SYSTEMATICS AND PHYLOGENY

Phylogenetic reconstruction of the genus *Tephrocactus* (Cactaceae) based on molecular, morphological, and cytogenetical data

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Abstract *Tephrocactus* comprises species mainly endemic to Argentina. Molecular phylogenetic analyses of all proposed species of the genus as well as classical (chromosome number, karyotype) and molecular cytogenetical techniques (DNA content, heterochromatin amount, rDNA genes) were conducted. Sequence data of two plastid DNA markers of *Tephrocactus* taxa were analyzed. Evolution of character states of cytogenetical and morphological (growth form, presence of leaves, glochids and tepal spiny mucrons, flower color) traits were reconstructed. Species show $x = 11$ with different ploidy levels ($2n = 22, 44, 66, 77, 242, 319$), small chromosomes, and symmetrical karyotypes. *Tephrocactus* was recovered as monophyletic with three main clades including 12 species, using molecular and morphological data. *Tephrocactus geometricus*, *T. halophilus*, and *T. paediophilus* are recognized as distinct species. Banding patterns showed $CMA^+/DAPI^-$ constitutive heterochromatin associated with nuclear organized regions. Heterochromatin amount ranged from 2.99% to 6.50%. The 18S-5.8S-26S ribosomal DNA (rDNA) sites coincided with the $CMA^+/DAPI^-$ signals. The 5S sites varied with ploidy levels of the taxa. DNA content (2C = 1.99–24.50 pg) had a significant and positive correlation with ploidy level and the number of rDNA genes. The ancestor is reconstructed to have been a dwarf shrub with strong articulation, glochids, and deciduous leaves, white, pink or pearly tepals without spiny mucrons, $2n = 22$, low DNA content, and one pair of each rDNA gene followed by three polyploidization events. *Tephrocactus* diversification has been associated with polyploidy and few cumulative small cryptic chromosomal changes.

Keywords ancestral state reconstruction; cytogenetics; DNA content; morphology; phylogeny; *Tephrocactus*

Supporting Information may be found online in the Supporting Information section at the end of the article.

■ INTRODUCTION

Cactaceae seem to have originated after the Eocene-Oligocene global drop in CO_2 (Arakaki & al., 2011). Radiation of its richest genera coincided with the expansion of aridity in North America during the late Miocene. Hernández-Hernández & al. (2014) suggested that diversification responded to the availability of new niches resulting from aridification and to the correlated evolution of novel growth forms and reproductive strategies. The family is monophyletic and includes four subfamilies: Cactoideae, Maihuenioideae, Opuntioideae, and Pereskioideae (Anderson, 2001; Nyffeler, 2002; Wallace & Gibson, 2002; Hunt & al., 2006; Mauseth, 2006). The subfamily Opuntioideae is monophyletic as well (Wallace, 1995; Nyffeler, 2002; Griffith & Porter, 2009; Hernández-Hernández & al., 2011). It is distributed from Canada to Patagonia (Schumann, 1897; Britton & Rose, 1919; Backeberg, 1966; Anderson, 2001; Stuppy, 2002; Hunt & al., 2006; Kiesling & al., 2008) and is distinguished by a number of synapomorphies: glochids (small, deciduous, barbed spines), woody funicular tissue surrounding the seed, calcium oxalate monohydrate druses and monoclinic cluster crystals in the outer stem hypodermis, and polyporate pollen grains with unusual exine structures (Robinson, 1974; Leuenberger, 1976; Gibson & Nobel, 1986; Stuppy, 2002; Hartl & al., 2007). Current classifications recognize 5 tribes, 15 genera, and 220–350 species (Anderson, 2001; Hunt & al., 2006).

The South American tribe Tephrocacteae sensu Hunt & al. (2006, including Austrocylindropuntieae and Pterocacteae) comprises spherical to terete-stemmed genera: *Austrocylindropuntia* Backeb., *Cumulopuntia* F.Ritter, *Maihueniopsis* Speg., *Pterocactus* K.Schum., *Punotia* D.R.Hunt, and *Tephrocactus* Lem. They show diverse life-forms, from geophytes, cushion plants, shrubs to columnar cacti, and seed anatomical structures diagnostic to differentiate them (Stuppy, 2002). Its phylogenetic relationships are not yet firmly understood (Hernández-Hernández & al., 2011; Ritz & al., 2012) as Tephrocacteae are part of a polytomy with the two major clades of Opuntioideae: the terete-stemmed (Cylindropuntieae sensu Hunt & al., 2006) and the mainly flat-stemmed (Opuntieae sensu Hunt & al., 2006).

Tephrocactus, previously treated as a subgenus of *Opuntia* (Lemaire, 1868), comprises small shrubs with reddish glochids, globose or cylindrical segments with moniliform

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branching, pericarpel with developed areoles, dry dehiscent fruits, and seeds generally with corky arils with aerenchymatic cells (Kiesling, 1984; Gilmer & Thomas, 1998; Stuppy, 2001; Hunt & al., 2006; Gorelick & Mahr, 2013). However, the number of its species varies depending on the authors and their generic circumscription from seven to twelve. Kiesling (1984) includes seven species endemic to Argentina, inhabiting dry, comparatively low (up to 2500 m a.s.l.), rocky or sandy places, that basically share sunken areolas, dry dehiscent fruits, and corky arils: *T. alexanderi*, *T. aoracanthus*, *T. articulatus*, *T. geometricus*, *T. halophilus*, *T. molinensis*, and *T. weberi* (suppl. Figs. S1, S2); in addition, he mentions another species, *T. paediophilus*, as possibly different from *T. aoracanthus*. Hunt & al. (2006) and Ritz & al. (2012), after molecular analyses, consider ten Argentinean species, some reaching Bolivia and Chile: the species recognized by Kiesling (1984), excluding *T. geometricus* considered a synonym of *T. alexanderi*, and four species that were described in other genera: *Austrocylindropuntia verschaffeltii*, *Cumulopuntia recurvata*, *Maihueniopsis nigrispina*, and *Puna bonnieae* (suppl. Figs. S1, S2). Morphologically, these taxa partially overlap with *Tephrocactus*: *Austrocylindropuntia verschaffeltii* has juicy indehiscent fruits, orange to red flowers, relatively large persistent leaves, and seeds with globular compact arils, but it has sunken areolas, and its habit is close to *T. weberi*. *Cumulopuntia recurvata* forms cushions, its seeds have hard, spherical and not auriculate arils, indehiscent and fleshy fruits, but it presents whitish flowers and grows at lower altitudes, as do *Tephrocactus* species. *Maihueniopsis nigrispina* possesses red flowers and seeds with hard, rugose but not auriculate (not possessing two lateral auricles as the other *Tephrocactus*) arils, grows in sandy places as *Tephrocactus* but at higher altitudes. *Puna bonnieae* has white to pink flowers, dry dehiscent fruits, and corky arils, but it presents pectinated spines, does not have glochids, has receptacles without developed areolas, and is a geophyte.

Tephrocactus members are commonly cultivated all over the world because of their size and attractive flowers; in addition, their stem fragments easily detach and, thus, can reproduce vegetatively with efficiency (Mandujano & al., 2007). In nature, agamic reproduction of clonal species results in more individuals and stability to the population than sexual reproduction, depending on the environmental conditions (Mandujano, 2007; Mandujano & al., 2007). Asexual reproduction is associated with polyploidy (Mable, 2004; Baldwin & Husband, 2013). Polyploidy evolves more commonly in diploid species that are capable of reproducing asexually because asexuality allows the persistence of polyploid genotypes even in cases where sexual reproduction is compromised because of meiosis abnormalities. Stebbins (1950) suggested that, in general, vegetative or clonal reproduction is a prerequisite for the evolution of polyploidy, although there are numerous exceptions.

Cytological studies have shown that polyploid cytotypes are common in the Opuntioideae (Pinkava, 2002), with the highest level in South American members (*Austrocylindropuntia*, *Miqueliopuntia*, *Tephrocactus*). However, chromosomes are among the characteristics of *Tephrocactus* that were never studied in detail, except for a few chromosome numbers reported so far (Yuasa & al., 1973; Las Peñas & al., 2009). In Cactaceae, most cytological studies provide chromosome counts, showing that its basic number is $x = 11$ (e.g., Cota & Philbrick, 1994; Bandyopadhyay & Sharma, 2000; Pinkava, 2002). There are few karyotypic studies available for South American cacti. However, those provide important information for addressing systematic and evolutionary problems in Cactaceae (Bandyopadhyay & Sharma, 2000; Las Peñas & al., 2008, 2009, 2014, 2017; Majure & al., 2012; Moreno & al., 2015a,b).

Chromosome banding and fluorescence *in situ* hybridization (FISH) have scarcely been applied to Cactoideae, Maihuenioideae, Opuntioideae (Las Peñas & al., 2009, 2017; Realini & al., 2014; Castro & al., 2016) and Pereskioideae (Las Peñas & al., 2008, 2014; Moreno & al., 2015a,b). Both techniques have provided important tools to understand genome organization and evolution in plants (e.g., Sumner, 1990; Jiang & Gill, 2006; Raskina & al., 2008). The physical mapping of tandem repeat genes (rDNA) with FISH resulted in useful markers for karyotype comparisons to evaluate relationships among species to better understand their divergence (Jiang & Gill, 2006; Raskina & al., 2008). Specifically, the 5S and 18-5.8-26S rDNA genes have been extensively utilized to establish chromosomal homeologies (e.g., Moscone & al., 1999; Adams & al., 2000; Taketa & al., 2005).

The nuclear DNA content per cell of cacti is mostly unknown (Bennett & Leitch, 2010). In Opuntioideae, there are some previous studies on the measurement of nuclear DNA content in species of *Consolea* and *Opuntia* (Palomino & Heras, 2001; Zonneveld & al., 2005; Negrón-Ortiz, 2007; Segura & al., 2007). The Cx value (DNA content per monoploid genome) in the species of the subfamily varies from 0.54 to 3.46 pg (Bennett & Leitch, 2010). Measurement of nuclear DNA content is a useful tool to study phylogenetic relationships between taxonomically related groups and to expand the understanding of genome size evolution (Ohri, 1998; Bennett & Leitch, 2005), as well as its correlates (Ohri, 1998; Knight & Ackerly, 2002).

Upon this background, the aim of this work was to study all proposed species of *Tephrocactus* in a molecular, cytological, and morphological context to produce a phylogenetic reconstruction of the group with the *trnK-matK* and *psbA-trnH* plastid DNA regions. In the phylogeny obtained, cytogenetical (karyotypes, heterochromatin amount, position of rDNA genes, DNA content) and morphological (growth form, presence of leaves, glochids and tepal spiny mucrons, flower color) characters were mapped onto the phylogenetic tree to cast light on their evolution.

■ MATERIALS AND METHODS

Taxon sampling. — In the present study, 19 species and varieties from 33 individuals were sampled representing all

purported species of *Tephrocactus* and four outgroups. Collection data are included in Table 1. Most sequences were here obtained and included in GenBank, whereas a few sequences were taken from previous studies (Table 1); when possible, the identification of voucher specimens of GenBank accessions was double-checked.

DNA extraction, amplification, and sequencing. — DNA was extracted using DNeasy Plant Mini Kit Qiagen (Qiagen, Hilden, Germany) according to the instructions and modifying the incubation time to 20 minutes. The phylogeny was constructed using two plastid DNA regions*: trnK-matK* (Hernández-Hernández & al., 2011) and *psbA-trnH* (Hamilton, 1999). Plastid data were used because the predominantly uniparental inheritance of plastid DNA provides insights into the origin of polyploid taxa (Palmer & al., 1988). The sequences were pre-aligned in MEGA v.6 (Tamura & al., 2013) using the Muscle algorithm with default settings; subsequently, sequence alignments were manually checked and optimized (data was submitted to TreeBASE, submission number S24532, https://www.treebase.org/treebase-web/search/studySearch.html).

Phylogenetic reconstruction. — Phylogeny reconstruction under parsimony was conducted with plastid DNA datasets using MEGA7 (Kumar & al., 2015). Heuristic searches included 1000 random addition replicates. Support for monophyly was determined by non-parametric bootstrapping (Felsenstein, 1985) on 1000 bootstrap replicates using the same criteria as the regular parsimony searches. Bayesian analysis was run with MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001; Nylander & al., 2004) with a model of sequence evolution selected by MrModeltest v.2.2 (Nylander, 2004), which implements the hierarchical likelihood ratio test (hLRT) and the Akaike information criterion (AIC). Tracer v.1.5 (Rambaut & Drummond, 2007) was used to determine whether the MCMC parameter samples were drawn from a stationary distribution, and adequate effective sample sizes for each parameter (ESS > 200) were reached. The tree was visualized with FigTree v.1.5.4 (http://tree.bio.ed.ac.uk/software/figtree/).

For the phylogenetic analysis based on molecular and morphological data, the matrix of molecular markers (*trnK-matK*, *psbA-trnH*) and the matrix of 15 morphological characters (Appendix 1) were used. Parsimony analyses were conducted with the program TNT v.1.1 (Goloboff & al., 2008). Optimal trees were searched using random addition sequences of Wagner trees, followed by the tree bisection-reconnection (TBR) algorithm, making 500 replications and saving up to 10 trees per replicate (command sequence: hold 5000; mult = tbr replic 500 hold 10). The resulting trees were used as starting points for a round of TBR branch swapping (command: bbreak = TBR). Support values were estimated using group frequencies under jackknifing.

Cytogenetic analyses. — Living plants were placed in earthenware pots in an equal-part mixture of sand and potting soil in the Experimental Garden of Museo Botánico (Córdoba, Argentina) to obtain adventitious roots. Preparations of metaphase chromosomes were done from root tips pretreated with 2 mM 8-hydroxyquinoline for 24 h at 4°C and fixed in 3 : 1 ethanol : acetic acid. For slide preparation, root tips were washed twice in distilled water (10 min each), digested with a solution of 2% cellulose (Sigma-Aldrich, Vienna, Austria) and 20% pectinase (from *Aspergillus niger*; Sigma-Aldrich) for 45 min at 37°C, and squashed in a drop of 45% acetic acid. After coverslip removal in liquid nitrogen, the slides were stored at −20°C.

For karyotype analyses, slide preparations were stained with Giemsa (Guerra, 1983), and permanent mounts were made with Entellan (Merck, Darmstadt, Germany). Ten metaphases of different individuals per species were photographed with a phase contrast optic Olympus BX61 with software CytoVision (Leica Biosystems) and camera JAI model CV-M4+ CL monochromatic. Photographs were used for measurements of the following features for each chromosome pair: short arm (s), long arm (l), and total chromosome length (c); the length of the satellite was added to that of its chromosome arm. The arm ratio (r = l / s) was then calculated and used to classify the chromosomes as recognized by Levan & al. (1964). In addition, mean chromosome length (C), mean total haploid chromosome length (THL) of the karyotype based on the mean chromosome lengths, and mean arm ratio (R) were calculated. Idiograms were based on the mean values. The chromosomes were arranged first into groups according to their increasing arm ratio and then according to decreasing length within each group. To merge the images, the free software ImageJ v.2 was employed (http://rsbweb.nih.gov/ij/). Karyotype asymmetry was estimated using the intrachromosomal (A_1) and interchromosomal (A_2) indices of Romero Zarco (1986).

For the CMA/DAPI banding, slides were stained with a drop of 0.5 mg/ml chromomycin A_3 (CMA) for 90 min, then stained with 2 µg/ml 4'-6-diamidino-2-phenylindole (DAPI) for 30 min (both stains from Sigma-Aldrich, Austria), and finally mounted in McIlvaine's buffer–glycerol (1 : 1) (Las Peñas & al., 2008). The amount of heterochromatin was expressed as a percentage of the total length of the haploid karyotype (%Ht).

For the fluorescence *in situ* hybridization (FISH), the protocol of Schwarzacher & Heslop-Harrison (2000) was used with the p*Ta*71 probe to identify the rDNA 18S-5.8S-26S loci (Gerlach & Bedbrook, 1979) labeled with biotin-14-dUTP by nick translation (Bionick, Invitrogen, Carlsbad, California, U.S.A.) and subsequently detected with avidin-FITC (Sigma-Aldrich). For analysis of the 5S rDNA loci, we used a specific probe from *Pereskia aculeata* (Las Peñas & al., 2011). These fragments were labeled with digoxigenin-11-dUTP (DIG nick translation mix, Roche Diagnostics, Mannheim, Germany) and detected with Anti-DIG-Rhodamine (Roche Diagnostics). The slides were mounted with antifade Vectashield (Vector Laboratories, Burlingame, California, U.S.A.) containing DAPI.

DNA content. — For every species, 1–3 individuals were measured, three runs each. DNA content was measured by

Table 1. Species examined in this study, vouchers, GenBank accessions (all sequences new), and cytogenetic methods applied.

Taxa	Vouchers	*psbA-trnH*	*trnK-matK*	2*n*	c-DNA
Tephrocactus alexanderi (Britton & Rose) Backeb.	Catamarca, Dpt. Tinogasta, Guanchín, *Las Peñas & al. 237*	MK286909	MK286925	x	x
	La Rioja, Dpt. Famatina, Altos del Carrizal, *Moreno s.n.*	MK286920	MK286926	x	x
T. aoracanthus (Lem.) Lem.	San Juan, Dpt. Ullún, Dique de Ullún, *Las Peñas & Uñates 3*	MK286921	MK286948	x	–
	San Juan, Dpt. Pocito, La Quebrada de la Lechuza, *Las Peñas & Uñates 642*	MK286910	MK286927	x	x
	Mendoza, Dpt. Las Heras, Villavicencio, *Las Peñas & al. 649*	MK286922	MK286928	x	–
T. articulatus (Pfeiff.) Backeb. var. *articulatus*	La Rioja, Dpt. Independencia, Route 74, km 1143, *Las Peñas 19*	MK286923	MK286929	x	x
	Córdoba, Dpt. Pocho, Chancaní, *Las Peñas 85*	–	–	x	–
	La Rioja, Dpt. Independencia, Cuesta de Guanchín, *Las Peñas & Uñates 566*	–	–	x	–
	South Africa, adventitious specimen, *Las Peñas 644*	MK286911	MK286930	x	x
T. articulatus var. *oligacanthus* (Speg.) Backeb.	La Rioja, Dpt. Independencia, near Patquia, *Las Peñas & Chiarini 209*	MK286912	MK286931	x	–
T. articulatus var. *strobiliformis* Backeb.	*Las Peñas 580*	MK286913	MK286932	x	x
T. bonnieae (D.J.Ferguson & R.Kiesling) Stuppy	Catamarca, Dpt. Tinogasta, Chaschuil, *Kiesling s.n.*	MK286907	MK286933	x	x
T. geometricus (A.Cast.) Backeb.	Catamarca, Dpt. Tinogasta, Cortaderas, *Las Peñas & al. 234*	MK286914	MK286934	x	x
	Catamarca, Dpt. Tinogasta, Cortaderas, *Las Peñas & al. 234bis*	–	–	x	–
T. halophilus (Speg.) Backeb.	San Juan, Dpt. Valle Fértil, Sierra Pie de Palo, *Las Peñas 572*	–	–	x	x
	San Juan, Dpt. Valle Fértil, near Sierra Pie de Palo, *Las Peñas 571*	MK286915	MK286935	x	x
T. molinensis (Speg.) Backeb.	Salta, Dpt. San Carlos, Los Molinos, *Las Peñas & Uñates 288*	MK286916	MK286936	x	x
	Salta, Dpt. Cafayate, N of Cafayate, *Las Peñas 577*	–	–	x	–
T. nigrispinus (K.Schum.) Backeb.	Jujuy, Dpt. Humahuaca, Tres Cruces, *Moreno 84*	MK286902	MK286937	x	–
	Jujuy, Dpt. Humahuaca, Iturbe, *Las Peñas 661*			x	x
T. paediophilus (A.Cast.) F.Ritter	La Rioja, Dpt. Chamical, Salina la Antigua, *Las Peñas 655*	MK286917	MK286938	x	x
T. recurvatus (Gilmer & Thomas) Hunt & Ritz	San Juan, Dpt. Iglesia, Los Médanos, *Las Peñas 182*	–	–	x	x
	San Juan, Dpt. Angaco, Villicúm, *Las Peñas 654*	MK286903	MK286939	x	–
T. verschaffeltii (F.A.C.Weber) Hunt & Ritz	Catamarca, Dpt. Ambato, Falda del Morro, *Cantero & al. 7268*	MK286900	MK286940	x	–
	La Rioja, Dpt. Chilecito, W from Sañogasta, *Las Peñas 659*	MK286924	MK286942	x	x
T. weberi var. *deminutus* Rausch	Salta, Dpt. San Carlos, Amblayo, *Las Peñas 579*	MK286918	MK286941	x	x
T. weberi (Speg.) Backeb. var. *weberi*	Salta, Dpt. San Carlos, Angastaco, *Las Peñas & Uñates 311*	–	–	x	–
	Salta, Dpt. San Carlos, Angastaco, *Las Peñas & Uñates 304*	–	–	x	x
	San Juan, Dpt. Valle Fértil, Sierra Pie de Palo, *Las Peñas & Uñates 357*	MK286919	MK286943	x	x
Pereskia aculeata	Misiones, Dpt. Oberá, Guaraní, *Barboza & al. 1036*	MK286905	MK286944	x	x
Brasiliopuntia schulzii	Corrientes, Dpt. Corrientes, Perichón, *Las Peñas 193*	MK286901	MK286945	x	x
Pterocactus tuberosus	Mendoza, Dpt. Lujan de Cuyo, Potrerillos, *Kiesling s.n.*	MK286906	MK286946	x	x
Opuntia quimilo	La Rioja, Dpt. Chamical, Chamical, *Las Peñas & al. 539*	MK286904	MK286947	x	x

2*n* = chromosome, banding, and FISH; c-DNA = DNA amount (the technique applied to each one indicated with x). En-dashes (–) indicate data not taken. All samples from Argentina unless indicated. Collection data include province, department, locality, collector and number. Voucher specimens were deposited in CORD.

Table 2. Cytogenetical features of *Tephrocactus* species.

Taxon	2*n*	*x*	KF	TLH	C (µm)	A_1	A_2	CMA^+/ NOR	% Ht	FISH 45S	FISH 5S	2C	Previous counts
T. alexanderi	22	2	11 *m*	23.85	2.17	0.17	0.09	2	4.55	2	2	2.98[E]	22 (Yuasa & al., 1974)
T. aoracanthus	242**	22	–	17.36	1.57	–	0.15	10	2.99	8	12	19.60	
T. articulatus var. *articulatus* (644)	88	8	–	25.45	2.31	–	0.10	–	–	2	8	8.35	88 (Yuasa & al., 1974)
T. articulatus var. *oligacanthus*	88**	8	–	24.12	2.20	–	0.08	–	–	2	8	–	
T. articulatus var. *strobiliformis*	77**	7	–	33.87	3.07	–	0.16	–	–	2	6	8.13	
T. bonnieae	44**	4	–	–	–	–	–	–	–	–	–	2.13[E]	
T. geometricus	22**	2	10 *m* + 1 *sm*	19.72	1.80	0.33	0.07	2	5.47	2	2	2.56	
T. halophilus	22**	2	11 *m*	28.03	2.53	0.38	0.14	2	6.00	2	2	2.17	
T. molinensis (577)	44**	4	–	27.90	2.54	–	0.04	4	5.07	4	4	6.63	
T. molinensis (288)	88**	8	–	–	–	–	–	–	–	–	–	–	
T. nigrispinus	44**	4	–	–	–	–	–	–	–	–	–	3.37[E]	
T. paediophilus	319*	29	–	–	–	–	–		–	10	12	24.50	ca. 330 (Yuasa & al., 1974)
T. recurvatus	44	4	–	39.50	1.80	0.23	0.20	2	3.10	2	4	1.99	44 (Las Peñas & al., 2009)
T. verschaffeltii	22*	2	11 *m*	16.27	1.40	0.12	0.09	2	5.80	2	2	2.14	44 (Yuasa & al., 1974)
T. weberi var. *deminutus*	22**	2	11 *m*	11.77	1.57	0.43	0.13	2	6.50	2	2	4.12	
T. weberi var. *weberi* (304)	44	4	–	–	–	–	–	–	–	–	–	3.42[E]	22, 33, 44 (Yuasa & al., 1974)
T. weberi var. *weberi* (357, 311)	22	2	11 *m*	17.22	2.51	0.26	0.29	4	4.01	4	4	6.79[E]	

Data are: 2*n*, somatic chromosome number; *x*, ploidy level; KF, karyotype formula; TLH, mean total haploid chromosome length; C, mean chromosome length; A_1, intrachromosomal asymmetry index; A_2 interchromosomal asymmetry index; CMA^+/NOR, number of chromosome pairs with CMA^+/$DAPI^-$ /NOR associated bands; %Ht, heterochromatin amount expressed as percentage of the haploid karyotype length; FISH, fluorescent in situ hybridization; 45S, number of rDNA 18S-5.8S-26S loci; 5S, number of rDNA 5S loci; [E], endopolyploidy; *, new cytotype; **, new chromosome number.

flow cytometry obtaining nuclear suspensions according to Doležel & al. (2007) with minor modifications. Briefly, small pieces of fresh leaves from the sample and the appropriate standard were co-chopped with a sharp razor blade in a glass petri dish containing 0.5 ml of Otto I solution (0.1 M citric acid 0.5% Tween 20) and 0.2 ml of 5% PVP (PVP 40, Sigma-Aldrich). Nuclear suspensions were then filtered through a 45-µm mesh nylon membrane and kept at room temperature for 10 to 60 min. After the addition of 0.5 ml of Otto II buffer (0.4 M $Na_2HPO_4 \cdot 12\ H_2O$), propidium iodide (50 µm/ml and RNAse (50 µm/ml) were added to stain DNA and avoid the labeling of double stranded RNA. Samples were kept at room temperature and analyzed after 10 min in a FACS Canto II Flow Cytometer (Becton-Dickinson, San José, California, U.S.A.) equipped with a 488 nm and a 633 nm laser. The emitted fluorescent light of the DNA dye (FL2) generates an electronic signal that can be recorded as height (FL2H) for the intensity of the staining as well as measured as pulse-area (FL2-A) and pulse-width (FL2-W) of the samples. Dot plots of FL2-A vs. time were used as a control of fluorescence emission during sample analysis. Doublets were excluded from the analysis using dot plots of FL2-A vs. FL2-W. Three DNA estimations were carried out for each plant (5000 or 10,000 nuclei per analysis) on three different days. Nuclear DNA content was calculated as: (Sample peak mean / Standard peak mean) × 2C DNA content of the standard (in pg). Cx values, representing the DNA content of one non-replicated monoploid genome with the chromosome number *x* (Greilhuber & al., 2005), were calculated as the 2C nuclear DNA content divided by ploidy level.

Statistical analyses. — Correlation analyses among all cytogenetic features obtained (suppl. Table S1) were

performed in InfoStat v.2012 (Di Rienzo & al., 2012) using the non-parametric Pearson test (Sokal & Rohlf, 1995).

Character mapping. — Cytogenetical and morphological characters are listed in the Table 2 and Appendix 1, respectively. Ancestral character state reconstructions with maximum-likelihood criterion (Mk1 model, in which all changes are equally probable) were conducted in Mesquite v.3.2 (Maddison & Maddison, 2017) using the concatenated (*trnK-matK* and *psbA-trnH*) data matrix in a pruned phylogenetic tree. The Bayesian analyses were performed with four independent Metropolis-coupled Markov chain Monte Carlo (MCMC) multistate and continuous random walk modules were implemented to analyze discrete and continuous states, respectively.

■ RESULTS

Phylogenetic analyses. — Our data matrix has 2282 molecular characters. Of this total, 2064 characters are constant and 141 variable characters are parsimony uninformative, leaving 77 parsimony informative characters. All the 15 morphological characters used are parsimony informative. The strict consensus tree obtained from plastid DNA sequences is well resolved (Fig. 1). The maximum parsimony and Bayesian analyses (Fig. 1) produced similar topologies. Bootstrap values and posterior probabilities (PP) are strong overall. *Tephrocactus* is recovered as monophyletic (PP = 1; Fig. 1). Three main clades are reconstructed: clade A (PP = 0.9) with two subclades: A1, with *T. molinensis*, *T. recurvatus*, and *T. bonnieae*; and A2, including *T. nigrispinus* and *T. verschaffeltii*; clade B, a smaller strongly supported clade (PP = 0.99), includes both varieties of *T. weberi*; and clade C (PP = 1), a larger, strongly supported clade including six species: *T. alexanderi*, *T. aoracanthus*, *T. articulatus*, *T. geometricus*, *T. halophilus*, and *T. paediophilus* (Fig. 1).

The strict consensus tree obtained from the combined matrix of plastid DNA sequences and morphological characters (Fig. 2) results in a well-supported topology with three major subclades very similar to the one obtained from the molecular phylogenetic analyses as presented above. The genus *Tephrocactus* is monophyletic and supported by two morphological synapomorphies: determined growth form and dwarf shrub habit. Clade A1 includes *T. molinensis*, *T. recurvatus* and *T. bonnieae*, grouped by molecular characteristics, with *T. molinensis* separated only by absence of spines and shrubby habit; *T. bonnieae* is remarkable for being the only species in this genus that lost its glochids. Clade A2 includes *T. nigrispinus* and *T. verschaffeltii*, sharing synapomorphic red tepals and molecular characters. Clade B, with

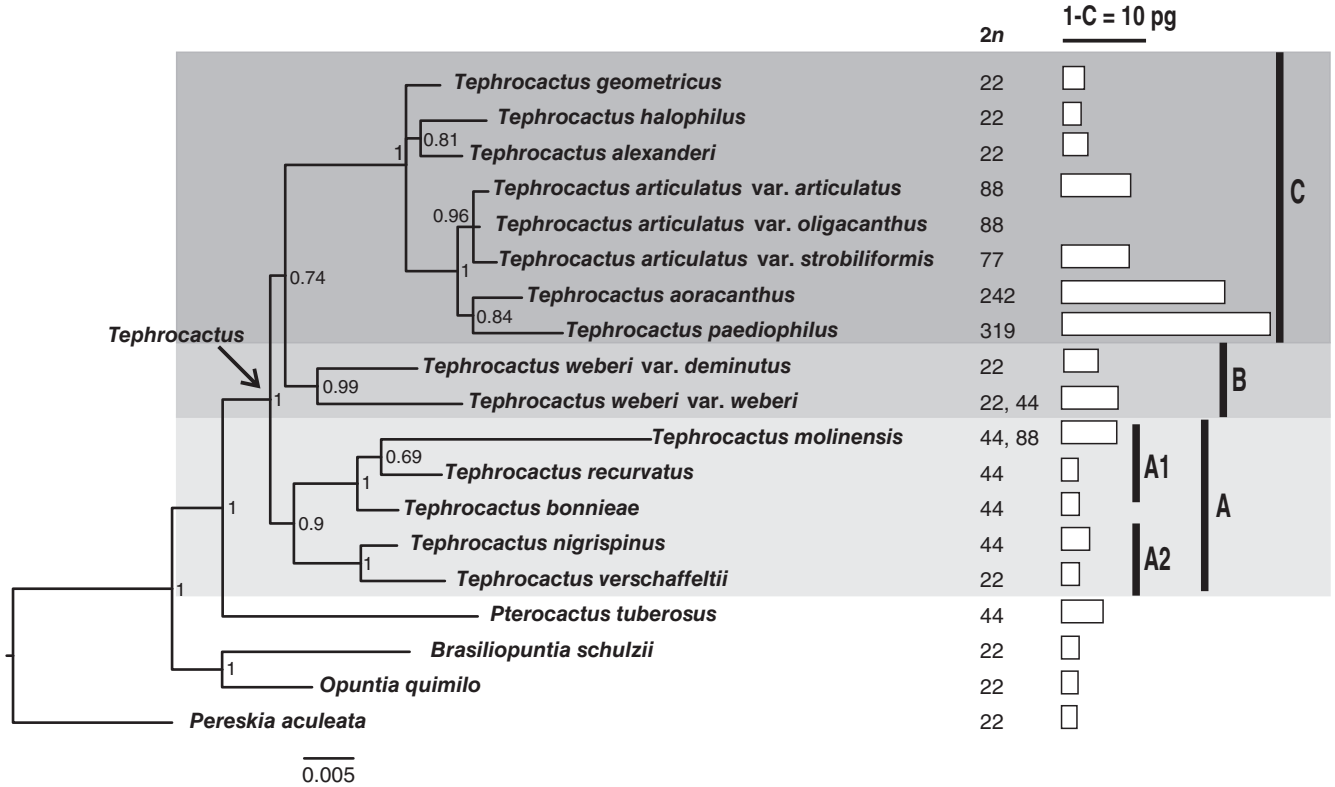

Fig. 1. Phylogenetic relationships of *Tephrocactus* obtained by Bayesian analysis of the combined *matK-trnK* and *psbA-trnH* markers. Numbers next to nodes indicate posterior probabilities from Bayesian inference analyses. Three main clades are reconstructed: clade A, with further subclades A1 and A2; clade B, a smaller, strongly supported clade; and clade C, a larger, strongly supported clade including six species. 2*n* column at the right indicates the chromosome numbers; white bars represent the corresponding 1C-values in pg for each species.

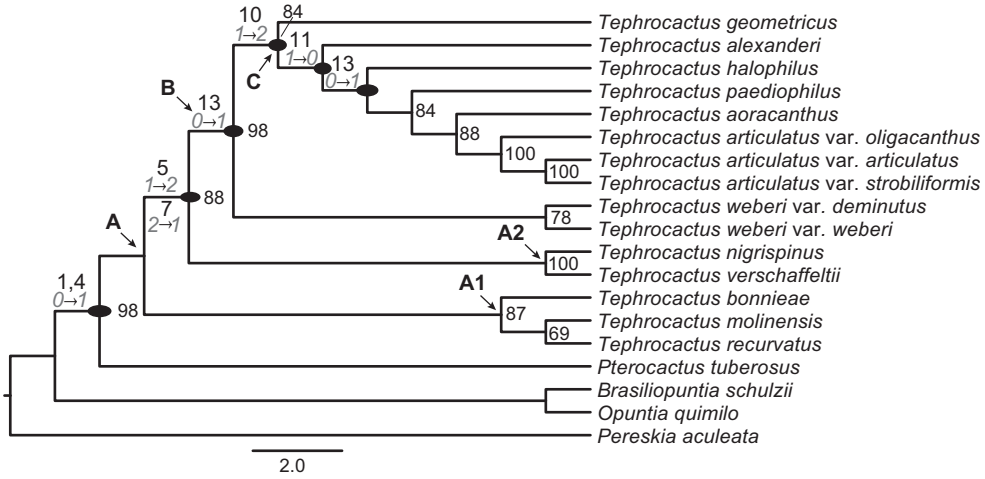

Fig. 2. Strict consensus tree of the phylogenetic reconstruction of *Tephrocactus* based on molecular and morphological data obtained with TNT. Numbers to the right of nodes indicate bootstrap values. Synapomorphies are indicated on the left of nodes with character numbers (Appendix 1) above and character states below (in italics).

T. weberi varieties, shares with clade A2 the yellow/red tepal color synapomorphy (Fig. 2). Finally, clade C has the same six species as in the analysis based on molecular data alone, which share articulated stem segments; except *T. geometricus* the other five species in this clade share white, pink, or pearly tepal colors, presence of tepal spine mucrons, and red/brown glochids (Fig. 2).

Finally, clade C has the same six species as in the analysis based on molecular data alone, which share articulated stem segments; except *T. geometricus* the other five species in this clade share white, pink, or pearly tepal colors, presence of tepal spine mucrons, and red/brown glochids (Fig. 2).

Cytogenetic analyses. — Karyotype features of each species studied are listed in Table 2, and the respective idiograms are displayed in Fig. 3. The absolute chromosome numbers are highly variable: $2n$ = 22, 44, 77, 88, 242 and 319 (Fig. 4), but as a whole, numbers are consistent within a taxon. The three varieties of the polyploid *T. articulatus* have different chromosome numbers: $2n$ = 77, 88 (Table 2). On the other hand, *T. weberi* var. *weberi* have populations with different ploidy levels ($2n$ = 22, 44) and *T. weberi* var. *deminutus* have exclusively diploid populations (Table 2).

The chromosomes are small with mean lengths (C) ranging from 1.40 µm (*T. verschaffeltii*) to 3.07 µm (*T. articulatus* var. *strobiliformis*). Total haploid karyotype length (TLH) varies from 11.77 µm (*T. weberi* var. *deminutus*) to 39.5 µm (*T. recurvatus*). The karyotypes of diploid taxa consist of *m* pairs and 0–1 *sm* pairs (Fig. 3); considering both centromere position and chromosome size variation they are symmetrical with slight variations within each species (A_1 ranges from 0.12 to 0.38 and A_2 from 0.04 to 0.29; Table 2).

With classical staining, no satellites are observed. On the other hand, all species show satellited chromosomes with molecular techniques in a number related to their ploidy level: one pair in $2x$, four pairs in $4x$, eight pairs in $8x$, 10 pairs in $22x$, and 12 pairs in $29x$; satellites are constantly located on the distal parts of short arms (Fig. 3). Banding patterns always show $CMA^+/DAPI^-$ constitutive heterochromatin associated with nuclear organized regions (NORs) in the satellited chromosomes (Table 2), which is the longest pair of the complement (Figs. 3, 5). Additional $CMA^+/DAPI^-$ pericentromeric bands are only found in *T. alexanderi* (Fig. 5A). The total amount of GC-rich

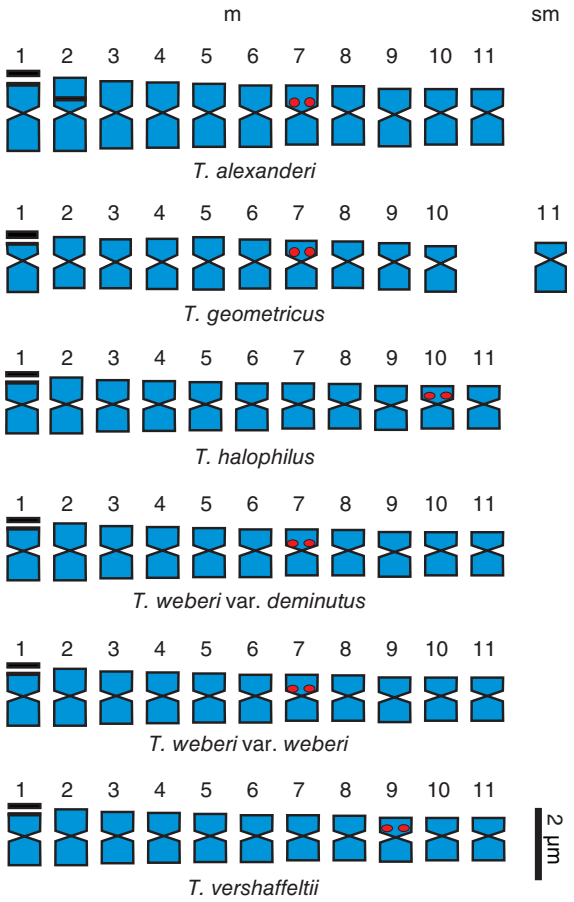

Fig. 3. Idiograms with physical location of repetitive segments in diploid *Tephrocactus*. 18S-5.8S-26S rDNA (black) and 5S rDNA (red). Pair 1 bears satellites in the short arm.

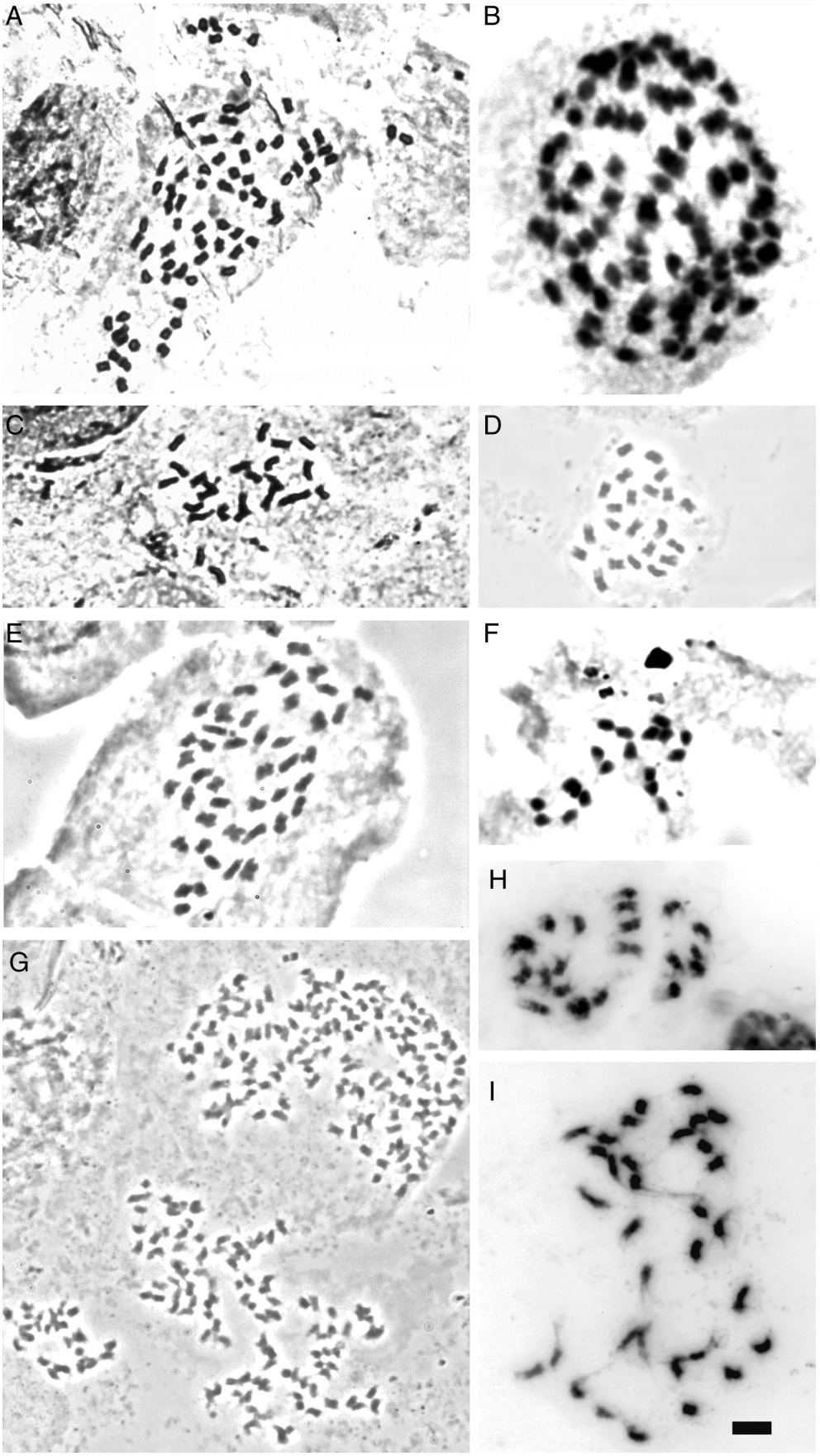

Fig. 4. Somatic metaphases of *Tephrocactus* with Giemsa staining. **A**, *T. articulatus* var. *oligacanthus* (2*n* = 88); **B**, *T. articulatus* var. *strobiliformis* (2*n* = 77); **C**, *T. alexanderi* (2*n* = 22); **D**, *T. geometricus* (2*n* = 22); **E**, *T. weberi* var. *weberi* (2*n* = 44); **F**, *T. weberi* var. *deminutus* (2*n* = 22); **G**, *T. paediophilus* (2*n* = 319); **H**, *T. halophilus* (2*n* = 22); **I**, *T. molinensis* (2*n* = 44). — Bar = 5 µm.

heterochromatin ranges from 2.99% to 6.50% of the total haploid karyotype length (Table 2).

The 18S-5.8S-26S rDNA sites in all species studied coincide with the $CMA^+/DAPI^-$ signals associated with the NOR bands described above (Table 2). The number of 5S sites varies from one pair in 2*x*, two pairs in 4*x*, four pairs in 8*x* to six pairs in 22*x*; they are always proximally located on short arms of different chromosome pairs (Figs. 3, 6).

In all studied taxa, the nuclear DNA content ranges from 2C = 1.99 pg (in *T. recurvatus*, 4*x* = 44) to 2C = 24.50 pg (in *T. paediophilus*, 22*x* = 319) (Table 2; Fig. 1). DNA content has a significant and positive correlation with the ploidy level ($r = 0.90$, $P = 0.001$) and the number of rDNA genes (45S: $r = 0.89$, $P < 0.01$; 5S: $r = 0.85$, $P < 0.01$). These are the only correlations found when comparing all chromosomic variables (suppl. Table S1). Two species exhibit endopolyploidy with 2C, 4C, and 8C cells in the same sample, with different percentages of nuclei in each ploidy level (Table 2).

Mapping of character evolution. — The mapping of cytogenetical characters onto the phylogeny reveals that the most probable ancestral chromosome number is 2*n* = 22 with three polyploidization events (Fig. 7). One event in clade A with all species on the tetraploid level (2*n* = 44). Another event in clade B with *T. weberi*, which has diploid and tetraploid populations. Finally, in some branches of clade C: the branch of *T. articulatus* and its varieties at the hexaploid, heptaploid and octoploid levels (2*n* = 66, 77, 88) and the branch with *T. aoracanthus* and *T. paediophilus* that showed docosaploid and nonaicosaploid levels with the highest numbers (2*n* = 242, 319, respectively).

Regarding the number of rDNA loci, the most probable ancestral condition is one pair of each gene. Afterwards, an amplification of the number of both loci would have occurred correlated with changes in the ploidy level (Fig. 7).

The DNA content suggests that the ancestral species has the lowest content whereas derived clades show the highest. The remaining cytological variables (C, THL, A_1, A_2) do not show a clear pattern in the phylogeny.

The mapping of the morphological characters indicates that the ancestor would have the following characters: dwarf shrub with strong articulation, glochids, and deciduous leaves, tepals without spiny mucrons and white, pink or pearly color. Yellow and red tepals are derived and are acquired in clade B (Fig. 8).

■ DISCUSSION

Phylogenetic analyses. — This is the first phylogeny that incorporates all purported species of *Tephrocactus*. A consensus tree combining morphological and molecular data shows a very close structure to the exclusively molecular tree. The early-diverging clade A1 included *T. bonnieae*, *T. recurvatus*, and *T. molinensis*, all up to now endemic Argentinean species with restricted ranges from the provinces of Catamarca, San Juan, and Salta, respectively. The morphological characteristics of clade C (presence of white, pink, or pearly tepal color, tepal spine mucrons, and red/brown glochids) were previously used to define the genus (Kiesling, 1984). However, *Tephrocactus* s.l. must include clades A and B, based on strong support of two (*trnK-matK*, *psbA-trnH*) molecular and 15 morphological characters supporting its monophyly (Figs. 1–2). *Tephrocactus molinensis*, in particular, has these morphological features typical of clade C, but molecular phylogenies suggest that it is not closely related to it (Griffith & Porter, 2009; Ritz & al., 2012; our data).

Previous data on different samples of species to study Opuntioideae phylogeny also indicate the monophyly of

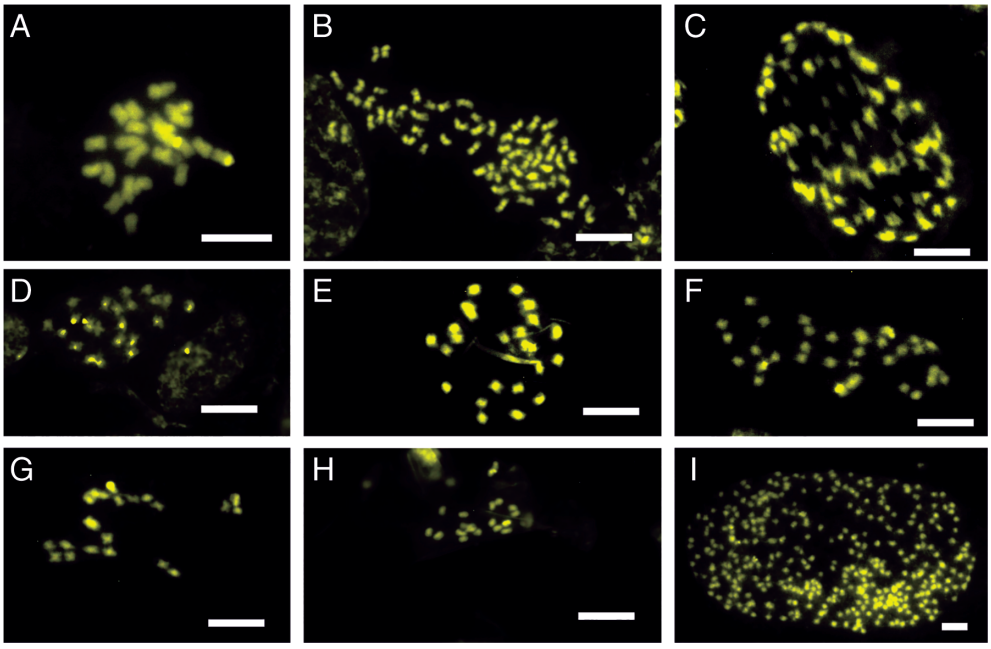

Fig. 5. Fluorochrome chromosome banding in *Tephrocactus* species. **A**, *T. alexanderi*; **B**, *T. articulatus* var. *oligacanthus*; **C**, *T. articulatus* var. *strobiliformis*; **D**, *T. geometricus*; **E**, *T. halophilus*; **F**, *T. molinensis*; **G**, *T. weberi* var. *weberi*; **H**, *T. weberi* var. *deminutus*; **I**, *T. paediophilus*. — Bars = 10 µm.

Tephrocactus and the inclusion of *Puna bonnieae* and *Maihueniopsis nigrispina* (Griffith & Porter, 2009; Ritz & al., 2012) and *Austrocylindropuntia verschaffeltii* and *Cumulopuntia recurvata* (Ritz & al., 2012), as members of the genus. These authors used a smaller sample, other plastid DNA and nuclear genes, and no morphological traits; thus, the structure of the obtained trees is slightly different. However, the close relationship among *T. articulatus*, *T. alexanderi*, and *T. aoracanthus*, between *T. molinensis* and *T. recurvatus*, and between *T. nigrispinus* and *T. verschaffeltii* is clear (Griffith & Porter, 2009; Ritz & al., 2012). At the same time, *T. weberi* has an unstable position in the different trees.

Tephrocactus is strongly monophyletic including 12 well-defined species. According to our results, *T. paediophilus* is a distinct species. Cytogenetically, it is unique, showing the highest ploidy level for the genus and the highest number for Cactaceae. It has the longest spines of Tephrocacteae, and possibly from the whole family, a trait probably associated with its polyploidy; morphologically, its spines are twisted, cylindrical, grooved, and located all around the stem segments, and spiny mucrons only on the upper tepals. It inhabits very salty environments from La Rioja Province (Argentina). Its closest relative is *T. aoracanthus*, with which it shares a different high chromosome number, high DNA content, and several loci for the rDNA genes. On the other hand, *T. aoracanthus* has shorter spines, which are rigid, straight, without grooves and located only on the upper half part of the stem segments, and tepals with strong spiny mucrons.

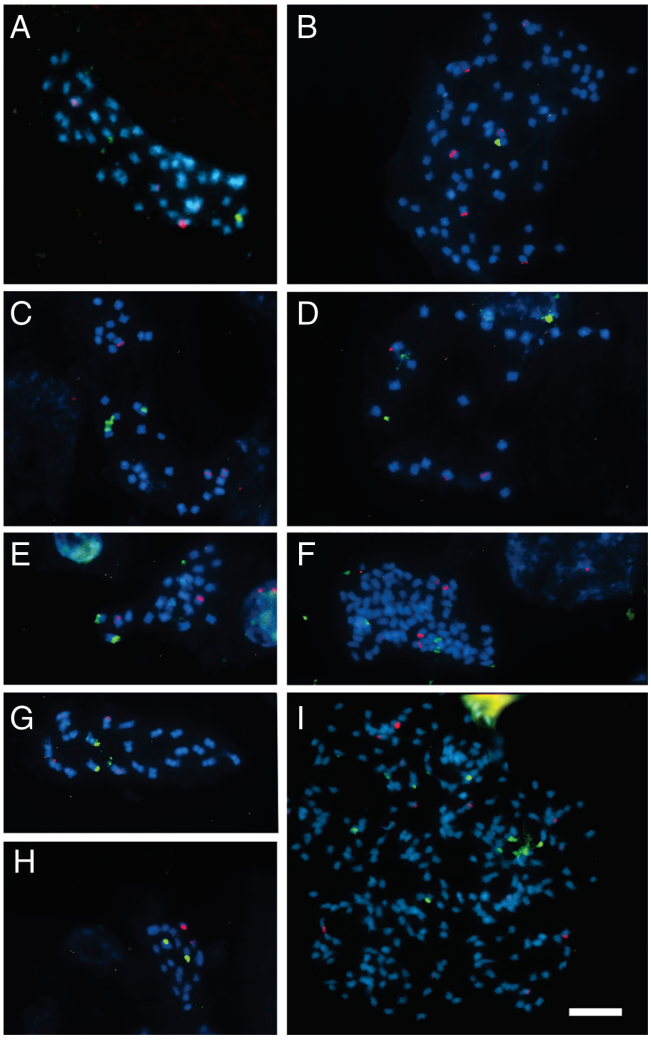

Fig. 6. Somatic chromosomes detected by FISH using 18S-5.8S-26S (green) and 5S rDNA (red) probes in *Tephrocactus* species. **A**, *T. articulatus* var. *oligacanthus*; **B**, *T. articulatus* var. *strobiliformis*; **C**, *T. alexanderi*; **D**, *T. geometricus*; **E**, *T. halophilus*; **F**, *T. molinensis*; **G**, *T. weberi* var. *weberi*; **H**, *T. weberi* var. *deminutus*; **I**, *T. paediophilus*. — Bar = 5 µm.

Tephrocactus geometricus is a valid species, as previously considered by Kiesling (1984) and supported by Anderson (2001) and Gorelick & Mahr (2013), although other authors interpreted it as a form of *T. alexanderi* (Hunt & al., 2006). Our phylogeny, the only one that included this species, clearly shows that molecular and morphological data separate it from *T. alexanderi* and *T. halophilus*. The same is true for *T. halophilus*, which appeared as a distinct species and was never analyzed phylogenetically before.

For *T. articulatus*, up to eight varieties have been accepted (Backeberg, 1966; Gorelick & Mahr, 2013). As it is the species with the widest distribution of the genus, more samples and varieties should be examined to understand their variability and validity. As *T. weberi* is phenotypically and cytologically variable, more samples have to be analyzed for the same purpose.

Cytogenetic analyses. — Almost all taxa examined were cytologically unknown (Table 2). All of them show the documented base number for Cactaceae of $x = 11$ (e.g., Bandyopadhyay & Sharma, 2000; Las Peñas & al., 2009, 2011, 2014, 2017). Interestingly, they present an euploid series in which diploids and tetraploids are the most frequent, followed by octoploids with one species and two varieties. Two species show higher numbers: $22x$ and $29x$. These numbers may have originated by autopolyploidy after processes of autoduplication. For *T. paediophilus*, the number $2n = 319$ is confirmed, a species previously reported with ca. 330 (Yuasa & al., 1973). Presently, this is the third-highest number reported for an angiosperm, after *Sedum suaveolens* (Crassulaceae) with $2n$ = ca. 640 (Uhl, 1978) and *Voanioala gerardii* (Arecaceae) with $2n$ = ca. 606 (Röser, 1994).

Nuclear DNA content data are here reported for *Tephrocactus* the first time. The Cx value of the diploid variety of *T. weberi* (var. *deminutus*) is higher than the tetraploid variety (var. *weberi*). Thus, genome downsizing occurred, as reported in *Maihuenia patagonica* (Phil.) Britton & Rose (Las Peñas & al., 2014) and in other angiosperms (Leitch & Bennett, 2004; Scaldaferro & al., 2012). There are few Cactaceae examined that present a range of Cx values from 0.54 to 3.46 pg (species of *Brasiliopuntia*, *Consolea*, *Escobaria*, *Maihuenia*, *Mammillaria*, *Opuntia*, *Pereskia*, *Rebutia*, and *Stetsonia*; Palomino & al., 1999; Zonneveld & al., 2005; Del Angel & al., 2006;

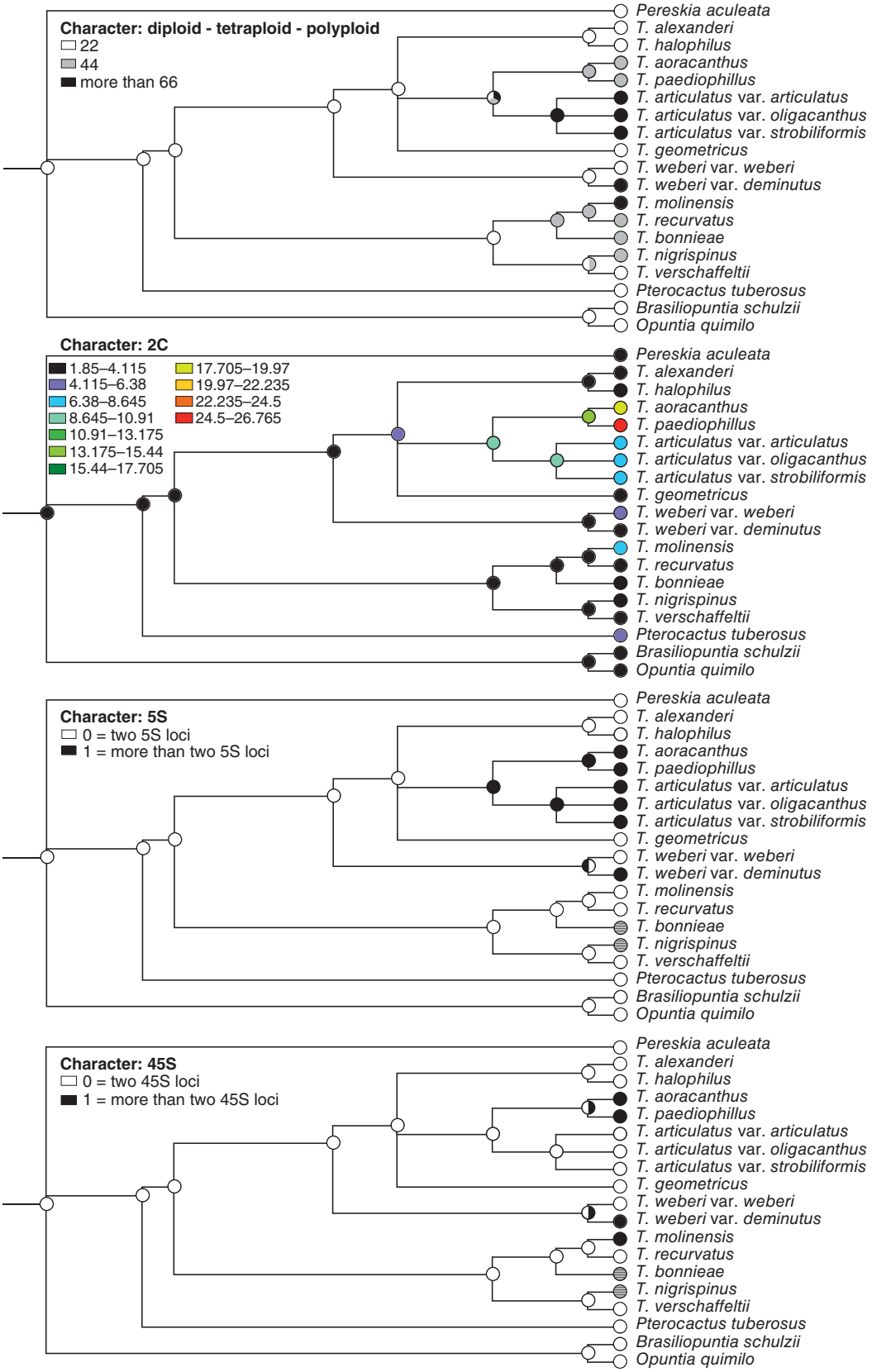

Fig. 7. Ancestral state reconstruction of cytogenetic traits under maximum parsimony. Characters are presented on an ultrametric topology of the Bayesian consensus tree. Light grey/dark grey circles indicate missing data.

Negrón-Ortiz, 2007; Las Peñas & al., 2014, 2017; Bauk & al., 2016). The average nuclear DNA content of *Tephrocactus* species is 2C = 6.59 pg (range 1.99–24.50). Certainly, this figure is higher than the ones reported for cacti, although most species show small C-values. This is due to the fact that *T. aoracanthus* and *T. paediophilus* have larger contents; both species are included in the range of large C-values for angiosperms according to Bennett & al. (2000). Leitch & Leitch (2008) suggested that species with small C-values are more evolutionarily flexible and able to colonize new and more diverse environments.

Thus, polyploidy is a relevant phenomenon in the genus *Tephrocactus*. It has been reported for around 25% of Cactaceae (cf. Pinkava, 2002; Majure & al., 2012; Las Peñas & al., 2017). Particularly, polyploidy seems to have played an important role in the evolution of Opuntioideae, in which *Tephrocactus* is included, with ca. 65% polyploids. In this genus, polyploidy is associated with asexual reproduction, as reported in other families (Mable, 2004; Baldwin & Husband, 2013). Clade C has the highest ploidy level and includes clonal species with stem segments that easily break and form new individuals. At the same time, seeds of these

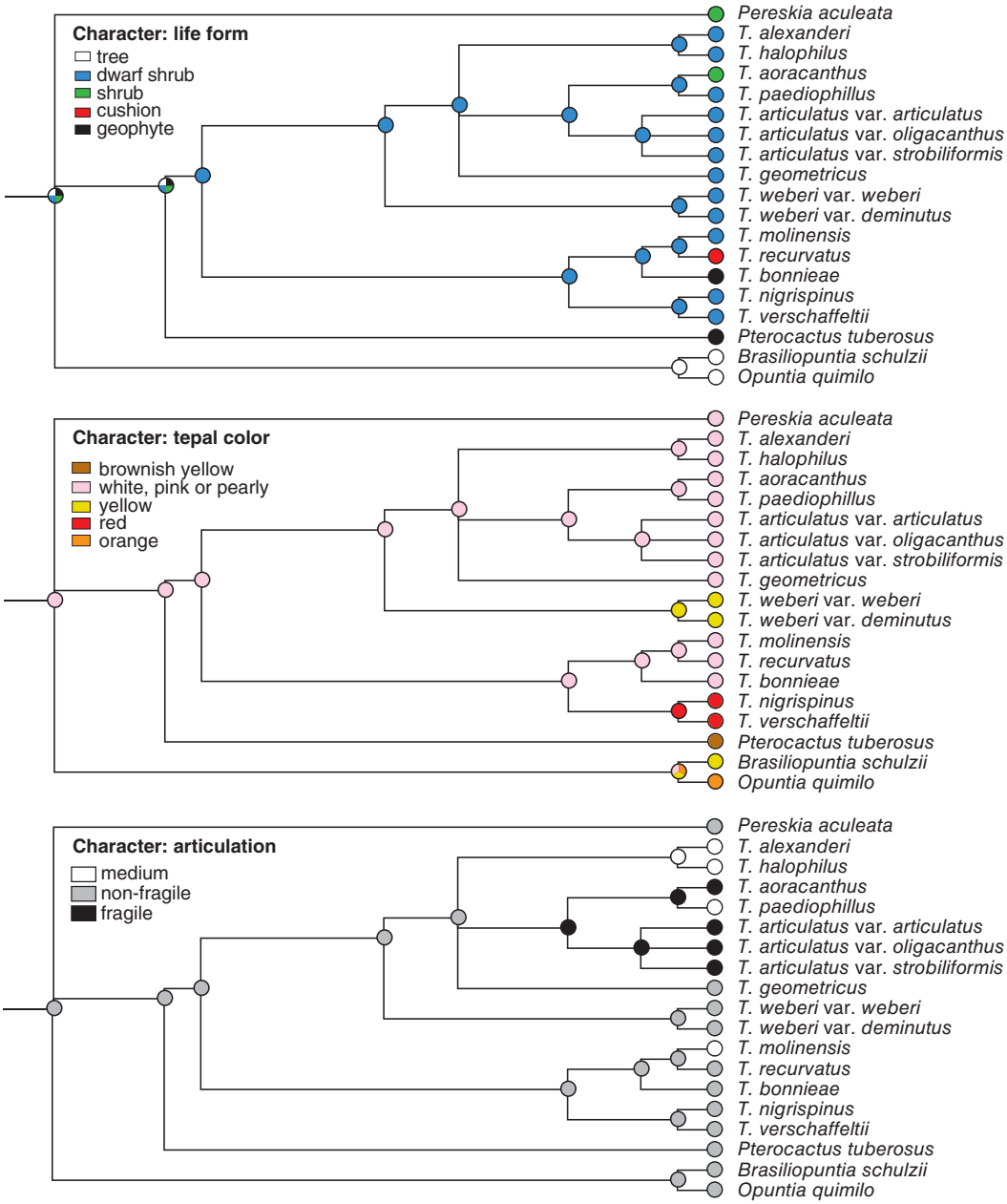

Fig. 8. Ancestral state reconstruction of morphological traits under maximum parsimony. Characters are presented on an ultrametric topology of the Bayesian consensus tree.

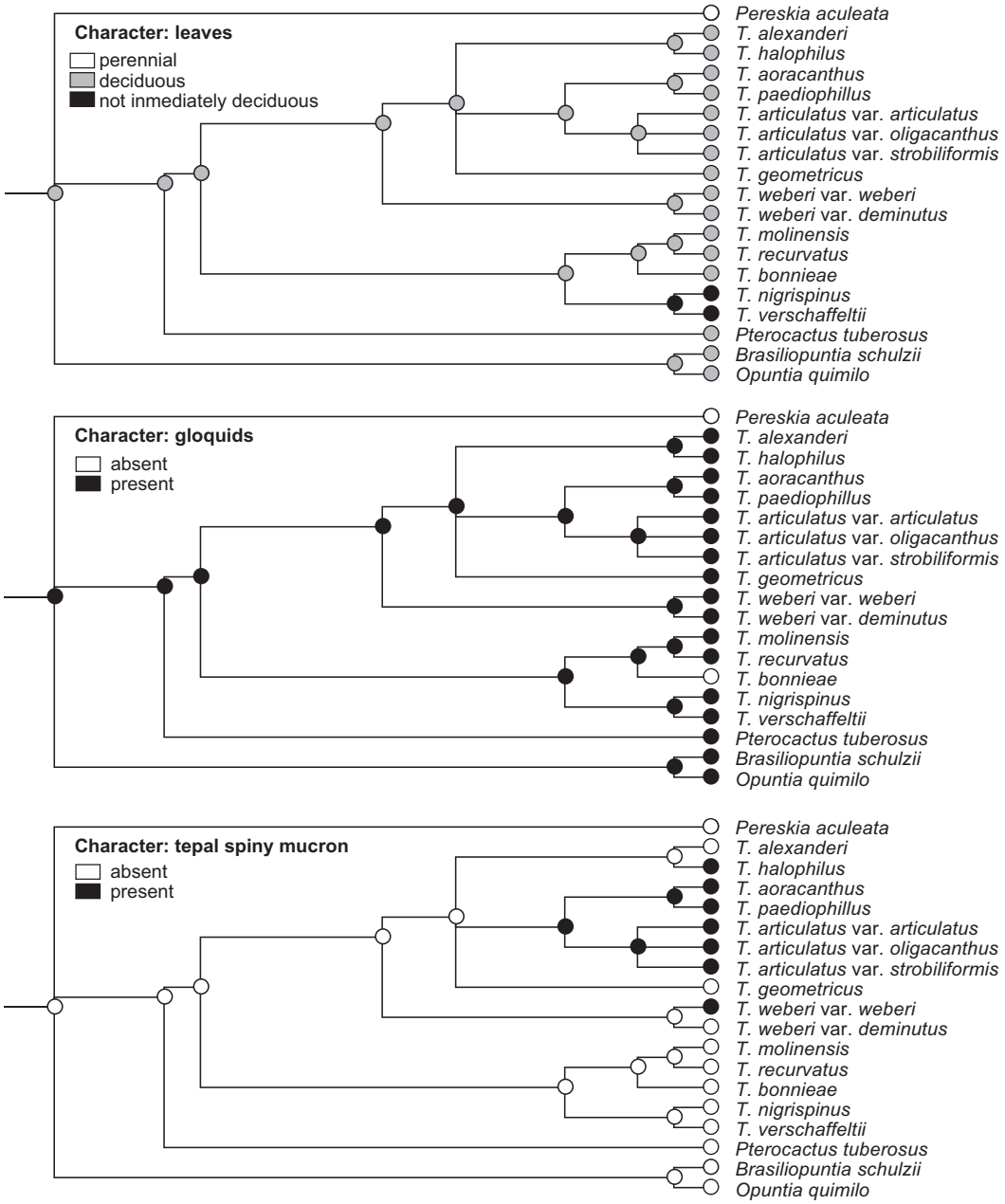

Fig. 8. Continued.

species germinate with difficulty (M.L. Las Peñas, unpub.). Vegetative reproduction was proposed as a prerequisite for the evolution of polyploidy (Stebbins, 1950; Baldwin & Husband, 2013).

All taxa analyzed have comparatively small chromosomes as it is common in cacti (Cota & Philbrick, 1994; Bandyopadhyay & Sharma, 2000; Las Peñas & al., 2008, 2009, 2011, 2014, 2017; Moreno & al., 2015a,b). At the same time, complements show rather homogeneous chromosome size. Karyotypes analyzed are the first reported in the genus and were performed only in the diploid species because of the small size and similarity of the chromosomes. They are mostly symmetrical and composed of *m* chromosomes, as in many Cactaceae (Cota & Philbrick, 1994; Bandyopadhyay & Sharma, 2000; Las Peñas & al., 2008, 2009, 2011, 2014, 2017; Moreno & al., 2015a,b). The presence of the longest chromosome pair with satellites attached on short arms was found in the species herein studied, as it is the rule in the scarce species examined so far (Las Peñas & al., 2008, 2009, 2011, 2014, 2017; Moreno & al., 2015a,b). Karyotype features do not allow individual species to be distinguished. Thus, chromosome stability has accompanied evolutionary divergence of the taxa studied, as detected in other families (e.g., Solanaceae: Stiefkens & al., 2010) and Cactaceae as well (Las Peñas & al., 2008, 2009, 2014, 2017; Moreno & al., 2015a,b).

The pattern of $CMA^+/DAPI^-$ band numbers and heterochromatin amounts are relatively conserved at the generic level in most angiosperms (e.g., Guerra, 2000). In species of *Tephrocactus*, like in the other few Cactaceae studied, the longest chromosome pair has $CMA^+/DAPI^-$ NOR-bearing bands. So far, these results suggest a chromosomic homeology in the family (Las Peñas & al., 2008, 2009, 2011, 2014, 2017; Moreno & al., 2015a,b; Castro & al., 2016).

Sites of 18S-5.8S-26S genes located in terminal regions of short chromosome arms are frequent in angiosperms (Acosta & al., 2016; Chiarini & al., 2017). This locus seems to coincide with a $CMA^+/DAPI^-$ NOR-associated band of the first pair in the few Cactaceae reported (Las Peñas & al., 2009, 2011, 2014, 2017; Moreno & al., 2015a,b; Castro & al., 2016). In the *Tephrocactus* species studied, with the only exception of *T. recurvatus*, the number of 18S-5.8S-26S genes increases with the ploidy level. On the other hand, the 5S rDNA loci have a variable position in the family (Las Peñas & al., 2011, 2014, 2017; Moreno & al., 2015a,b; Castro & al., 2016). A positive correlation is detected between DNA content, chromosome number, and number of rDNA genes. This correlation may be related to the recent origin of the polyploids in *Tephrocactus*.

Character mapping. — Cytological and morphological character mapping and high-throughput sequencing approaches are valuable for providing insights into the origin of diverse karyotype features in angiosperms (e.g., Lan & Albert, 2011; Acosta & al., 2016; Chiarini & al., 2017; Deanna & al., 2018).

The *Tephrocactus* ancestor is clearly diploid. The diversification of the genus was accompanied by polyploidy in all clades. In clade A, species are diploid, tetraploid or octoploid, and in clade B they are diploid or tetraploid. The derived clade C is the most variable in ploidy levels (from $2x$ to $29x$), reaching the highest numbers. The increase in ploidy level in the different clades is associated with an increase in DNA content and numbers of rDNA genes.

Some morphological features used to distinguish species (growth form, leaves, spines, glochid color) are homoplastic. The ancestral, and most conserved, life-form is dwarf shrub; only *T. aoracanthus* is a shrub, *T. recurvatus* a cushion, and *T. bonnieae* a geophyte, all autapomorphies of these species. The ancestor and the basal clades A and B have a non-fragile stem articulation, whereas clade C has the fragile stem synapomorphy. Deciduous leaves are conserved, except in clade B in which they are not immediately deciduous. The lack of glochids is an autapomorphy of *T. bonnieae*, and tepal spiny mucrons is a synapomorphy of clade C.

■ CONCLUSIONS

Tephrocactus is strongly monophyletic including 12 well-defined species. Morphologically, it can be characterized as forming small clumps with globose to cylindrical stem segments often easily detached, with moniliform branching, apical flowers usually pearly white to pink, generally with sunken areolas, dry fruits, and seeds with corky auriculated arils (except *T. recurvatus* and *T. verschaffeltii*). The great diversification in the genus has been associated with polyploidy and few chromosome rearrangements visible with conventional staining, i.e., large duplications, pericentric inversions, and reciprocal translocations of segments of unequal size. In this sense, cumulative small and cryptic structural changes are proposed to play an important karyoevolutionary role in Cactaceae. The ancestor would have these characters: diploid ($2n = 22$, $x = 11$), with a small genome ($2C < 1.85$ pg), two asyntenic rDNA loci, dwarf shrub with strong articulation, glochids, deciduous leaves, and white, pink or pearly tepals without spiny mucrons. Other flower colors are derived and acquired only in clade B.

■ AUTHOR CONTRIBUTIONS

Design of the research: MLLP; performance of the research: MLLP, RK, GB; data analysis, collection, or interpretation: MLLP, RK, GB; plant collection: MLLP, RK; writing the manuscript: MLLP, RK, GB. — MLLP, https://orcid.org/0000-0003-4244-7807; GB, https://orcid.org/0000-0002-1915-9949

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Appendix 1. Morphological characters and coding used in character mapping of *Tephrocactus*.

	Character														
Species	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Tephrocactus alexanderi	2	1	1	1	1	1	0	2	1	1	2	0	1	0	2
T. aoracanthus	2	1	2	1	1	1	0	2	1	2	3	2	1	1	2
T. articulatus var. *articulatus*	2	1	1	1	1	1	1	3	1	2	0	2	0	1	2
T. articulatus var. *oligacanthus*	2	1	1	1	1	1	0	3	1	2	1	2	0	1	2
T. articulatus var. *strobiliformis*	2	1	1	1	1	1	1	0	1	2	4	2	0	1	2
T. bonnieae	1	1	3	1	1	1	0	2	0	0	2	1	1	0	2
T. geometricus	2	1	1	1	1	1	1	2	1	2	2	1	0	0	2
T. halophilus	2	1	1	1	1	1	0	1	1	2	2	0	1	1	2
T. molinensis	2	1	1	1	1	1	1	0	1	2	2	0	1	0	2
T. nigrispinus	0	1	1	2	0	3	0	1	1	1	4	1	1	0	1
T. paediophilus	2	1	1	1	1	1	0	4	1	2	0	0	1	1	1
T. recurvatus	1	1	4	1	0	1	0	2	1	1	2	1	1	0	1
T. verschaffeltii	2	1	1	2	0	3	0	1	1	1	1	1	0	0	2
T. weberi var. *deminutus*	2	1	1	1	1	2	0	1	1	2	1	1	1	1	2
T. weberi var. *weberi*	2	1	1	1	1	2	0	1	1	2	1	1	1	0	2
Brasiliopuntia schulzii	2	0	0	1	0	2	0	2	1	1	3	1	1	1	2
Opuntia quimilo	2	0	0	1	0	4	0	2	1	1	3	1	0	0	2
Pereskia aculeata	1	0	2	0	0	1	0	2	0	0	1	1	1	0	0
Pterocactus tuberosus	1	1	4	1	1	0	0	2	1	1	1	1	1	0	1

0: root (0 slightly tuberous, 1 tuberous, 2 fibrous); **1: growth form** (0 indeterminate, 1 determinate); **2: life form** (0 tree, 1 dwarf shrub, 2 shrub, 3 cushion, 4 geophyte); **3: leaves** (0 perennial, 1 deciduous, 2 not immediately deciduous); **4: pericarp** (0 juicy indehiscent, 1 dry dehiscent); **5: tepal color** (0 brownish yellow, 1 white, pink or pearly, 2 yellow, 3 red, 4 orange); **6: spines** (0 present, 1 absent); **7: spine type** (0 absent, 1 needle like, 2 subulate, 3 flat, 4 cylindrical, grooved); **8: glochids** (0 absent, 1 present); **9: glochid color** (0 absent, 1 pink, yellow or white, 2 red brown); **10: stem segment form** (0 globose to ovate, 1 cylindrical, 2 globose, 3 ovate, 4 sub-cylindrical); **11: articulation** (0 medium, 1 non-fragile, 2 fragile); **12: pericarpel spines distribution** (0 absent, 1 present); **13: tepal spiny mucron** (0 absent, 1 present); **14: aril consistence** (0 present, 1 hard, 2 corky, 3 papery).