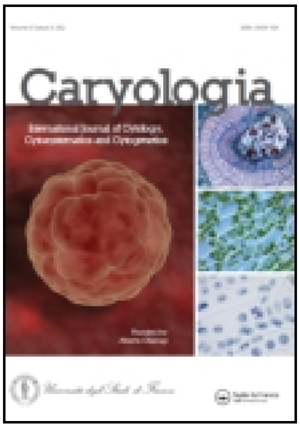

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Chromosome number, meiotic behavior and pollen fertility of six species of Chrysolaena (Vernonieae, Asteraceae)

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Chromosome number, meiotic behavior and pollen fertility of six species of *Chrysolaena* (Vernonieae, Asteraceae)

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Chromosome number, meiotic behavior and pollen fertility were determined for six species of the genus *Chrysolaena* H. Rob. (Vernonieae, Asteraceae). All the species had a base chromosome number of $x = 10$, with somatic numbers that ranged between $2n = 20$ and $2n = 60$. The chromosome number of *Chrysolaena obovata* (Less.) Dematt. ($2n = 4x = 40$) and a new cytotype for *C. lithospermifolia* (Hieron.) H.Rob. ($2n = 4x = 40$) are reported for the first time. Normal meiotic behavior was observed for the diploid species *C. platensis* (Spreng.) H. Rob., *C. propinqua* (Hieron.) H. Rob. and *C. flexuosa* (Sims) H. Rob. However, the polyploid taxa *C. obovata*, *C. lithospermifolia* (tetraploid) and *C. cognata* (Less.) Dematt. (hexaploid) showed many meiotic irregularities such as multivalent formation, chromosomes outside plate, early segregation of chromosomes, laggards and bridges in anaphase and telophase. Pollen fertility was high in all species, however, including in the polyploids. Polyploidy is more common in *Chrysolaena* than in any other New World Vernonieae so far reported and appears to be an important factor in the evolution of this genus.

Keywords: Compositae; cytogenetic; cytology; polyploidy; South America; *Vernonia*

Introduction

The Vernonieae Cass. is one of the largest tribes of Compositae (Asteraceae), with about 1700 species distributed throughout the tropical regions of Asia, Africa and America. It is also considered to be one of the most complex tribes in the family from both biological and taxonomic viewpoints (Keeley et al. 2007). Most species have been traditionally included in the huge core genus *Vernonia* Schreb, traditionally ascribed ca. 1000 species. However, in a series of studies on the New World Vernonieae, Robinson (1999) greatly reduced the size of the genus and now most species of *Vernonia* are restricted to eastern North America. South American species previously in *Vernonia* were placed in new genera, one of which is the genus *Chrysolaena* H. Rob. (Subtribe Vernoniinae).

The genus *Chrysolaena* differs from other New World Vernonieae by the presence of sericeous or velutinous indumentum on the leaves, anthers with glandular apical appendages, styles without a basal node and cypselas with glands (Robinson 1988). The genus can also be separated from the other American genera by pollen morphology, and especially by the base chromosome number of $x = 10$ (Dematteis 2002). This chromosome number is very common in the Old World Vernonieae, but is mostly absent in American species (Dematteis 1997). As presently delimited, *Chrysolaena* comprises 18 species geographically concentrated in southern Brazil and northern Argentina, with some species extending to Uruguay, Paraguay, Bolivia and Peru (Dematteis 2009). The distinctions among *Chrysolaena* species are relatively clear; however, they vary considerably in chromosome number due to polyploidization (Ruas et al. 1991; Dematteis 1997, 2007; Oliveira et al. 2007).

Chromosome studies reveal that chromosome numbers range from $2n = 20$ to $2n = 80$ and there is variation among species in cytotypes as well as ploidy levels (Ruas et al. 1991; Dematteis 1997). For example, in *C. platensis* (Spreng.) H. Rob. there are diploid ($2n = 20$), tetraploid ($2n = 40$) and octoploid ($2n = 80$) populations (Dematteis 1997). Likewise, *Chrysolaena flexuosa* (Sims) H. Rob. has both diploid and tetraploid cytotypes, while *C. sceptrum* (Chodat) Dematt. includes hexaploid and octoploid populations (Dematteis 2009). The most extreme chromosome variation is found in *C. cognata* (Less.) Dematt., with diploid, tetraploid, hexaploid and even odd-number polyploids, such as $2n = 5x = 50$, which would be the result of hybridization between different cytotypes (Dematteis 2002). Chromosome number, meiotic behavior and pollen fertility of six species of *Chrysolaena* were examined in this study. The results are discussed in relation to the taxonomy and evolution of the genus.

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Materials and methods

Material was obtained from natural populations in different localities within Argentina, Bolivia and Uruguay (Table 1). Voucher specimens are deposited in the herbarium of Instituto de Botánica del Nordeste (CTES).

Meiosis was studied in young inflorescences fixed in lactic acid–ethanol (1:5) and refrigerated until examined. Pollen mother cells were macerated and squashed using 2% acetocarmine. Permanent slides were prepared using Euparal. Photographs were taken through a Zeiss Axioplan microscope with a Cannon Power Shot A640 camera.

Pollen fertility was estimated by staining with carmine-glycerin (1:1). Uniformly stained pollen grains were considered fertile while the non-stained grains were scored as sterile. Approximately 300–500 pollen grains were analyzed per species.

Results

The six species of *Chrysolaena* investigated here all had a base chromosome number of $x = 10$. There were also polyploids of differing ploidy levels (Table 1). In *Chrysolaena platensis* (Figure 1A), *C. propinqua* (Figure 1E) and *C. flexuosa* (Figure 1H), all the populations were diploid with $2n = 20$. On the other hand, *Chrysolaena obovata* (Figure 1J) and *C. lithospermifolia* were tetraploid with $2n = 40$, while *C. cognata* was hexaploid with $2n = 60$ (Figure 1N).

The meiotic behavior of the analyzed species is presented in Tables 2 and 3. In *Chrysolaena platensis*, *C. propinqua* and *C. flexuosa*, due to their diploid condition, there was a preponderance of bivalents at diakinesis, but univalents were also observed in some cells (Figure 1B, E). Only in *C. propinqua* did 100% of the cells form bivalents at this stage (Figure 1H). In general, meiosis was normal in these three species, but some irregularities did occur. As seen in Figure 1C some chromosomes were off-plate and precocious chromosome segregation occurred during metaphase I along with lagging chromosomes and bridges in anaphase I (Figure 1D, G, I). For example, in *C. flexuosa* chromosome bridges (without fragments) were also observed at anaphase II in 16.66% of the cells.

In the tetraploid species, *C. obovata*, a high frequency of bivalents were observed at diakinesis (Figure 1J), along with occasional univalents and tetravalents (Figure 1K). At metaphase I off-plate univalents (Figure 1M) and early segregation of chromosomes were also observed while at anaphase I there were between one and four chromosome bridges per cell (with or without fragments) (Figure 1L). In *C. lithospermifolia*, on the other hand, at metaphase I there were off-plate chromosomes and in anaphase II and telophase II (Figure 1Q–R) there were also lagging chromosomes and bridges that, in some cases, occurred in the same cell (Figure 1R). Also both bivalents and tetravalents were observed (Figure 1Q) in addition to off-plate chromosomes in metaphase I and irregular second meiotic division, in both anaphase II and telophase II.

Hexaploid individuals of *C. cognata* showed bivalents and many configurations having multivalents at diakinesis (Figure 1N). The meiotic behavior also revealed irregularities in meiosis I and II, such as univalents outside the plate, early segregation of chromosomes in metaphase I, lagging chromosomes (Figure 1P) and a variable number of bridges per cell in anaphase I and anaphase II (Figure 1O). The pollen fertility was relatively high in all species, between 89.27% and 98.04% (Table 3). *Chrysolaena cognata* recorded the lowest percentage of fertility (89.27%), while *C. platensis* showed the highest (98.04%).

Discussion

The chromosome number $2n = 20$ found in *C. platensis*, *C. propinqua* and *C. flexuosa* and the cytotype with $2n = 60$ observed in *C. cognata* are consistent with the chromosome counts recorded in previous cytological studies (Angulo & Dematteis 2009; Dematteis 1997,

Table 1. Examined specimens and somatic chromosome numbers ($2n$) observed in six species of *Chrysolaena*.

Species	$2n$	Location and voucher specimens
C. cognata (Less) Dematt.	$2n = 6x = 60$	Argentina. Chaco. Dept. 1° de Mayo. Colonia Benitez. High fields near the village entrance. *M. Dematteis 3029* (CTES).
C. flexuosa (Sims) H. Rob.	$2n = 2x = 20$	Argentina. Misiones. Dept. Capital. Low fields near the Zaimán stream. *M. Dematteis*, *R. Salas and A. Vega 3035* (CTES).
C. lithospermifolia (Hieron.) H. Rob.	$2n = 4x = 40^{+}$	Argentina. Corrientes. Dept. San Roque. Joint routes 123 and 12, sandy soil, high. *M. Dematteis and M. Seo 2469* (CTES).
C. obovata (Less) Dematt.	$2n = 4x = 40^{*}$	Bolivia. Dept. Santa Cruz. Prov. Ñuflo de Chávez. 3 km NW of San Javier, way to the tannery. *M. Dematteis*, *J. P. Coulleri*, *E. Meza Torres and A. Vega 3475* (CTES).
C. platensis (Spreng.) H. Rob.	$2n = 2x = 20$	Argentina. Misiones. Dept. General Manuel Belgrano. 2.2 km S of Dos Hermanas. Low fields. *M. Dematteis*, *R. Salas and A. Vega 3083* (CTES).
C. propinqua (Hieron.) H. Rob. var. *propinqua*	$2n = 2x = 20$	Argentina. Misiones. Dept. Capital. Low fields near the Zaimán stream. *M. Dematteis*, *R. Salas and A. Vega 3038* (CTES).

*First chromosome count for the taxon⁺New cytotype for the taxon.

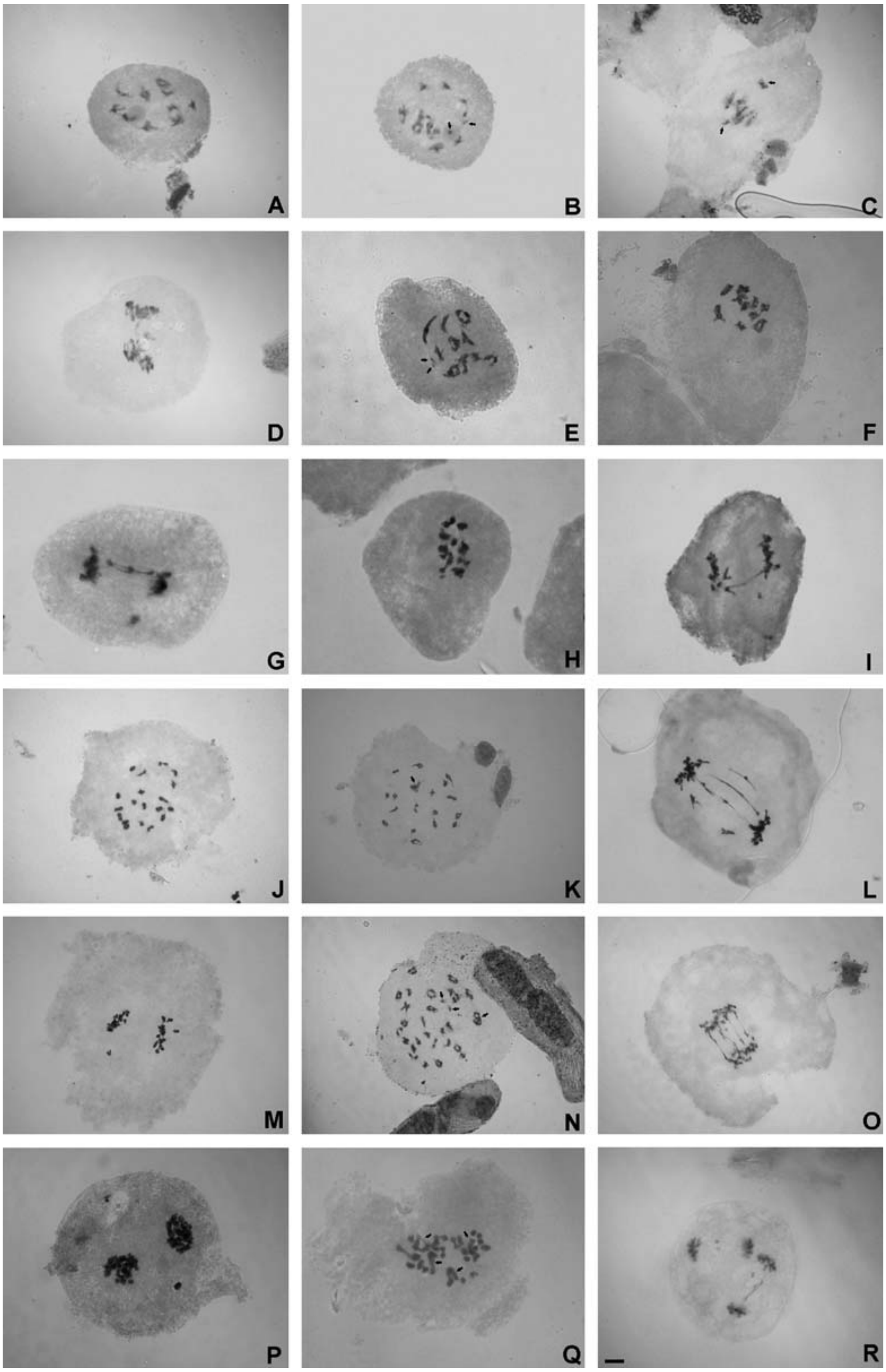

Figure 1. Meiotic behavior in *Chrysolaena*. (A–D) *C. flexuosa*: (A) diakinesis, 10II; (B) diakinesis, 9II + 2I (arrows); (C) off-plate chromosomes in metaphase I (arrows); (D) chromosome bridge with fragment in anaphase I. (E–G) *C. platensis*: (E) diakinesis, 9II + 2I (arrows); (F) diakinesis, 10II; (G) anaphase I with one bridge and fragment. (H–I) *C. propinqua*: (H) diakinesis, 10II; (I) bridge and fragment in anaphase I. (J–M) *C. obovata*: (J) diakinesis, 20II; (K) diakinesis, 18II + 1VI (arrows); (L) bridges and lagging chromosomes in anaphase I; (M) off-plate chromosome in metaphase II. (N–P) *C. cognata*: (N) diakinesis, 26II + 1VI + 2I (arrows); (O) bridges in anaphase I; (P) lagging chromosome in anaphase I. (Q–R) *C. lithospermifolia*: (Q) diakinesis, 5IV + 1III + 2II + 13I (arrows indicate tetravalent); (R) bridges and lagging chromosomes in anaphase II. Scale = 5 µm.

Table 2. Number of pollen mother cells analyzed (N°) and percentage of meiotic irregularities found in six species of *Chrysolaena*.

Phase	*C. platensis*		*C. flexuosa*		*C. propinqua*		*C. obovata*		*C. cognata*		*C. lithospermifolia*	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Metaphase I												
Regular	107	82.31	177	94.65	150	92.02	84	71.79	78	91.76	219	89.75
Precocious migration	1	0.77	1	0.53	2	1.23	2	1.71	3	3.53	2	0.82
Off-plate chromosomes	22	16.92	9	4.82	11	6.75	31	26.5	4	4.71	23	9.43
Total	130	100	187	100	163	100	117	100	85	100	244	100
Anaphase/telophase I												
Regular	69	93.25	79	77.45	98	76.56	75	72.12	67	87.01	164	96.48
Lagging	2	2.7	4	3.92	9	7.03	6	5.77	4	5.19	3	1.76
Bridges with fragment	1	1.35	4	3.92	2	1.56	4	3.84	2	2.6		
Bridges without fragment	2	2.7	15	14.71	19	14.85	19	18.27	4	5.2	3	1.76
Total	74	100	102	100	128	100	104	100	77	100	170	100
Metaphase II												
Regular							35	75.55			42	91.3
Precocious migration												
Off-plate chromosomes							9	20.45			4	8.8
Total							44	100			46	100
Anaphase/telophase II												
Regular			15	83.34					13	86.67	85	74.56
Lagging											24	21.05
Bridges with fragment											2	1.76
Bridges without fragment			3	16.66					2	13.33	3	2.63
Total			18	100					15	100	114	100

Table 3. Meiotic configurations and pollen fertility (%) in six analyzed species of *Chrysolaena*.

Species	Configuration	No. cells	%	Pollen grains	Pollen fertility
C. cognata	30 II	4	66.66	382	89.27
	26 II + 1 VI + 2 I	1	16.67		
	25 II + 2 IV + 2 I	1	16.67		
C. flexuosa	10 II	17	94.44		92.10
	9 II + 2 I	1	5.56	342	
C. lithospermifolia	20II	2	66.66	589	90.66
	5IV + 1III + 2II + 13I	1	33.34		
C. obovata	20 II	10	43.48	475	97.46
	18 II + 1 IV	6	30.44		
	16 II + 2 IV	7	26.08		
C. platensis	10 II	14	87.5	357	98.04
	9 II + 2 I	2	12.5		
C. propinqua	10 II	13	100	526	95.06

2002, 2009; Dematteis & Fernandez 2000). *Chrysolaena obovata* has not been previously studied and consequently this constitutes the first chromosome report for the species. In *C. lithospermifolia* ($2n=40$) a new tetraploid cytotype was discovered for a population from Argentina. A previous study of this species showed a count of $2n=20$ (Dematteis 1998a), indicating that there are two different ploidy levels within *C. lithospermifolia*.

A similar result has been reported for *C. platensis* with diploid ($2n=20$), tetraploid ($2n=40$) and octoploid ($2n=80$) cytotypes (Dematteis 1997). *Chrysolaena flexuosa* has also been reported to have diploid and tetraploid populations, and *C. sceptrum* was found to have both hexaploid and octoploid populations (Dematteis 2009). The greatest variation in chromosome number was observed in *C. cognata* where there are diploid, tetraploid and hexaploid cytotypes (Dematteis 2002). Difference in ploidy level did not prevent clear identification of species, however. There were some morphological differences: for example, the hexaploid plants were generally taller than those that were tetraploid, which were, in turn, taller than the diploids. This increase in size with increasing ploidy level is known as the gigas effect, and is characteristic of the polyploidization process (Stebbins 1971). The size differences were not uniform enough, however, to accurately predict ploidy level from morphology alone.

Meiotic counts ranged from $2n=20$ to $2n=60$ across species with regular meiotic behavior in the majority of cases. There was a prevalence of 10 II at diakinesis in the diploid species, *C. platensis*, *C. propinqua* and *C. flexuosa*. In the polyploids *C. cognata*, *C. obovata* and *C. lithospermifolia* a variable number of multivalents were observed, however, suggesting possible autopolyploidy. Chromosome numbers for *C. sceptrum* (Chodat) Dematt. ($2n=80$), *C. simplex* (Less.) Dematt. ($2n=40$) and *C. verbascifolia* (Less.) H.Rob. ($2n=20$) also support a base number of $x=10$ for *Chrysolaena*, as suggested previously (Dematteis 1998b, 2002; Salles de Mello et al. 2010). This is a common base number for several genera from the Old World, although polyploidy does not appear to be common (Jones 1979).

In the New World, *Vernonanthura* H. Rob. have counts of $n=17$ and $n=34$, while counts for *Lessingianthus* H. Rob. include $n=16$, 32, 64 (Dematteis 2002; Angulo & Dematteis 2012), some of which likely represent polyploids. However, in *Chrysolaena* polyploidy occurs much more frequently than elsewhere in American genera. Additionally, a base number of $x=10$ has been reported for a few other New World genera (i.e. *Critoniopsis* Sch. Bip. and *Lepidaploa* Cass.) but it is not common. Most of the New World groups show basic numbers that range between $x=14$ and $x=17$. In some cases, these numbers are specific for a genus or section, and are generally considered among the most valuable phylogenetic characters (Dematteis 2002).

The only American genera of Vernonieae with a base number lower than $x=13$ are *Acilepidopsis* H. Rob. and *Mesanthophora* H. Rob., which present $x=9$ and are not related to *Chrysolaena* (Dematteis & Robinson 1997; Dematteis & Salgado 2001). These two genera have triporate pollen grains, clearly differing from *Chrysolaena*, which always shows tricolporate grains (Keeley & Robinson 2009; Angulo & Dematteis 2010).

The chromosome numbers observed in this study, together with those reported previously for other American genera, indicate that polyploidy has been of notable importance in the evolution of the New World Vernonieae.

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