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Cytology and embryology of the pompom weed, *Campuloclinium macrocephalum* (Eupatorieae, Asteraceae)

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Abstract

Campuloclinium macrocephalum (Less.) DC. is a perennial herb widely distributed in the New World, but introduced to South Africa, where it is commonly called "pompom weed". This species is considered one of the most important weeds from Brazil and it has been included among the plant invaders of South Africa. Results of the meiotic and embryological analyses of six populations of *C. macrocephalum* are reported in this paper. The microsporogenesis analysis revealed five triploid ($2n=3x=30$) and one diploid ($2n=2x=20$) populations with a basic chromosome number $x=10$. The diploid specimens showed regular meiotic behavior, but the triploid plants presented irregular chromosome pairing which result in the formation of univalents, bivalents and trivalents at diakinesis. In consequence, laggard chromosomes, unbalanced nuclei and micronuclei were observed in subsequent phases of meiosis. The embryological analysis showed that the triploid specimens of *C. macrocephalum* have embryo sac development from a nucellar cell (apospory), which indicates that these specimens are apomictic. Almost all cases of apomixis found in tribe Eupatorieae are diplosporous apomixis. *Campuloclinium macrocephalum* constitutes the second species of the tribe and the first of the genus with apospory as reproductive system. The aposporous apomixis combined with the presence of xylopodium would be two important factors responsible for the invasiveness of *C. macrocephalum*.

Keywords: Apomixis; Spospory; *Eupatorium*; Triploid; Weed

1. Introduction

Campuloclinium macrocephalum (Less.) DC. is a perennial herb widely distributed in the New World, from Mexico to Argentina (Cabrera, 1974). In southern South America, its distribution area includes Brazil, Bolivia, Paraguay, Uruguay and the north of Argentina (Freire, 2008). In the 1970s, this taxa was introduced to South Africa, where it is commonly called "pompom weed", probably in reference to the large size of the flower heads.

This species is considered a medicinal plant in Paraguay where it is mostly used as anti-inflammatory and sedative (Vega et al., 2008). However, *C. macrocephalum* has been recognized by Holm et al. (1979) as one of the most important weeds of Brazil and it was also included by Henderson (1995, 2007) and Foxcroft et al. (2007) among the plant invaders of South Africa. *Campuloclinium macrocephalum* was probably introduced to South Africa about 30 years ago, where it has invaded undisturbed climax grasslands, wetlands and roadsides in several provinces, reducing the grazing potential of grasslands and impacting on biodiversity (Wells et al., 1986; Klein and Neser, 1999). Due to the absence of natural enemies of *C. macrocephalum*, several species of thrips from South America are currently being tested as a potential biological control agent against this invasive weed in South Africa (Mound and Pereyra, 2008).

In recent years, several studies on the biology of *C. macrocephalum* have been published. Cortadi and Gattuso (1994) provided a biochemical and anatomical characterization. Vega et al. (2008) determined the chemical composition of the

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Table 1
Populations of *C. macrocephalum* analyzed.

Population	Voucher
A	Paraguay. Dept. Amambay: Parque Nacional Cerro Corá. Around the monument to Mariscal López. *M. Dematteis and A. Schinini 883* (CTES).
B	Argentina. Corrientes: Dept. Mercedes. 8 km W of Felipe Yofre, sandy hills. *M. Dematteis and M. Seo 2467* (CTES).
C	Argentina. Corrientes: Dept. Capital. Campus of the Universidad Nacional del Nordeste. *G. E. Farco 1* (CTES).
D	A. Argentina. Corrientes: Dept. San Roque. 5.3 km S of San Roque, next to the road. *M. Dematteis* et al. *2762* (CTES).
E	B. Argentina. Corrientes: Dept. Bella Vista. 10 km N of the bridge on the river Santa Lucia. *G. E. Farco 4* (CTES).
F	C. Argentina. Corrientes: Dept. Saladas. Route 117, next to the access to Saladas. *G. E. Farco 5* (CTES).

species, while Marzinek and Oliveira (2010) determined the structure and ontogeny of the pericarp. Recently, Goodall et al. (2010) tested the allelopathy to explain the invasiveness of *Campuloclinium macrocephalum* in the South African grassland biome. Despite these studies, all the remaining areas of the biology of *C. macrocephalum* are still poorly known.

From the cytological viewpoint, *C. macrocephalum* has been previously analyzed and almost all the chromosome studies indicate $2n=20$, $2n=40$ or $2n=ca.40$. Turner et al. (1979) found $n=$ca.20II for specimens from the province of Santa Fe (Argentina). A meiotic analysis on a population of Buenos Aires (Argentina) recorded 19II+1I or 20II+1I (Galiano and Hunziker, 1987). The most recent analysis recorded $2n=20$ and $2n=40$ for two samples from Paraguay, suggesting that the species is cytologically heterogeneous, having populations with different ploidy levels (Dematteis et al., 2007). However, at the present relatively little is known regarding the meiotic behavior and the reproduction of *C. macrocephalum*.

In this paper was carried out a meiotic and embryological analysis of different populations of *C. macrocephalum*, in order to contribute data that would aid our understanding of the reproduction of this species.

2. Material and methods

2.1. Plant materials

The six populations of *C. macrocephalum* analyzed in this study were collected in Argentina and Paraguay (Table 1). The voucher specimens were deposited in the herbarium of the Instituto de Botanica del Nordeste (CTES). The sources of the samples are summarized in Table 1.

2.2. Meiotic analysis

Meiosis was studied in floral buds fixed in a 5:1 lactic acid–ethanol solution, transferred to 70% ethanol and then stored at 4 °C for subsequent examination. The anthers were macerated and stained with 2% lacto-propionic orcein, and permanent preparations were made using Euparal as mounting media. The slides were examined using a Zeiss Axioplan microscope and the images were obtained with a Nikon digital camera. The analysis included the chromosome count, determination of the ploidy level, analysis of the meiotic behavior and establishment of the type of polyploidy in all six populations.

2.3. Embryological studies

Buds for the embryological studies were fixed in FAA (formaldehyde–acetic acid–ethanol) solution for 48 h and stored in 70% ethanol at 4 °C. Longitudinal sections of buds in different stages were prepared according to the standard techniques. Permanent microscope slides were obtained by

Table 2
Meiotic configurations observed in six populations of *C. macrocephalum*.

Population	2n	Ploidy level	Meiotic configurations	Percentage	Number of cells
A	30	3x	2I+2II+8III	50	2
			20I+2II+1IV	50	
B	30	3x	4I+8II+1IV+1VI	20	5
			11I+5II+3III	20	
			6I+6II+4III	20	
			3I+3II+7III	20	
			8I+5II+4III	20	
C	30	3x	3II+8III	25	4
			1I+7II+5III	25	
			2I+8 II+4III	25	
			9II+4III	25	
D	30	3x	10I+10II	5.55	15
			3I+9II+3III	5.55	
			7I+7II +3III	16.66	
			11I+8II+1III	5.55	
			7I+10II+1III	5.55	
			8I+8II +2III	5.55	
			7I+9II+3III	5.55	
			4I+10II+2III	5.55	
			5I+8II+3III	16.66	
			5I+11II +1III	5.55	
			7I+8II+2III	5.55	
			6I+6II+4III	5.55	
			6I +9II+2III	5.55	
E	30	3x	4I+13II	5.55	18
			6I+6II +4III	11.11	
			10I +7II+2III	5.55	
			4I+10II+2III	5.55	
			4I+3II+6III	5.55	
			3I+5II+5III	5.55	
			8I+8II+2III	5.55	
			6I+8II+2III	11.11	
			2I+5II+6III	5.55	
			1I+10II+3III	5.55	
			3I+6II+5III	11.11	
			5I+8II+3III	5.55	
			5I+5II+5III	5.55	
			6I+5II+4III	5.55	
			4I+4II+6III	5.55	
F	20	2x	10II	100	18

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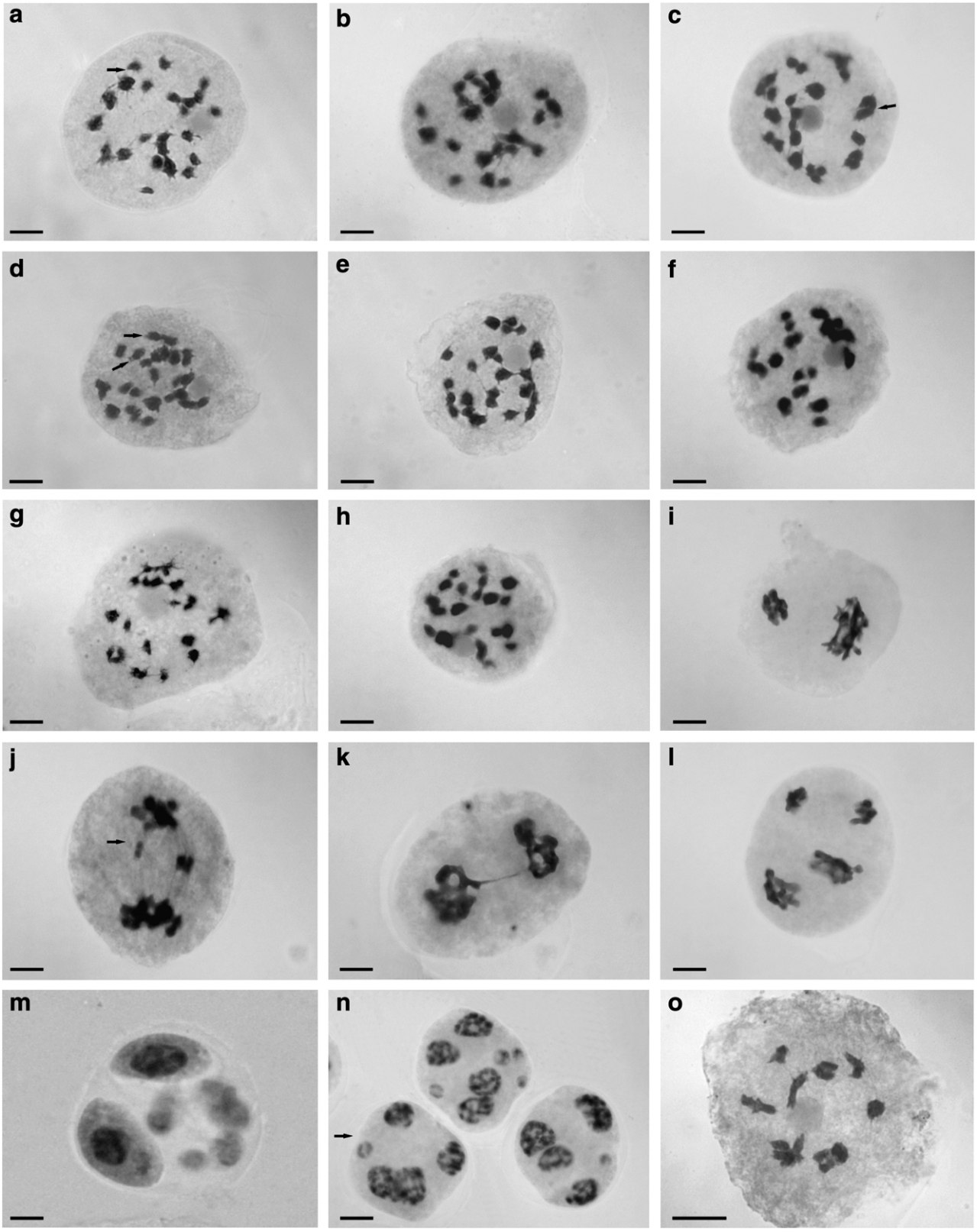

Fig. 1. Microsporogenesis in triploid and diploid specimens of *C. macrocephalum*. (a–n) Meiotic division in population D (triploid), (o) diakinesis in population F (diploid). (a) 11I+8II+1III; (b) 5I+8II+3III; (c) 3I+9II+3III; (d) 10I+10II and showing microsatellites; (e) 8I+8II+2III; (f) 1I+10II+3III; (g) 3I+6II+5III; (h) 7I+7II+3 III; (i) chromosome distribution at anaphase I; (j) anaphase I showing three lagging chromosomes; (k) anaphase I showing a bridge; (l) telophase II showing differences in the nuclei in formation; (m) polyploid with two normal microspores and six micro-microspores; (n) polyads; (o) diploid cytotype, 10II. Scale bar=5 μm.

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dehydration through an ethanol series with a rinsing pre-impregnant. Techniques proposed by Johansen (1940) were used for paraffin infiltration, after which the material was imbedded in Histoplast ®. Sections of 10–12 µm were cut with a rotary microtome and stained with Astra blue-safranin (Luque et al., 1996) before being mounted with synthetic Canada Balsam. Observations and photography were performed using a Leica DM LB2 microscope, equipped with a digital camera.

2.4. Pollen fertility

The evaluation of the pollen viability was done using the acetic carmine-glycerol technique proposed by Marks (1954). The pollen grains were stained for about 12 h and at least 200 grains for each sample were analyzed to establish the pollen viability. Well-filled pollen grains with stained nuclei were considered fertile while unstained pollen grains were regarded as sterile.

3. Results

3.1. Meiotic behavior

The results of the meiotic analysis from the six populations of *C. macrocephalum* are detailed in Table 2. Five samples (populations A–E) were triploid with somatic chromosome number $2n=30$, while only one population was diploid having $2n=20$ (population F).

The diploid plants showed a regular behavior with exclusive formation of 10 bivalents at diakinesis and without irregularities in successive phases of meiosis (Fig. 1). However, the triploid populations showed a wide variation of meiotic configurations within each sample (Fig. 1a–h). The number of univalents ranged from 2 to 11, the bivalents from 2 to 13, while the trivalents varied between 1 and 8 in the different populations examined (Table 2).

Due to the different meiotic configurations, the triploid populations showed a great number of irregularities at metaphase I and anaphase I (Table 3). In populations A, B and C 100% of cells in metaphase I had chromosomes outside of the division plane. Populations D and E showed a smaller proportion of irregular metaphase plates, namely 89.44% and 84.85% respectively.

In anaphase I, the triploid populations showed regular behavior in 42.59% to 69.17% of the cells. The remaining cells in anaphase I displayed a wide variation of irregularities (Fig. 1i–k) such as chromosome bridges (1.61%–8.19%), bridges and fragments (0.00%–7.68%), laggard chromosomes (25.70%–47.59%) and laggard chromosomes accompanied by bridges and fragments (0.00%–2.56%). Laggard chromosomes and fragments led to the formation of micronuclei at the first and second meiotic divisions. Micronuclei gave rise to microcytes at the tetrad stage, which in turn developed into small, sterile pollen grains (Fig. 2).

Meiosis resulted in a high frequency of irregular meiotic products such as dyads, triads and tetrads having a variable number of microcytes, occasionally with micronuclei (Fig. 1m–n). The percentage of irregular tetrads in triploid populations varied between 23.70% and 75.72%, while in the diploid sample it was always regular (Table 3). The proportion of regular and abnormal tetrads was determined in all the specimens, except in population A, where the sample had no florets at this stage. The tetrads were classified into different types (1 to 35), according to the number and size of the microspores and micronuclei. The different types of meiotic products are described in Table 4. All the meiotic products observed are shown in Fig. 2.

Table 3
Percentage of meiotic abnormalities in different meiotic phases.

	A	B	C	D	E	F
Metaphase I						
Regular (%)	0	0	0	10.52	15.15	100
Chromosomes outside plate (%)	100	100	100	89.44	84.85	0
Number of cells analyzed	36	28	40	57	66	50
Anaphase I						
Regular (%)	42.59	52.98	63.70	69.17	51.28	100
Laggards (%)	47.59	42.55	34.69	25.70	34.64	
Bridges with fragment (%)	1.63	0	0	2.80	7.68	0
Bridges without fragment (%)	8.19	3.73	1.61	2.33	3.84	0
Laggards+bridges+fragments		0.74			2.56	
Number of cells analyzed	75	134	124	386	78	50
Tetrads						
Regular (%)	–	24.27	43.01	76.30	46.54	100
Irregular (%)	–	75.72	36.98	23.70	53.46	0
Number of cells analyzed		103	93	249	318	50
Pollen fertility						
Fertile (%)	25.25	32.04	51.67	58.42	57.40	99.35
Sterile (%)	74.74	67.95	43.32	41.57	42.60	1.36
Number of cells analyzed	891	1554	538	1027	1242	514

Pollen fertility of the triploid populations ranged widely between 25.25% and 58.42%, while it was 99.35% in the diploid sample. Variation was also observed in the size of pollen grains, which ranged from very small (micrograins) to double the size of the regular pollen.

3.2. Female gametophyte development

The embryological analysis of population C showed that the individuals possess one anatropous ovule per floret. Megasporogenesis was observed in the early developmental stages, while the complete sequence of the megagametogenesis process was studied in more advanced phases.

The complete sequence of the meiotic division of the megaspore mother cell, which is clearly distinguished from the remaining cells by the presence of a large vacuole (Fig. 3a) was studied. The first division of the megaspore mother cell (Fig. 3b), which is always transverse, resulted in a dyad. Subsequently, in

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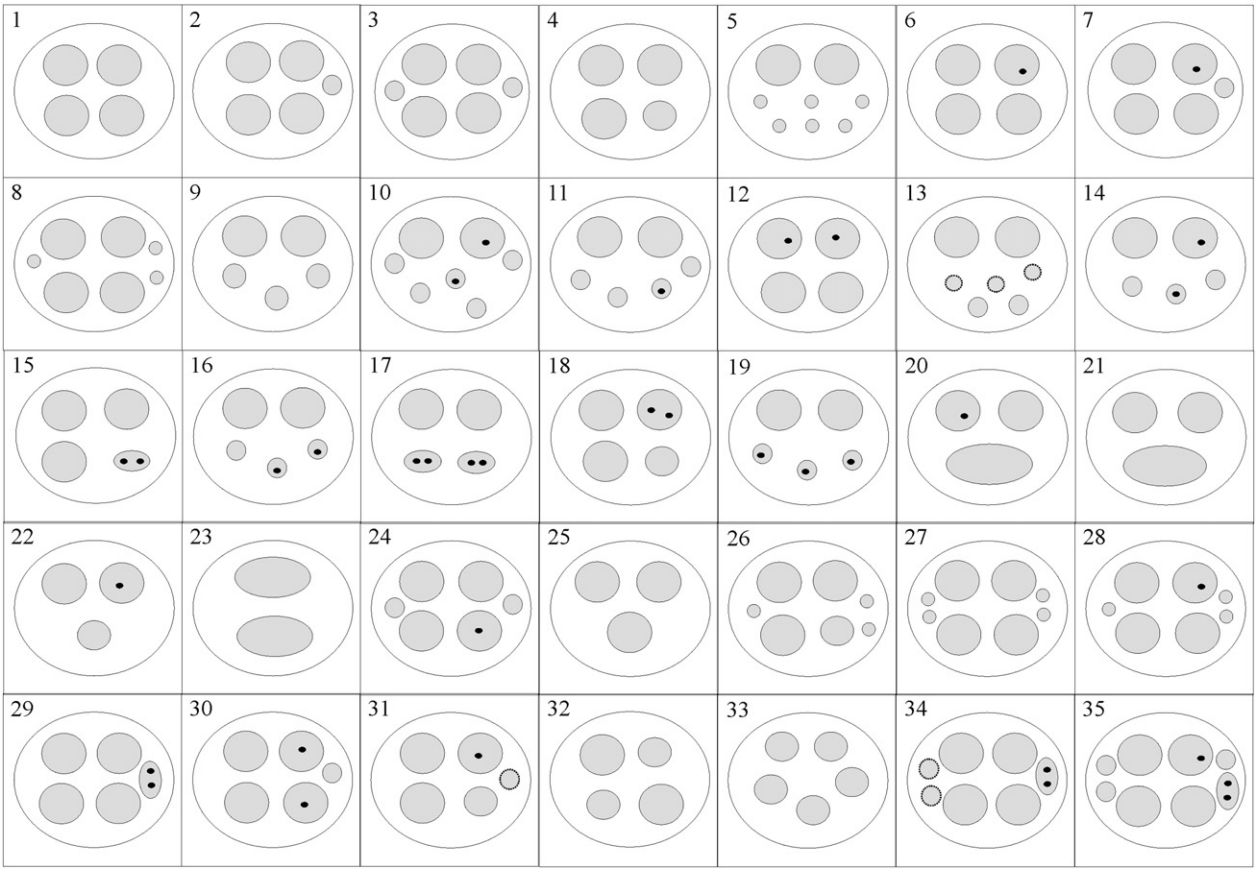

Fig. 2. Microspore types present in the triploid populations of *C. macrocephalum.*

the second division, it produced a tetrad of four megaspores which are arranged in a linear arrangement.

Most of the megaspores degenerate immediately after the second meiotic division. The megaspore is displaced by other cells, in general from the nucellus (Fig. 3i). The nuclear cell develops an aposporic embryo sac type *Hieracium*. The ovules with two embryo sacs, one sexually deteriorated and the other aposporic well developed are shown in Fig. 3 g–h.

4. Discussion

From the cytological viewpoint, the tribe Eupatorieae is relatively poorly known. At present chromosome numbers of about 467 taxa of the tribe have been reported. This represents little more than 20% of the species (Cave, 1960, 1965; Fedorov, 1969; Moore, 1973, 1974, Moore and Moore, 1977; Goldblatt, 1981, 1984, 1988; Goldblatt and Johnson, 1990, 1991, 1994, 1996, 2003, 2006). Several basic chromosome numbers have been reported for this tribe, ranging between x=4 and x=25, although the most frequent are $x=10$ and $x=17$ (Watanabe et al., 1995). The ancestral number of the tribe seems to be $x=10$, and the remaining chromosome numbers are thought to have derived from a combination of polyploidy and aneuploidy (Wulff et al., 1996).

The cytogenetic analysis of *C. macrocephalum* revealed the existence of diploid ($2n=20$) and triploid ($2n=30$) populations within the species. The chromosome number $2n=20$ observed here is in agreement with a previous analysis of specimens from Paraguay (Dematteis et al., 2007), but this constitutes the first diploid population recorded from Argentina. Almost all the previous chromosome studies have reported $2n=40$ or $2n=$ca.40 for *C. macrocephalum* (Turner et al., 1979; Galiano and Hunziker, 1987; Dematteis et al., 2007). The number $2n=30$ found in our study thus constitutes a new chromosome number and ploidy level for *C. macrocephalum*.

The occurrence of trivalents at diakinesis of microsporogenesis in the triploid individuals suggests that they are autopolyploid. The existence of triploid populations could be explained by fertilization of a reduced gamete with an unreduced gamete of a sexual or partially sexual diploid population. However, the most simple mechanism would be hybridization among diploid and tetraploid specimens. Previous chromosome studies have reported both, diploid and tetraploid specimens, and consequently this hypothesis could be possible. A similar case has been reported in *Campovassouria bupleurifolia* (DC.) R.M. King et H. Rob., but in this species no diploid populations had been found, and all samples studied thus far had been triploid (Coleman and Coleman, 1984).

The microsporogenesis of triploid species of Eupatorieae analyzed to date, such as *C. bupleurifolia* and *Chromolaena callilepis* (Sch.Bip. ex Baker) R.M. King et H.Rob. in general are quite similar. As in *C. macrocephalum*, aberrations in

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Table 4
Different types and percentages of meiotic products in four triploid populations of *C. macrocephalum*.

Type	Description	B	C	D	E
1	4 microspores (regulat tetrads)	25.00	43.01	76.30	46.54
2	4 microspores+1 microcyte	35.00	23.65	9.63	23.58
3	4 microspores+2 microcytes	11.00	3.22	0.40	7.23
4	3 microspores+1 reduced microspore	0.00	3.22	0.00	0.00
5	2 microspores+6 microcytes	0.00	1.07	0.00	0.00
6	4 microspores. 1 with micronucleus	11.00	10.75	8.43	9.43
7	4 microspores. 1 with micronucleus+1 microcyte	5.00	4.30	0.00	4.71
8	4 microspores+3 microcytes	2.00	2.15	0.00	0.00
9	2 microspores+3 microcytes	0.00	1.07	0.00	0.31
10	2 microspores. 1 with micronucleus. + 5 microcytes. 1 with micronucleus	0.00	1.07	0.00	0.00
11	2 microspores+4 microcytes. 1 with micronucleus	0.00	1.07	0.00	0.00
12	4 microspores. 2 with micronuclei	3.00	3.22	0.00	1.57
13	2 microspores+5 microcytes. 3 without cytoplasm	0.00	1.07	0.00	0.00
14	2 microspores. 1 with micronucleus+3 microcytes. 1 with micronucleus	0.00	1.07	0.00	0.00
15	3 microspores+1 microcyte with 2 micronuclei	3.00	0.00	0.00	0.00
16	2 microspores+3 microcytes. 2 with micronuclei	2.00	0.00	0.00	0.00
17	2 microspores+2 microcytes with 2 micronuclei	1.00	0.00	0.00	0.00
18	3 microspores. 1 with 2 micronuclei+1 reduced microspore	1.00	0.00	0.00	0.62
19	2 microspores+3 microcytes with micronuclei	1.00	0.00	0.00	0.00
20	2 microspores. 1 with micronucleus+1 large microspore	0.00	0.00	0.88	0.00
21	2 microspores+1 large microspore	0.00	0.00	1.60	0.00
22	2 microspores. 1 with micronucleus+1 small microspore	0.00	0.00	0.40	0.00
23	Dyad	0.00	0.00	0.40	0.00
24	4 microspores. 1 with micronucleus+2 microcytes	0.00	0.00	0.40	0.31
25	Triad	0.00	0.00	1.20	0.00
26	3 microspores+1 small microspore+3 microcytes	0.00	0.00	0.00	1.88
27	4 microspores+4 microcytes	0.00	0.00	0.00	0.62
28	4 microspores. 1 with micronucleus+3 microcytes	0.00	0.00	0.00	0.31
29	4 microspores+1 microcyte with two micronuclei	0.00	0.00	0.00	0.62
30	4 microspores. 2 with micronuclei+1 microcyte	0.00	0.00	0.00	0.31
31	3 microspores. 1 with micronucleus+1 small microspore+1 microcyte without cytoplasm	0.00	0.00	0.00	0.62
32	2 microspores+2 small microspores	0.00	0.00	0.00	0.31
33	5 medium sized microspores	0.00	0.00	0.00	0.31
34	4 microspores+1 microcytes with two micronuclei+2 microcytes without cytoplasm	0.00	0.00	0.00	0.31
35	4 microspores. 1 with micronucleus+4 microcytes. 1 with two micronuclei	0.00	0.00	0.00	0.31
	Total of cells	100	93	249	318

chromosome pairing during diakinesis and metaphase have been observed, forming univalents, bivalents, trivalents and higher multivalents (Bertasso-Borges and Coleman, 2005). The irregularities in the chromosome pairing found in *C. macrocephalum* produce a high reduction of pollen fertility. These observations suggest that sexual reproduction would be very rare or absent in the triploid material studied.

However, triploid and other odd polyploids species of Eupatorieae commonly have a high percentage of seed viability through the mechanism of apomixis. Apomixis is usually defined as a natural process that allows clonal reproduction through seeds, avoiding meiosis and fertilization, resulting in offspring that are genetically identical to the maternal plant (Nogler, 1984).

The first case of apomixis found in the tribe was detected by Holmgren (1919) in *Eupatorium glandulosum* Kunth. Further studies such as those carried out by Fryxell (1957), Sullivan (1976) and Sparvoli (1960) determined the occurrence of apomixis in other species of Eupatorieae from the northern hemisphere. Apomixis was also detected in six species of the tribe from South America: *Ageratina riparia* (Regel) R.M. King et H. Rob. (Sparvoli, 1960), *Chromolaena callilepis* (Coleman and Coleman, 1984), *C. squalida* (DC.) R.M. King et H. Rob. (Coleman and Coleman, 1988), *C. odorata* (Lam.) R.M. King et H. Rob. (Coleman, 1989), *Gyptis tanacetifolia* (Czapik, 1996) D.J.N. Hind et Flann (Rozenblum, Maldonado and Waisman, 1988) and *Praxelis pauciflora* (Kunth) R.M. King et H. Rob. (Bertasso-Borges and Coleman, 1998).

There are two principal types of apomixis, adventitious embryony and gametophytic apomixis. Adventitious embryony occurs when an embryo is formed directly from a cell of the nucellus or integument of the ovule without the occurrence of the megagametophyte generation (Nogler, 1984). In gametophytic apomixis there are two distinct mechanisms for producing an unreduced embryo sac, diplospory, in which the embryo sac originates from the megaspore mother cell by mitosis or modified meiosis, and apospory, in which the embryo sac is formed from a somatic cell of the ovule, generally of the nucellus. Almost all cases of apomixis detected at the present in the tribe Eupatorieae are diplosporous apomixis (Bertasso-Borges and Coleman, 2005). The single species in which apospory has been recorded is *Campovassouria bupleurifolia.* Thus *C. macrocephalum* constitutes the second species of the tribe and the first taxa of the genus with this reproductive

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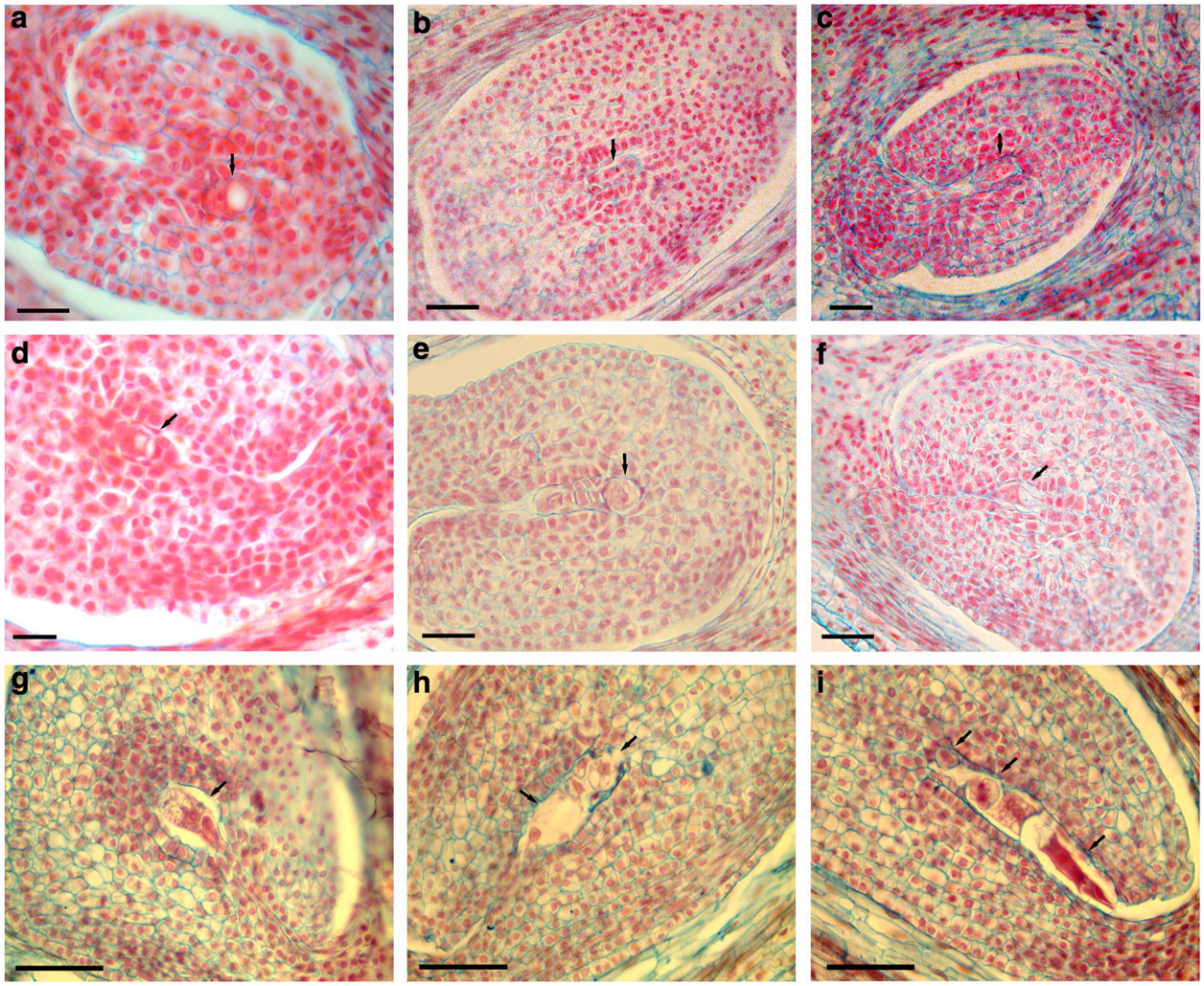

Fig. 3. Megasporogenesis and megagametogenesis in a triploid population of *C. macrocephalum*. (a) Megaspore mother cell (MMC) with a large vacuole; (b) MMC in anaphase I; (c) MMC in telophase I; (d) dyad; (e) four megaspores degenerate and a nuclear cell developing towards the chalaza to form the aposporic embryo sac; (f) differentiated cells towards micropyle and the four megaspores degenerate; (g) sexual embryo sac developing; (h) embryo sac (below) and rest of cells (above); (i) aposporic embryo sac (above). Scale bar = 5 μm.

mechanism. Apomixis was recorded in 2.9% of the genera of the Asteraceae family. However, embryological studies in most of the genera of the family are limited, so it is possible that the number of apomictic species in this family is much larger (Czapick, 1996).

Among the most striking features of gametophytic apomixis is its strong correlation with polyploidy. In a list with more than 126 genera (Carman, 1997), less than a handful are reported to include diploids (Nogler, 1984; Mogie, 1992; Schranz et al., 2006). The odd ploidy levels (triploid, pentaploid) are often reliable predictors of the presence of apomixis (Asker and Jerling, 1992; Koltunow, 1993). Explanations for the association between gametophytic apomixis and polyploidy are numerous and varied; they include mechanisms that focus on the ecology of polyploids and apomicts (Stebbins, 1950), the genetic consequences of polyploidy and apomixis (Manning and Dickson, 1986), the genetic basis of apomixis (Mogie, 1992), and the shared role of unreduced gamete formation (Harlan and De Wet, 1975).

The inheritance of gametophytic apomixis has since been reported to be associated with the transfer of either a single locus or a small number of loci in most of the systems studied to date (Ozias-Akins, 2006; Schallau et al., 2010). In the aposporous grasses *Pennisetum* (Sherwood et al., 1994), *Panicum* (Savidan, 1983), and *Brachiaria* (Valle et al., 1994), apomixis segregates as a single dominant locus. Simple dominant inheritance also has been reported for apospory in the dicotyledonous genera *Ranunculus* (Nogler, 1984) and *Hieracium* (Bicknell et al., 2000). Some of these studies have shown that the region controlling aposporous apomixis has reduced recombination, with multiple markers and potentially multiple genes falling within the linkage group (Ozias-Akins, 2006). There is consistent evidence suggesting that most of the alleles controlling apomixis are dominant (Whitton et al., 2008).

Apomictic species also are almost invariably perennials, and they often use a vegetative mechanism of asexual reproduction, such as stolon or rhizome growth. Thus, in the field, through a combination of apomixis and vegetative division, apomicts can

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form large clonal stands, and these may persist through long periods of time (Bicknell and Koltunow, 2004). *Campuloclinium macrocephalum* does not reproduce vegetatively, but has a woody subterranean base that constitutes a xylopodium. It acts as a carbohydrate storage organ allowing the species to resist fire, drought and herbicide damage. When the aerial parts of the plant are damaged by fire or die in the dry season, the underground xylopodium that is well-protected against overheating by the soil layers, sprout repeatedly. Thus the presence of a xylopodium, combined with aposporous apomixis, are two important factors that contribute to the invasiveness of *C. macrocephalum*.

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References

Asker, S.E., Jerling, L., 1992. Apomixis in Plants. CRC, Boca Raton. pp. 298.

Bertasso-Borges, M.S., Coleman, J.R., 1998. Embryology and cytogenetics of *Eupatorium pauciflorum* and *E. intermedium* (Compositae). Genetics and Molecular Biology 21, 507–514.

Bertasso-Borges, M.S., Coleman, J.R., 2005. Cytogenetics and embryology of *Eupatorium laevigatum* (Compositae). Genetics and Molecular Biology 28, 123–128.

Bicknell, R.A., Koltunow, A.M., 2004. Understanding apomixis: recent advances and remaining conundrums. The Plant Cell 16, 228–245.

Bicknell, R.A., Borst, N.K., Koltunow, A.M., 2000. Monogenic inheritance of apomixis in two *Hieracium* species with distinct developmental mechanisms. Heredity 84, 228–237.

Cabrera, A.L., 1974. Compositae. In: Burkart, A. Flora Ilustrada de Entre Ríos 6, 106–540.

Carman, J.G., 1997. Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory, and polyembryony. Biological Journal of the Linnean Society 61, 51–94.

Cave, M.S., 1960. Index to plant chromosome numbers, I (1–4). Carolina Botanical Society Berkeley. The University of North Carolina Press, Chapel Hill.

Cave, M.S., 1965. Index to plant chromosome numbers, II (5–9). The University of North Carolina Press, Chapel Hill, Carolina Botanical Society Berkeley.

Coleman, J.R., 1989. Embryology and cytogenetics of apomictic hexaploid *Eupatorium odoratum* L. (Compositae). Revista Brasileira de Genetica 12, 803–817.

Coleman, J.R., Coleman, M.A., 1984. Apomixis in two triploid Brazilian species of *Eupatorium*: *E. bupleurifolium* and *E. callilepis*. Revista Brasileira de Genetica 7, 549–567.

Coleman, J.R., Coleman, M.A., 1988. Embryology and cytogenetics of apomictic triploid *Eupatorium squalidum* DC. (Compositae). Revista Brasileira de Genetica 11, 129–148.

Cortadi, A.A., Gattuso, M.A., 1994. Caracterización anatómica e histoquímica de *Eupatorium macrocephalum* Less., *E. inulaefolium* H.B.K. y *E. subhastatum* Hook. et Arn. (Asteraceae). Dominguezia 11, 32–42.

Czapik, R., 1996. Problems of apomictic reproduction in the families Compositae and Rosaceae. Folia Geobotanica 31, 381–387.

Dematteis, M., Molero, J., Ângulo, M.B., Rovira, A.M., 2007. Chromosome studies on some Asteraceae from South America. Botanical Journal of the Linnean Society 153, 221–230.

Fedorov, A., 1969. Chromosome numbers of flowering plants. Reimpr. 1974. O. Koeltz Sci. Publ, Koeningstein.

Foxcroft, L.C., Rouget, M., Richardson, D.M., 2007. Risk assessment of riparian plant invasions into protected areas. Conservation Biology 21, 412–421.

Freire, S.E., 2008. Tribu Eupatorieae (Asteraceae). In: Zuloaga, F.O., Morrone, O., Belgrano, M. (Eds.), Catálogo de las Plantas Vasculares del Cono Sur de América del Sur: Argentina, Sur de Brasil (Paraná, Santa Catarina y Rio Grande do Sul), Chile, Paraguay y Uruguay. Monographs in Systematic Botany from the Missouri Botanical Garden 114, 1277–1302.

Fryxell, P.A., 1957. Mode of reproduction of higher plants. Botanical Review 23, 135–233.

Galiano, N.G., Hunziker, J.H., 1987. Estudios cariológicos en Compositae IV. Vernonieae y Eupatorieae. Darwiniana 28, 1–8.

Goldblatt, P., 1981. Index to plant chromosome numbers 1975–1978. Monographs in Systematic Botany from the Missouri Botanical Garden 5. Saint Louis.

Goldblatt, P., 1984. Index to plant chromosome numbers 1979–1981. Monographs in Systematic Botany from the Missouri Botanical Garden 8. Saint Louis.

Goldblatt, P., 1988. Index to plant chromosome numbers 1984–1985. Monographs in Systematic Botany from the Missouri Botanical Garden 23. Saint Louis.

Goldblatt, P., Johnson, D.E., 1990. Index to plant chromosome numbers 1986–1987. Monographs in Systematic Botany from the Missouri Botanical Garden 30. Saint Louis.

Goldblatt, P., Johnson, D.E., 1991. Index to plant chromosome numbers 1988–1989. Monographs in Systematic Botany from the Missouri Botanical Garden 40. Saint Louis.

Goldblatt, P., Johnson, D.E., 1994. Index to plant chromosome numbers 1990–1991. Monographs in Systematic Botany from the Missouri Botanical Garden 51. Saint Louis.

Goldblatt, P., Johnson, D.E., 1996. Index to plant chromosome numbers 1992–1993. Monographs in Systematic Botany from the Missouri Botanical Garden 58. Saint Louis.

Goldblatt, P., Johnson, D.E., 2003. Index to plant chromosome numbers 1998–2000. Monographs in Systematic Botany from the Missouri Botanical Garden 94. Saint Louis.

Goldblatt, P., Johnson, D.E., 2006. Index to plant chromosome numbers 2001–2003. Monographs in Systematic Botany from the Missouri Botanical Garden 106. Saint Louis.

Goodall, J., Witkowski, E.T.F., Ammann, S., Reinhardt, C., 2010. Does allelopathy explain the invasiveness of *Campuloclinium macrocephalum* (pompom weed) in the South African grassland biome? Biological Invasions 12, 3497–3512.

Harlan, J.R., De Wet, J.M.J., 1975. On Ö. Winge and a prayer: the origins of polyploidy. Botanical Review 4, 361–390.

Henderson, L., 1995. Plant invaders of southern Africa. Plant Protection Research Institute, Handbook No 5. Agricultural Research Council, Pretoria. pp. 176.

Henderson, L., 2007. Invasive, naturalized and casual alien plants in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). Bothalia 37, 215–248.

Holm, L.G., Pancho, J.V., Herberger, J.P., Plucknett, D.L., 1979. A Geographical Atlas of World Weeds. John Wiley and Sons, New York. pp. 391.

Holmgren, I., 1919. Zytologische studien uber die fortpflanzung bei den gattungen *Erigeron* und *Eupatorium*. Svenska Vetenskapsakademiens Årsbok 59, 1–118.

Johansen, D.A., 1940. Plant Microtechnique. McGraw-Hill, New York.

Klein, M., Neser, S., 1999. Population explosion of the floral kind. Pl Protect News 54, 912.

Koltunow, A.M., 1993. Apomixis: embryo sacs and embryos formed without meiosis or fertilization in ovules. Plant Cell 5, 1425–1437.

Luque, R., Sousa, H.C., Kraus, J.E., 1996. Métodos de coloração de Roeser (1972)- modificado-E Kropp (1972), visando a substituição do azul de astra por azul de alcião 8GS ou 8GX. Acta Botanica Brasileira 10, 199–212.

Manning, J.T., Dickson, D.P.E., 1986. Asexual reproduction, polyploidy, and optimal mutation rates. Journal of Theoretical Biology 118, 485–489.

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Marks, G.E., 1954. An acetic-carmine glycerol jelly for use in pollen fertility counts. Stain Technology 29, 277.

Marzinek, J., Oliveira, D.M.T., 2010. Structure and ontogeny of the pericarp of six Eupatorieae (Asteraceae) with ecological and taxonomic considerations. Anais da Academia Brasileira de Ciencias 82, 279–291.

Mogie, M., 1992. The Evolution of Asexual Reproduction. Chapman & Hall, London. pp. 276.

Moore, R.L., 1973. Index to plant chromosome numbers 1967–1971. Regnum Vegetabile 90. Utrecht.

Moore, R.L., 1974. Index to plant chromosome numbers 1972. Regnum Vegetabile 91. Utrecht.

Moore, R.L., Moore, R.L., 1977. Index to plant chromosome numbers 1973–1974. Regnum Vegetabile 96. Utrecht.

Mound, L.A., Pereyra, V., 2008. *Liothrips tractabilis* sp.n. (Thysanoptera, Phlaeothripinae) from Argentina, a potential biocontrol agent of weedy *Campuloclinium* (Asteraceae) in South Africa. Neotropical Entomology 37, 63–67.

Nogler, G.A., 1984. Gametophytic apomixis. In: Johri, B.M. (Ed.), Embryology of Angiosperms. Springer, Berlin, pp. 474–518.

Ozias-Akins, P., 2006. Apomixis: developmental characteristics and genetics. Critical Reviews in Plant Sciences 25, 199–214.

Rozenblum, E., Maldonado, S., Waisman, C.E., 1988. Apomixis in *Eupatorium tanacetifolium* (Compositae). American Journal of Botany 75, 311–322.

Savidan, Y., 1983. Genetics and utilization of apomixis for the improvement of guinea grass (Panicum maximum Jacq.). In: Lexington, K.Y., Smith, J.A., Hayes, V.W. (Eds.), Proceedings of the 14th International Grasslands Congress (1981). Westview Press, Boulder, pp. 182–184.

Schallau, A., Arzenton, F., Johnston, A.J., Hähnel, U., Koszegi, D., Blattner, F.R., Altschmied, L., Haberer, G., Barcaccia, G., Bumlein, H., 2010. Identification and genetic analysis of the apospory locus in *Hypericum perforatum* L. The Plant Journal 62, 773–784.

Schranz, E.M., Kantama, L., De Jong, H., Mitchell-Olds, T., 2006. Asexual reproduction in a close relative of Arabidopsis: a genetic investigation of apomixis in Boechera (Brassicaceae). New Phytologist 171, 425–438.

Sherwood, R.T., Berg, C.C., Young, B.A., 1994. Inheritance of apospory in buffelgrass. Crop Science 34, 1490–1494.

Sparvoli, E., 1960. Osservazioni cito-embriologiche in *Eupatorium riparium*. II. Megasporogenesi e sviluppo del gametofito femminile. Annali di Botanica 26, 481–504.

Stebbins, G.L., 1950. Variation and Evolution in Plants. Columbia University Press, New York. pp. 643.

Sullivan, V.I., 1976. Diploidy, polyploidy and agamospermy among species of *Eupatorium* (Compositae). Canadian Journal of Botany 54, 2907–2917.

Turner, B.L., Bacon, J., Urbatsh, L., Simpson, B., 1979. Chromosome numbers in South American Compositae. American Journal of Botany 66, 173–178.

Valle, C.B., Glienke, C., Leguizamon, G.O.C., 1994. Inheritance of apomixis in *Brachiaria*, a tropical forage grass. Apomixis Newsletter 7, 42–43.

Vega, M.R.G., Carvalho, M.G., Vieira, I.J.C., Braz-Filho, R., 2008. Chemical constituents from the Paraguayan medicinal plant, *Eupatorium macrocephalum* Less. Journal of Natural Medicine 62, 122–123.

Watanabe, K., King, R.M., Yahara, T., Ito, M., Yokoyama, J., Suzuki, T., Graneford, D.J., 1995. Chromosomal cytology and evolution in Eupatorieae (Asteraceae). Annals of the Missouri Botanical Garden 82, 581–592.

Wells, M.J., Balsinhas, A.A., Joffe, H., Engelbrecht, V.M., Harding, G., Stirton, C.H., 1986. A catalogue of problem plants in southern Africa. Memoirs of the Botanical Survey of South Africa 53, 1–658.

Whitton, J., Sears, C.J., Baack, E.J., Ottoy, S.P., 2008. The dynamic nature of apomixis in the angiosperms. International Journal of Plant Science 169, 169–182.

Wulff, A.F., Hunzinker, J.H., Escobar, A., 1996. Estudios cariológicos en Compositae. VII. Darwiniana 34, 213–231.

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