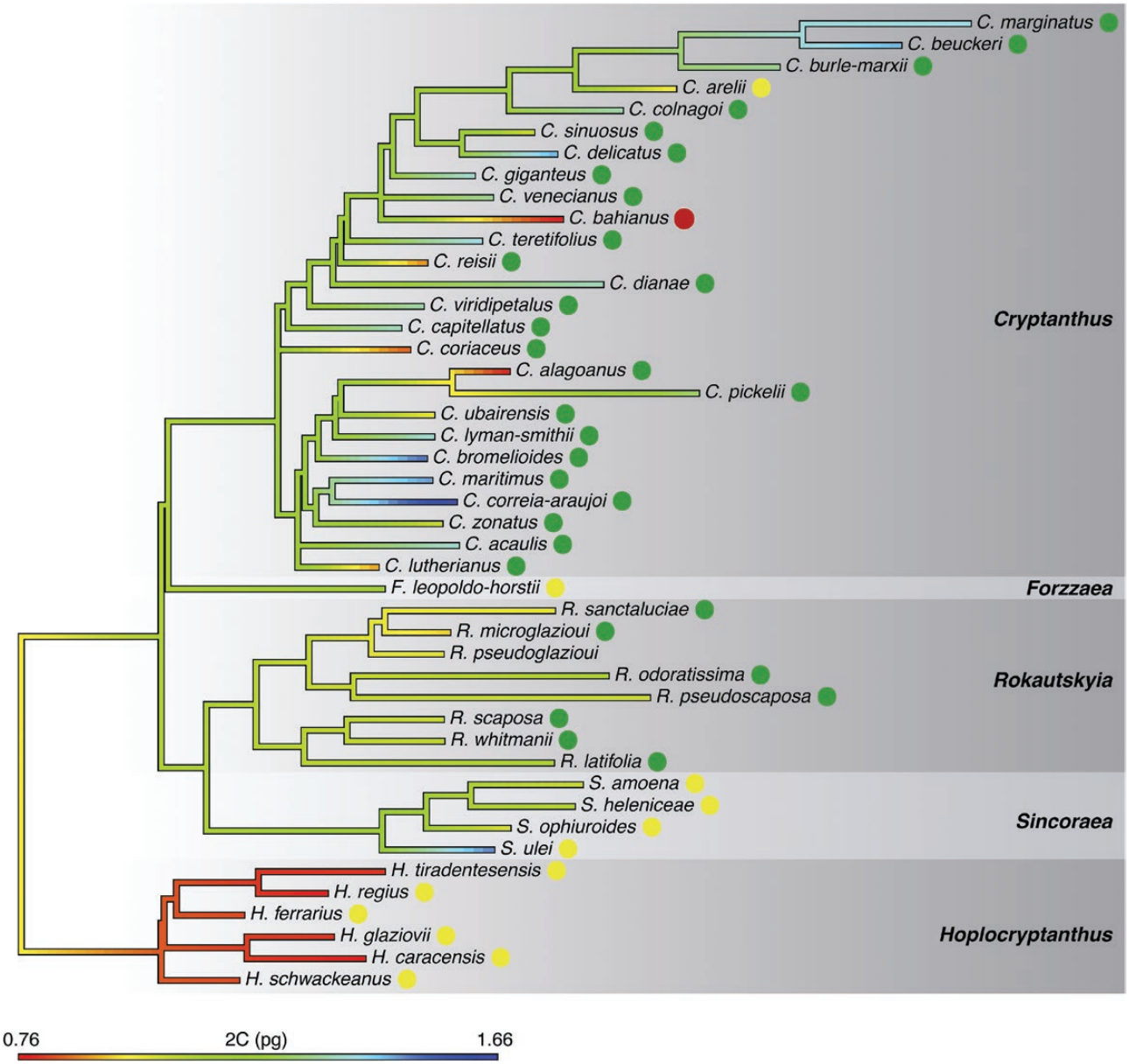

Figure 2. Maximum likelihood reconstruction of the evolution of DNA content (2C) in cryptanthoid complex, based on relationships revealed by AFLP molecular phylogeny (Cruz *et al.*, 2017). The habitat of the species is specified through circles close to the species names: green – Atlantic Forest; yellow – campos rupestres; red – Caatinga. The bar indicates the DNA content (2C) variation.

the DNA 2C-values had standard deviations below 0.05 among different individuals of the same species.

The reconstruction of character states showed an intermediate value of the ancestral genome size for *Cryptanthus* (Fig. 2). There was only a weak to intermediate phylogenetic signal ($\lambda = 0.425$) for the distribution of the genome size on the phylogenetic tree for the genus. This relatively low phylogenetic signal of genome sizes occurred due to the considerable variation and relative randomness in the distribution of the DNA content among *Cryptanthus* spp. (Fig. 2). On the other hand, clusters formed by species of *Hoplocryptanthus, Forzzaea, Rokautskia and Sincoraea* showed a more evident phylogenetic pattern for genome size, in which there was an increase of the DNA content for the last three genera (Fig. 2). Despite the considerable variation in the distribution of the DNA content observed for *Cryptanthus*, in general the clusters formed in this group had a tendency to larger genome sizes than those in the other genera (Fig. 2).

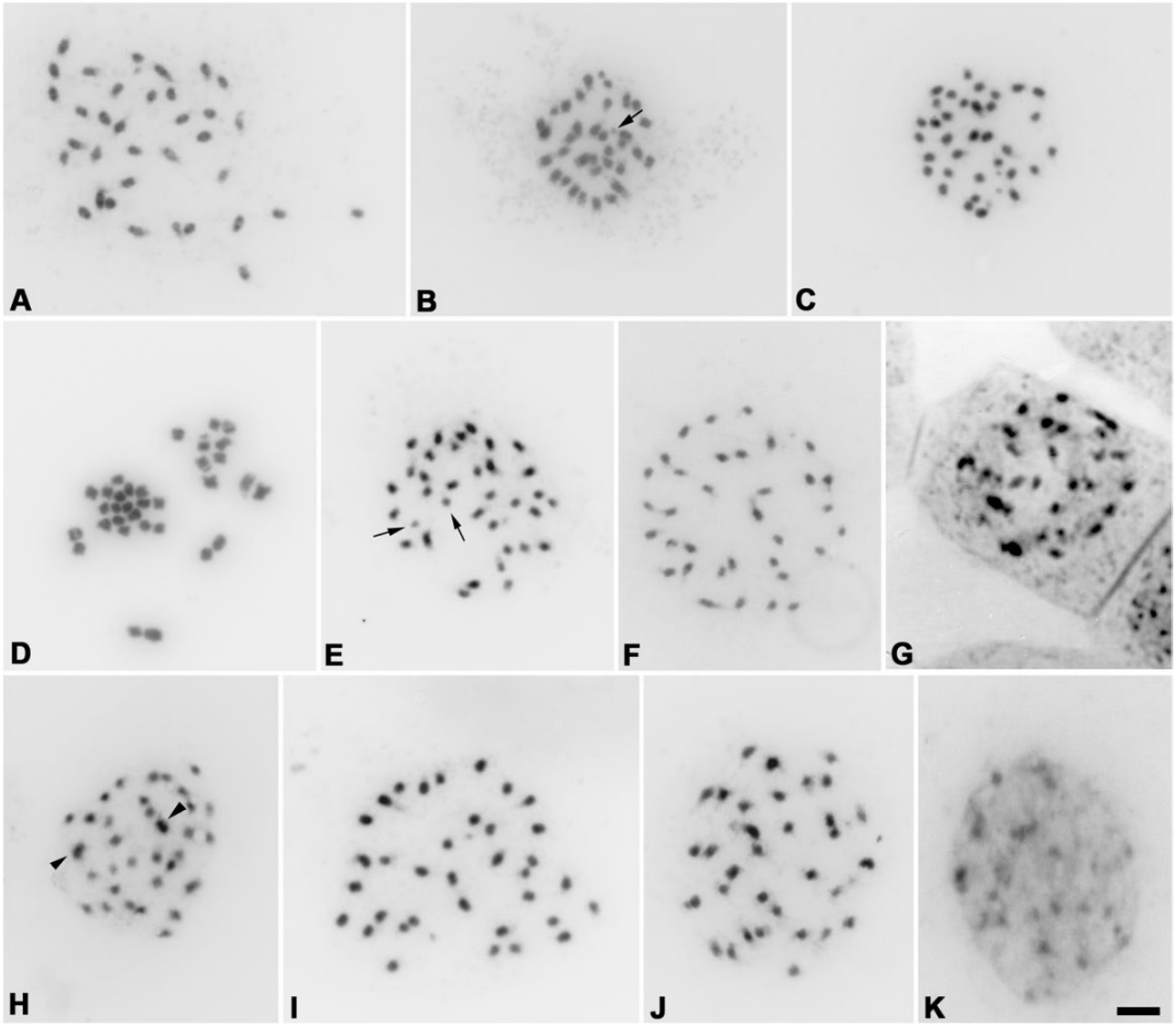

Figure 3. Metaphase cells of cryptanthoid complex species stained with DAPI (A–J): A, *Cryptanthus acaulis* (2*n* = 34); B, *C. bahianus* (2*n* = 34); C, *C. burle-marxii* (2*n* = 34); D, *C. correia-araujoi* (2*n* = 34); E, *C. dianae* (2*n* = 32); F, *Hoplocryptanthus ferrarius* (2*n* = 34); H, *Rokautskyia pseudoglazioui* (2*n* = 32); I, *C. lutherianus* (2*n* = 34); J, *C. zonatus* (2*n* = 34) or stained with Giemsa: G, *C. marginatus* (2*n* = 32); K, Interphase nuclei of *R. pseudoglazioui*. Arrows indicate B chromosomes in: B, *C. bahianus*; E, *C. dianae*. Arrowheads (in H) indicate the largest chromosomes found in the karyotype of *R. pseudoglazioui*. Scale bar = 5 μm.

Regarding the cytogenetic data, eight *Cryptanthus* spp., one *Hoplocryptanthus* sp. and one *Rokautskyia* sp. had small, predominantly metacentric and submetacentric chromosomes. Seven species had the diploid number 2*n* = 2*x* = 34: *C. acaulis* (Lindl.) Beer (Fig. 3A), *C. bahianus* (Fig. 3B), *C. burle-marxii* Leme (Fig. 3C), *C. correia-araujoi* (Fig. 3D), *Hoplocryptanthus ferrarius* Leme, S.Heller & Zizka (Fig. 3F), *C. lutherianus* I.Ramírez (Fig. 3I) and *C. zonatus* (Vis.) Beer (Fig. 3J). Three species had the diploid number 2*n* = 2*x* = 32: *C. dianae* Leme (Fig. 3E), *C. marginatus* (Fig. 3G) and *Rokautskyia pseudoglazioui* Leme, S.Heller & Zizka (Fig. 3H). In addition, supernumerary B chromosomes were found in *C. bahianus* (2*n* = 34 + 1–3 B; Fig. 3B) and *C. dianae* (2*n* = 32 + 3B; Fig. 3E).

In species with 2*n* = 34, the sizes of type A chromosomes ranged from 1.01 to 2.74 μm, and in species with 2*n* = 32 the sizes ranged from 1.02 to 2.91 μm. In the case of *R. pseudoglazioui*, the presence of two larger chromosomes is noteworthy (Fig. 3H, arrowheads) when compared to the

remaining chromosomes of the complement. In turn, B chromosomes were small, ranging from 0.74 µm in *C. bahianus* to 0.76 µm in *C. dianae*.

DISCUSSION

In this study, we investigated the variation of the genome sizes in the cryptanthoid complex, a conspicuous group of Bromeliaceae. DNA 2C-values of < 2.80 pg, considered as very small genome sizes among plant groups (Leitch & Bennett, 2007; Dodsworth *et al.*, 2015; Vu *et al.*, 2017; Pellicer *et al.*, 2018), were observed in all species analysed here. Also, we analysed the evolution of genome size in the cryptanthoid complex, considering the phylogenetic relationships among the genera of the group and conducting cytogenetic analysis in a bromeliad group with several particular cytogenetic features.

Genome size and chromosome number diversity in the cryptanthoid complex

Chromosome numbers indicate that *Cryptanthus*, *Hoplocrytanthus* and *Rokautskyia* are predominantly diploid, with the prevalence of $2n = 34$ (reported here for seven of the ten analysed species listed in Table 1 and five additional species listed in Table S1). A single case of a polyploid species, with $2n = 54$, was reported by Lindschau (1933) for *C. beuckeri* É.Morren and was not confirmed by a later report on the same species by Marchant (1967), who reported $2n = 34$ for the same species. As highlighted previously by Brown & Gilmartin (1986) and Gitaí, Horres & Benko-Iseppon (2005), possible mistakes associated with the work of Lindschau (1933) may be due to the manual section technique used by that author. The primary concern regarding this technique relies on the fact that metaphase chromosomes are often positioned in different planes in three-dimensional nuclei. Thus, the chromosomes counting in stained cuts can lead to either over- or under-estimation of their number (Gitaí *et al.*, 2014). Therefore, the counts of Lindschau (1933) will not be considered in the present work.

In general, despite the conserved ploidy, the genome sizes in plant groups evolve in a highly dynamic and bidirectional process that can result in significant variation also among homoploid species (Bennett & Leitch, 2005; Loureiro *et al.*, 2010). In this study, a variation of 2.13-fold in genome size was observed, even when considering species with the same chromosome number, such as *C. bromelioides* Otto & A.Dietr. ($2n = 34$; 2C = 1.52 pg) and *Hoplocryptanthus schwackeanus* Leme, S.Heller & Zizka ($2n = 34$; 2C = 0.82 pg), with a 1.85-fold difference in genome size. Such differences in 2C-values among related homoploid species are widely accepted as a species-specific attribute (Šmarda & Bureš, 2006).

Amplification and/or deletion of satellite repeats and the differential accumulation of transposable elements (TEs) may be causative agents of such differences, which are mainly related to the variation in genome sizes among closely related species (Ågren & Wright, 2011), as observed previously in other plant groups (Bennetzen, Ma & Devos, 2005; Lim *et al.*, 2006; Vitte & Bennetzen, 2006; Pellicer *et al.*, 2018). Moreover, a few TE families or even a single category, can be prevalent and account for the variation in genome sizes between closely related species (Grover & Wendel, 2010), also considering niche-associated speciation (Serrato-Capuchina & Matute, 2018).

Species of the cryptanthoid complex (Leme *et al.*, 2017) have genome sizes in a range similar to that observed within *Cryptanthus*. Despite this, a significant variability was observed considering chromosome basic numbers, with *Hoplocryptanthus* and *Forzzaea* having $x = 16$, 17 or 18 and *Sincoraea* having $x = 25$, in which 2C values measured in the present work ranged from 1.02 pg for *S. ophiuroides* Leme, S.Heller & Zizka (with $2n = 50$) to 1.5 pg in *Sincoraea ulei* Leme, S.Heller & Zizka (no chromosome counts available). Our measurements also included a polyploid species, *Sincoraea amoena* Leme, S.Heller & Zizka (with $2n = 100$), but with similar genome size (2C = 1.04 pg) to the known diploid related species (Fig. 2). However, the available genome size measurement for a species of the cryptanthoid complex with $2n = 50$, *Orthophytum saxicola* (Ule) L.B.Sm. (Ramírez-Morillo & Brown, 2001), revealed a considerably smaller genome (2C = 0.64 pg). Nevertheless, the similar 2C-values comparing preliminary data from *Orthophytum* and the most representative data for the cryptanthoid complex do not indicate the elimination of genomic material, instead pointing to rearrangements from an ancestor with the base number $x = 25$, towards the generation of a lower chromosome number ($x = 17$). Such a chromosome number diversity in the proposed cryptanthoid group indicates that, in its current configuration, this group may not be monophyletic. Further studies using more sensitive techniques including FISH (fluorescence in situ hybridization) may shed light on this still unsolved question regarding the origin of the secondary basic number $x = 17$.

Genome size evolution and molecular phylogeny

The reconstruction of the genome sizes of the phylogenetic tree for the cryptanthoid complex did not show a clear pattern of covariance among species in *Cryptanthus*. This indicates that, at least for this genus, genome size is not phylogenetically conserved.

The lack of correlation between the variation of genome sizes and the phylogenetic relationships among closely related species was already observed in other monocots, e.g. Orchidaceae (Jersáková *et al.*, 2013; Šmarda *et al.*, 2014). In the *Rokautskia*, *Sincoraea* and *Hoplocryptanthus* clades, the genome sizes within genera are more conserved, as also observed for Asteraceae and Dipterocarpaceae (Andrés-Sanchez *et al.*, 2013; Ng *et al.*, 2017). Therefore, the observed genome size variation in the cryptanthoid complex cannot be directly related to phylogenetic relationships. The genome sizes on the cryptanthoid complex experienced processes of downsizing and upsizing, probably due to different evolutionary processes such as natural selection (Leitch & Bennett, 2004), deletion-biased DNA repair (DSB), transposon-mediated unequal homologous recombination (Schubert & Vu, 2016) and niche genome size constraints (Knight *et al.*, 2005).

When tracing character transitions on the phylogenetic tree, genome sizes were significantly larger in *Cryptanthus* than in the other genera, with numerous shifts occurring, most of which pointing to an increase of DNA content. Thus, in *Cryptanthus*, there is a large variation and no evident pattern of distribution of the genome sizes among and within the clusters, resulting in a low phylogenetic signal. Even using a molecular phylogenetic tree sufficiently representative for the group, once it covers the whole range of estimated genome sizes, it is still challenging to make further considerations on the ancestral states and possible directions of genome size evolution in *Cryptanthus*.

Besides, the genome size variation observed between *Rokautskyia*, *Sincoraea* and *Hoplocryptanthus* could be associated with the geographical ranges, once the species distribution is split between the states of Espírito Santo (*Rokautskyia*), Bahia (*Sincoraea*) and Minas Gerais (*Hoplocrytanthus*). Moreover, these species show peculiar morphological traits in response to different habitats (Ramírez-Morillo, 1996; Leme, 2007; Leme & de Paula, 2009; Leme *et al.*, 2017) and, therefore, this pattern of distribution could be interpreted as an environmental adaptation. Given that genome size has been described as an important adaptive character that lies at the intersection between phenotype and genotype (Oliver *et al.*, 2007), such tendencies deserve some attention.

Genome size and habitat preferences

Genome size and various environmental parameters (such as habitat, temperature, precipitation and elevation) have been the focus of several studies. For instance, in a study of *Lasiocephalus* Willd. ex Schltdl. (Asteraceae), Dušková *et al.* (2010) observed a strong correlation between several environmental factors (e.g. height, habitat and growth form) and genome size. Additionally, Veselý *et al.* (2012) observed that genome size evolution in geophytes is closely related to their ecology (e.g. growth in humid conditions) and phenology. In the same way, our data indicate an association between genome size and habitat preferences (campos rupestres and Atlantic Forest), where the existing variables of these environments may have shaped the historical biogeography and the genetic diversity of the studied species (Carnaval *et al.*, 2009; Martins, 2011; Cruz *et al.*, 2017). Considering that the ecology of plant species is determined by sets of traits, many of which are constrained by the genome size, the amount of nuclear DNA may determine the range of conditions in which a plant can evolve (Loureiro *et al.*, 2010). This pattern seems to be clear for the cryptanthoid complex, in which most of the species from the Atlantic Forest have larger genome sizes than those from campos rupestres.

The rocky outcrop areas in the Brazilian campos rupestres shelter a vegetation type considered to be peculiar (Porembski & Barthlott, 2000). Many of these species have specific features that allow their survival in nutrient impoverished soils, seasonal water deficit, high solar exposure, strong winds and environments influenced by rocks of chemically poor origin (Oliveira *et al.*, 2015; Schaefer *et al.*, 2016; Silveira *et al.*, 2016). Such conditions have been key constraints to the selection of species that strive in such habitats (Giulietti, Pirani & Harley, 1997; Porembski *et al.*, 1998). On the other hand, the Atlantic Forest is characterized by a strong seasonality, sharp environmental gradients and orographic driven rainfall, forming a diverse landscape that includes open, mixed and closed evergreen, semi-deciduous and deciduous forests (Tabarelli *et al.*, 2010). In summary, Atlantic Forest and campos rupestres differ markedly in nutrient and water availability, taking into account that the amount of available nutrients (e.g. nitrogen and phosphorus) and water in campos rupestres are lower than in Atlantic Forest habitats. Moreover, campos rupestres show considerably different climate conditions, such as lower rates of precipitation and higher incidence of sunlight (Oliveira *et al.*, 2015; Almeida *et al.*, 2018; Bomfim *et al.*, 2018; Winbourne *et al.*, 2018).

Considering the contrasting environmental conditions of between Atlantic Forest and campos rupestres, it seems to be reasonable that such a difference in the respective habitats may have contributed in shaping genomes sizes in the cryptanthoid complex, as observed in the wide variation of DNA content among species from the Atlantic Forest, in contrast to a more stable pattern of the species of the rocky outcrops in campos

rupestres. Cells need nitrogen and phosphorus to make nucleic acids, and thus species with larger genomes need more of these nutrients than other with smaller genomes, making them less competitive in nutrient poor environments (Pellicer *et al.*, 2018). Therefore, since Atlantic Forest species of the cryptanthoid complex are favoured by greater nutrient availability, it makes sense that they also have larger genomes. Furthermore, the remaining Atlantic Forest fragments present different topographies, which are important aspects in availability of the nutrients (Bomfim *et al.*, 2018). Thus, this is probably related to the larger variable genome size in this environment. The genome size is directly related with ecological and/or morphological traits (Suda *et al.*, 2015; Pellicer *et al.*, 2018) and in the cryptanthoid complex it is probably related to the production of smaller and more numerous seeds and ovules by *Hoplocryptanthus* spp. (Leme *et al.*, 2017), possibly indicating a developmental constraint for the species with larger genomes (Beaulieu *et al.*, 2007).

Within a given biological group, species with larger genomes tend to be under-represented in environments with extreme conditions (Knight *et al.*, 2005), as observed for *Hoplocryptanthus* spp. in campos rupestres. The genome size seems to affect the adaptability of plant species, considering that larger genome sizes seem to be maladaptive through its constraints on plant physiology, failing to adapt to variable habitats (Pandit, White & Pocock, 2014), such as the studied species from the Atlantic Forest. On the other hand, smaller genomes are associated with a smaller cell size, faster rates of mitosis and meiosis, faster germination and thus reduced generation times, which result in adaptation to time-limited environments (Pandit *et al.*, 2014), as may likely be the case of the studied species of *Hoplocryptanthus* in campos rupestres. Species of *Sincoraea* and *Forzzaea* do not follow the same tendency as observed for *Hoplocryptanthus*; instead, their genome sizes are larger like other bromeliads from xeric environments, such as *Deuterocohnia* Mez, *Dyckia* Schult. & Schult.f. and *Encholirium* Mart. ex Schult. & Schult.f. (Moura *et al.*, 2018). This could be related to different adaptations, considering that species of these genera have, for example, larger and less numerous seeds than *Hoplocryptanthus* (Leme *et al.*, 2017).

When comparing the species of the *Rokautskyia* and *Cryptanthus* clades, which occur in the Atlantic Forest, there is a difference in terms of the average genome size. Despite the importance of the environmental factors in shaping genome sizes, their variation of DNA content seems to be also influenced by phylogenetic relationships, at least to some extent, given that species of *Cryptanthus* and *Rokautskyia* had different amounts of genomic DNA, regardless of the habitat type. It has been shown that the genome size influences a wide array of characteristics, e.g. radiation and ecological behaviour in plant communities (Bennett, 1987; Bennett & Smith, 1991), which may be true for the species of the cryptanthoid complex, considering their different habitats.

Considerations relating to cytogenetic features

Bromeliaceae show considerable variation in chromosome number ($2n$ = 18 to $2n$ = 200), but x = 25 has been suggested as the most probable base number. *Cryptanthus*, *Hoplocryptanthus* and *Rokautskyia* are the only bromeliad groups with the suggested base number of x = 17 ($2n$ = 32, 34, 36), which is considered derived among Bromelioideae (Gitaí *et al.*, 2014). The variation of the diploid number in this group may be related to dysploidy events, which are often described in different plant groups, including other taxa commonly occurring on campos rupestres, e.g. Xyridaceae (Benko-Iseppon & Wanderley, 2002) and Asteraceae (Salles-de-Mello *et al.*, 2010). As in other plant groups, whole-genome duplication (WGD) can be clearly recognizable in Bromeliaceae (reviewed by Gitaí *et al.*, 2014). In angiosperms, the polyploid populations may undergo post-polyploid genome diploidization, gradually generating a functionally diploid-like genome through chromosomal rearrangements that frequently result in decreasing dysploid changes (Mandáková & Lysak, 2018). This may be true for the cryptanthoid complex, in which most species have $2n$ = 34. For example, in the present work, the considerable difference in the size of one chromosome pair in *R. pseudogaziovii* ($2n$ = 32) may be indicative of a decreasing dysploidy event.

The presence of B chromosomes was previously described for *Cryptanthus* and other Bromeliaceae (Cotias-de-Oliveira *et al.*, 2000; Gitaí *et al.*, 2005; Ceita *et al.*, 2008; Gitaí *et al.*, 2014), as described here for *C. dianae*. Such supernumerary chromosomes have been reported for *Cryptanthus* spp. with small (*C. bahianus*; 2C = 0.76 pg, $2n$ = 34, 1-3B) and large genomes (*C. dianae* 2C = 1.36 pg, $2n$ = 32, 1-3B; *C. praetextus*, 2C = 1.35 pg, $2n$ = 32, 32, 1-2B) (see Gitaí *et al.*, 2014). The presence of B chromosomes in Bromeliaceae has been considered an indication of active speciation processes related to chromosomal rearrangements (Gitaí *et al.*, 2014), also possibly indicating hybridization processes, as reported for many cereal species (Jones & Ruban, 2019).

CONCLUSIONS

The reconstruction of character states indicated that intermediate genome sizes might represent the ancestral condition for the major phylogenetic clusters, providing evidence of both decreases and increases in genome sizes through the evolution of the cryptanthoid complex at the diploid level. Besides, the occupation process of different habitats seems to have played a prominent role in the genome size evolution among the species of the cryptanthoid complex, especially considering the reported decreasing in DNA contents associated with the occupation of the campos rupestres by *Hoplocryptanthus* spp. The variation of the chromosome numbers and the presence of B chromosomes in the complex deserve additional studies to elucidate the events linked to the karyoevolution of the group, in which dysploidy seems to play an important role. These findings, together with the inclusion of morphological, ecological and genetic data in future analyses, may help to explain the establishment of the cryptanthoid complex species in different habitats such as the Atlantic Forest domain and campos rupestres and a better understanding of the population dynamics in the group.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Chromosome numbers reported in the literature for cryptanthoid complex species as compared to 2C genome sizes given in Table 1. Counts within parenthesis (carried out by Lindschau, 1933) were based on the microtome section technique that lead to misinterpretations of chromosome numbers, being therefore excluded from the discussion of the present data. [K]Kew Gardens database (Ramírez-Morillo & Brown, 2001); Gitaí *et al.* (2014). Chromosome counts between parenthesis (generated by Lindschau, 1933) considered 'non-reliable'.