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Research Article

Cytotaxonomy and geographic distribution of cytotypes of species of the South American genus *Chrysolaena* (Vernonieae, Asteraceae)

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ABSTRACT Understanding speciation and biodiversity patterns in plants requires knowledge of the general role of climate in allowing polyploids to escape competition and persist with their diploid progenitors. This is a particularly interesting issue in widespread species that present multiple ploidy levels and occur across a heterogeneous environment. *Chrysolaena* (Vernonieae, Asteraceae) is a cytogenetically very diverse genus, with significant interspecific and intraspecific ploidy level variation and with continuous distribution across South America. No previous studies have summarized chromosome count data of *Chrysolaena* or addressed the cytogeography of the genus. Ploidy level of *Chrysolaena* species was determined by chromosome counting during mitosis and/or meiosis; the geographic distribution of cytotypes was examined and the correlations between the distribution of particular cytotypes and current ecological conditions were evaluated. A total of 43 new chromosome counts and five ploidy levels ($2x$, $4x$, $6x$, $7x$, $8x$) were reported. The chromosome number of *C. cordifolia* ($2n = 7x = 70$) and a new cytotype for *C. propinqua* var. *canescens* ($2n = 4x = 40$) are reported for the first time. Three geographic areas with high diversity of cytotypes and species were detected. The results obtained do not suggest a clear distribution pattern that depends on climatic factors for *Chrysolaena* populations. However, a geographic pattern was identified in the distribution of ploidy levels, with diploid species presenting a more restricted distribution than polyploid species.

Key words: chromosome numbers, environmental factors, geographic pattern, polyploidy.

1 Introduction

Polyploidy, the duplication of entire sets of chromosomes, is a central feature in the evolution and diversification of vascular plants, driving genomic novelty and acting as a mode of immediate and sympatric speciation (Husband et al., 2013; Soltis et al., 2015). Variation in ploidy level may occur not only between species of the same genus, but also within a single species (Muñoz-Pajares et al., 2018). Recent estimates suggest that 12–16% of species display cytotype variation (Soltis et al., 2007; Rice et al., 2015). Although polyploidy is a major mechanism of speciation in plants, the factors that contribute to polyploid establishment and persistence remain unsolved (Ramsey & Schemske, 1998, 2002). The adaptive significance of polyploidy to explain the geographic distribution of cytotypes is difficult to determine. Despite the rarity of polyploid formation and the occurrence of different cytotypes in different ecological niches, the prevalence of polyploids in the plant kingdom suggests that they may have a fitness advantage over their progenitors, and that genome duplication has an adaptive role (Stebbins, 1950; Muñoz-Pajares et al., 2018). The different plant species are distributed on Earth according to their climatic and edaphic requirements, and to the opportunities they have had to reproduce and expand in their geographical area (Cabrera, 1971). A key to understanding speciation and biodiversity patterns in plants is to test if climate plays a role in allowing polyploids to escape competition and persist with their diploid progenitors (Husband et al., 2013). However, recent studies have proposed alternative patterns, such as dispersal capabilities or other life-history traits that could better explain long-term persistence of polyploids, rather than shifts to new climatic niches (Glennon et al., 2014).

The role of polyploidy in the formation of the hyper-diversity of the Neotropics has been little studied (Paule et al., 2017). Cytogeographic patterns may unravel significant amounts of diversity by identifying multiple chromosomal races within a single taxonomic species; therefore, those patterns contribute to the conservation of rare species and ecological restoration (Soltis et al., 2007). This is a particularly interesting issue if the species is widespread, presents multiple ploidy levels and occurs across a heterogeneous environment. *Chrysolaena* H. Rob. (Vernonieae, Asteraceae) is a South American genus formed by 18 species geographically concentrated in southern Brazil and northern Argentina, and with some species extending to Uruguay, Paraguay, Bolivia and Peru. Most species have a wide geographic distribution, such as *C. propinqua* (Hieron.)

H. Rob., *C. flexuosa* (Sims.) H. Rob., *C. lithospermifolia* (Hieron.) H. Rob., *C. verbascifolia* (Less.) H. Rob., *C. platensis* (Spreng.) H. Rob. and *C. cognata* (Less.) Dematt. However, there are some endemic species, such as *Chrysolaena nicolackii* H. Rob., *C. dusenii* (Malme) Dematt., *C. sceptrum* (Chodat.) Dematt., *C. guaranitica* Dematt., *C. candelabrum* (Chodat.) Dematt., and *C. cordifolia* Dematt. (Robinson, 1999; Dematteis, 2009; Via do Pico, 2015).

Despite the morphological characteristics (inflorescence shape, pollen type, microcharacters) taken into account for the circumscription of the genus (Robinson, 1988), chromosome number is probably one of the most important characters to separate *Chrysolaena* from the other genera of the Vernonieae tribe, since there are no other American genera with a basic number $x = 10$, which is considered primitive and typical of the Old World's Vernonieae (Dematteis, 2009; Keeley & Robinson, 2009).

Chromosome number determinations of *Chrysolaena* have been frequently published. The first information about chromosome numbers in the genus was provided by Nuñez (Cabrera, 1944), followed by the works of Jones (1979), Galiano & Hunziker (1987), Hunziker et al. (1990), and Ruas et al. (1991). Many of these chromosome numbers were reported under nomenclatural combinations in the genus *Vernonia* Schreb., from which *Chrysolaena* was segregated (Robinson, 1988; Dematteis, 2009). The most comprehensive investigations have been conducted in recent years (Dematteis, 1996, 1997a, 1997b, 2002, 2007, 2009; Angulo & Dematteis, 2009a, 2009b; Oliveira et al., 2012; Via do Pico, 2015; Via do Pico & Dematteis, 2012, 2013, 2014, 2017; Echeverría & Camadro, 2017). These studies revealed that *Chrysolaena* is a cytogenetically very diverse genus, with significant interspecific and intraspecific chromosomal variation. Species with ploidy levels ranging from diploid ($2n = 2x = 20$) to octoploid ($2n = 8x = 80$) have been reported, including some species with odd chromosome numbers (Dematteis, 2009; Via do Pico & Dematteis, 2012, 2013, 2014, 2017). Meiotic studies suggest an autopolyploid origin (Via do Pico & Dematteis, 2012; Via do Pico, 2015). Most of the analyzed species present at least two cytotypes (see Table 1); however, some species have been little studied and have few records, in some cases only one, such as *C. cristobaliana* ($2n = 7x = 70$) (Via do Pico & Dematteis, 2014). For *C. simplex*, different basic chromosome numbers have been reported, $x = 10$ (typical of *Chrysolaena*) (Ruas et al., 1991) and $x = 16$ (typical of *Lessingianthus*) (Oliveira et al., 2012).

The distribution of the genus *Chrysolaena* is continuous in South America, meaning that different species and different cytotypes of a species can coexist in sympatry, especially those species that have a wide geographical distribution (Dematteis, 2009; Via do Pico, 2015). Since the distribution area of *Chrysolaena* comprises a great diversity of climates and ecological regions, the geographic segregation of the cytotypes could be the result of adaptive differences of diploids and polyploids to the heterogeneity of environmental factors. Different cytotypes within a polyploid species frequently show marked differences in geographical distribution; therefore, the study of distribution patterns of ploidy levels can provide insights into the long-term action of evolutionary processes (Husband et al., 2013).

No previous studies have summarized the data of all chromosome counts in *Chrysolaena* or addressed their cytogeographic distribution. To address these issues, the present study determined ploidy level by chromosome counts during mitosis and/or meiosis and examined the geographical distribution of cytotypes from natural populations of *Chrysolaena*. The aims of this study were: (i) to increase the cytological records for the species, by providing unpublished chromosome numbers; (ii) to present a review of all the vouchers for published counts; (iii) to verify if there are cytogeographic distribution patterns in the species distribution range; and (iv) to evaluate if there are correlations between the distribution of particular cytotypes and current ecological conditions.

2 Material and Methods

2.1 Materials

For the cytological studies, flower buds and seeds were collected from natural populations from different localities in Argentina, Brazil, Paraguay and Uruguay, during 2009–2018. The specimens collected were deposited in the herbarium of the Instituto de Botánica del Nordeste (CTES) Corrientes, Argentina.

Previous chromosome counts were considered in cases where the identity of the species could be corroborated and they had geographic coordinates or detailed descriptions of the collection sites. The counts made by Jones (1979) and Hunziker et al. (1990) on *Chrysolaena cognata* and *C. flexuosa,* and the counts made by Oliveira et al. (2012) on *C. simplex* were not considered for the cytogeographic and statistical analyses, since these specimens have a different basic chromosome number from the typical of the genus ($x = 10$). However, the contribution of these authors was taken into account and the chromosome counts are reported in the table of previously published data (Table 1).

2.2 Methods

2.2.1 Chromosome counts

The chromosome counts of the different entities were made from mitotic and/or meiotic preparations. The specimens analyzed and the information on their origin and collector data are detailed in Table 2. The mitotic and meiotic preparations were analyzed with a conventional microscope and the photographs were taken with a Zeiss Axioplan microscope equipped with a Cannon Power Shot A640 camera. To obtain the mitotic preparations, seeds were germinated in Petri dishes. Once the rootlets emerged and presented an adequate length, they were pre-treated with 0.002 M 8-hydroxyquinoline at room temperature, for about 4–5 h. Then, the roots were fixed in absolute ethyl alcohol: acetic acid (3:1) for 24–72 h. The staining was performed using Feulgen's technique. For the study of the meiotic chromosomes, young capitula were fixed in a mixture of ethyl alcohol and lactic acid in a 5:1 ratio for 72 h, and were preserved in 70% alcohol at 4 °C until analyzed. Anthers were macerated and squashed using 2% lacto-propionic orcein. Permanent slides were prepared using Euparal.

2.2.2 Geographic distribution of cytotypes and climatic variables

Maps were made detailing the geographic location of *Chrysolaena* populations using the software Diva-Gis version 7.5.0.0 (Hijmans et al., 2004). For this, the ploidy level information obtained in this study was used together with ploidy levels from other populations published in previous studies (Table 1). For

Table 1 Meiotic and/or mitotic chromosome numbers, ploidy level, locality details, and voucher information of *Chrysolaena* species published

Species	*n*	2*n*	Ploidy	Voucher	References
C. cognata (Less.) Dematt.	ca. 17*	-	-	Brazil. Parana State. *Jones 22712* (GA).	Jones, 1979
	34*	-	-	Brazil. Sao Paulo State. *Jones 22619* (GA).	Jones, 1979
	ca. 34+2-3Bs*	-	-	Brazil. Minas Gerais State. *Jones 22667* (GA).	Jones, 1979
	ca. 34*	-		Brazil. Sao Paulo State. *Jones 22678* (GA).	Jones, 1979
	34*	-		Brazil. Parana State. *Jones 22696* (GA).	Jones, 1979
	34*	-		Brazil. Parana State. *Jones 22699* (GA).	Jones, 1979
	ca. 34*	-		Brazil. Parana State. *Jones 22715* (GA).	Jones, 1979
	34*	-		Brazil. State of Rio de Janeiro. *Jones 22673* (GA).	Jones, 1979
	-	20	2*x*	Argentina. Corrientes. Dept. Saladas, road to Mburucuyá. 28° 15' 14" S–58° 30' 13" W. 70 m.a.s.l. *Dematteis 523* (CTES).	Dematteis, 1997a
	-	40	4*x*	Argentina. Misiones. Dept. San Ignacio, house of H. Quiroga. 27° 15' 54" S–55° 33' 11" W. 155 m.a.s.l. *Dematteis 513* (CTES).	Dematteis, 1997a
	-	40	4*x*	Argentina. Misiones. Dept. San Ignacio, Teyú Cuaré. 27° 17' 8" S–55° 35' 12" W. 192 m.a.s.l. *Dematteis 516* (CTES).	Dematteis, 1997a
	-	40	4*x*	Argentina. Entre Ríos. Dept. Concordia, Concordia, Rivadavia Park. 31° 22' 5" S–57° 59' 59" W. 27 m.a.s.l. *Krapovickas & Cristóbal 46576* (CTES).	Dematteis, 1997a
	-	60	6*x*	Argentina. Chaco. Dept. 1° de Mayo, Margarita Belén. 27° 15' 25" S–58° 58' 59" W. 57 m.a.s.l. *Dematteis & Solis Neffa 501* (CTES).	Dematteis, 1997a
	20	40	4*x*	Paraguay. Dept. Amambay. Chirigüelo. 22° 38' 51" S–55° 52' 51" W. 481 m.a.s.l. *Dematteis & Schinini 857* (CTES, FCQ).	Dematteis, 2002
	-	50-60	5*x*-6*x*	Argentina. Misiones. Dept. Candelaria. 9 km of the route 12, on the road to Oberá. 27° 28' 5" S–55° 41' 52" W. 150 m.a.s.l. *Krapovickas & Cristóbal 46950* (CTES).	Dematteis, 2002
	30	60	6*x*	Argentina. Misiones. Dept. Candelaria. Route 15, 21 km E of Santa Ana. 26° 35' 26" S–54° 31' 4" W. 295 m.a.s.l. *Dematteis 592* (CTES).	Dematteis, 2002
	10	20	2*x*	Argentina. Corrientes. Dept. Mburucuyá. Route 6, 3 km W from Manantiales. 27° 59' 50" S–58° 12' 13" W. 75 m.a.s.l. *Dematteis* et al. *972* (CTES).	Dematteis, 2002
	-	20	2*x*	Argentina. Misiones. Dept. Guaraní. Predio Guaraní, Paraíso stream. 27° 6' 31" S–54° 6' 13" W. 345 m.a.s.l. *Tressens* et al. *5801* (ASU, CTES, LPB).	Dematteis, 2002
	-	80	8*x*	Argentina. Misiones. Dept. General Manuel Belgrano. Campina de Américo. 26° 16' 26" S–53° 50' 18" W. 638 m.a.s.l. *Dematteis & Krapovickas 1918* (CTES).	Angulo & Dematteis, 2009a
	-	40	4*x*	Argentina. Misiones. Dept. San Ignacio. Teyú Cuaré. 27° 16' 50" S–55° 33' 35" W. 139 m.a.s.l. *Dematteis* et al. *2594* (CTES).	Angulo & Dematteis, 2009a
	30	-	6*x*	Argentina. Chaco. Dept. 1° de Mayo. Colonia Benitez. 27° 20' 12" S–58° 57' 19" W. 54 m.a.s.l. *Dematteis 3029* (CTES).	Via do Pico & Dematteis, 2012
	-	80 + 0-4Bs	8*x*	Argentina. Misiones. Dpto. General Manuel Belgrano. Campina de Americo, graveyard. 26° 16' 28" S–53° 41' 59" W. 813 m.a.s.l. *Dematteis* et al. *3053* (CTES).	Via do Pico & Dematteis, 2013

Continued

Table 1 Continued

Species	*n*	2*n*	Ploidy	Voucher	References
	-	40	4*x*	Uruguay. Dpto. Tacuarembó, Gruta de los Helechos, 10 km NW from Tacuarembó. 31° 38’ 22” S—56° 02’ 07” W. 187 m.a.s.l. *Dematteis* et al. *3761* (CTES).	Via do Pico & Dematteis, 2013
	-	40	4*x*	Argentina. Misiones. Dpto. San Ignacio. House of H. Quiroga. 27° 15’ 4” S—55° 33’ 2” W. 68 m.a.s.l. *Dematteis* et al. *3040* (CTES).	Via do Pico & Dematteis, 2013
C. cristobaliana Dematt.	-	70 + 0-7Bs	7*x*	Paraguay. Amambay. Pedro Juan Caballero. Near neighborhood commune. 22° 35’ 8” S—55° 43’ 17” W. 621 m.a.s.l. *Dematteis* & *Vega 4283* (CTES).	Via do Pico & Dematteis, 2014
C. flexuosa (Sims) H. Rob.	ca. 17*	-	-	Uruguay. Maldonado. *Marchesi s.n.* (GA).	Jones, 1979
	ca. 30-32*	-	-	Argentina. Buenos Aires. Pdo. Tandil. Tandil, hills in front Golf Club. *Hunziker* & *Gamerro 11551* (LPB, SI).	Hunziker et al., 1990
	-	40	4*x*	Brazil. Rio Grande do Sul. Guaíba. s. d. 30° 5’ 5” S—54° 20’ 41” W. 159 m.a.s.l. *Ruas 5626* (FUEL).	Ruas et al. 1991
	20	40	4*x*	Uruguay. Dept. Artigas, Tomás Gomensoro. 30° 33’ 32” S—57° 23’ 41” W. 103 m.a.s.l. *Dematteis* et al. *491* (CTES).	Dematteis, 1997a
	-	40	4*x*	Uruguay. Dept. Cerro Largo, route 8, 385 km. 31° 9’ 21” S—54° 6’ 34” W. 165 m.a.s.l. *Dematteis* et al. *496* (CTES).	Dematteis, 1997a
	-	40	4*x*	Argentina. Corrientes. Dept. Mercedes, route 123, 159 km. 29° 16’ 25” S—57° 57’ 27” W. 114 m.a.s.l. *Dematteis* et al. 490 (CTES).	Dematteis, 1997b
	20	40	4*x*	Uruguay. Department Rivera. Camino a Tacuarembó, route 5. 31° 9’ 21” S—55° 39’ 13” W. 213 m.a.s.l. *Dematteis* et al. *493* (CTES, MA, MEXU).	Dematteis, 2002
	-	20	2*x*	Paraguay. Dept. Cordillera. 25 km de Tacuara, camino a Ybyturuzú, 1 km from río Yhú. 25° 15’ 47” S—57° 0’ 46” W. 93 m.a.s.l. *Duré* et al. *44/99* (BCN, CTES, PY).	Dematteis et al., 2007
	-	20	2*x*	Uruguay. Dept. Lavalleja. 11 km S from Minas, stony ground. 34° 24’ 39” S—55° 15’ 28” W. 209 m.a.s.l. *Dematteis* & *Schinini 1671* (CTES).	Angulo & Dematteis, 2009b
	10	-	2*x*	Argentina. Misiones. Dept. Capital. Near A° Zaimán. 27° 26’ 51” S—55° 54’ 16” W. 59 m.a.s.l. *Dematteis* et al. *3035* (CTES).	Via do Pico & Dematteis, 2012
	-	40 + 0-2Bs	4*x*	Uruguay. Dpto. Artigas. Ruta 3, 20 km W de Artigas, way to Tomás Gomensoro. 30° 26’ 57” S—56° 36’ 57” W. 210 m.a.s.l. *Dematteis* et al. *3703* (CTES).	Via do Pico & Dematteis, 2013
	-	40	4*x*	Uruguay. Dpto. Tacuarembó. 14 km NE from Tacuarembó, 1.7 km after Gruta de los Helechos. 31° 37’ 30” S—55° 02’ 16” W. 216 m.a.s.l. *Dematteis* et al. *3750* (CTES).	Via do Pico & Dematteis, 2013
	10	20	2*x*	Argentina. Corrientes. Dept. General Paz. Field near the route. 27° 26’ 47” S—57° 20’ 01” W. 6 m.a.s.l. *Via do Pico* et al. *45* (CTES).	Via do Pico & Dematteis, 2013
	-	60	6*x*	Argentina. Buenos Aires. Partido Gral. Pueyrredón, Sierra de Los Padres. 37° 34’ 16” S—57° 28’ 04” W. 6 m.a.s.l. *Echeverría, M.L. 301*; N°: 8615 (BAL).	Echeverría & Camadro, 2017
	-	60	6*x*	Argentina. Buenos Aires. Partido Balcarce, Sierra La Barrosa. 37° 53’ 40.7” S—58° 16’ 14” W. 103 m.a.s.l. *Echeverría, M.L. 302*; N°: 8616 (BAL).	Echeverría & Camadro, 2017

Continued

Table 1 Continued

Species	*n*	2*n*	Ploidy	Voucher	References
	-	60	6*x*	Argentina. Buenos Aires. Partido Balcarce, Sierra La Chata. 37° 52' 13.1" S–58° 22' 40" W. 113 m.a.s.l. *Echeverría, M.L. 303*; N°: 8617 (BAL).	Echeverría & Camadro, 2017
	-	60	6*x*	Argentina. Buenos Aires. Partido Balcarce, Sierra La Bachicha. 37° 47' 52" S–58° 08' 36" W. 86 m.a.s.l. *Echeverría, M.L. 304*; N°: 8618 (BAL).	Echeverría & Camadro, 2017
C. lithospermifolia (Hieron.) H. Rob.	10	20	2*x*	Argentina. Corrientes. Dept. Saladas. Río San Lorenzo. 28° 6' 49" S–58° 46' 14" W. 56 m.a.s.l. *Dematteis & Solís Neffa 503* (CTES, LP).	Dematteis, 1998
	-	20	2*x*	Argentina. Corrientes. Dept. Bella Vista. 10 km S from Bella Vista, Arroyo Toropí. 28° 35' 27" S–59° 2' 57" W. 64 m.a.s.l. *Angulo 8* (CTES).	Angulo & Dematteis, 2009b
	20	-	4*x*	Argentina. Corrientes. Dept. San Roque. Routes junction 123 y 12. 28° 51' 19" S–58° 44' 3" W. 68 m.a.s.l. *Dematteis & Seo 2469* (CTES).	Via do Pico & Dematteis, 2012
	10	20	2*x*	Argentina. Corrientes. Dept. Saladas. San Lorenzo creek. 28° 06' 50" S–58° 46' 13" W. 53 m.a.s.l. *Via do Pico* et al. *4* (CTES).	Via do Pico & Dematteis, 2013
C. obovata (Less.) Dematt.	10	-	2*x*	Brazil. Goiás. Serra do Caiapó. 17° 15' 0" S–51° 28' 59" W. 875 m.a.s.l. *Irwin & Soderstrom 6973* (MBM).	Dematteis, 2009
	20	-	4*x*	Bolivia. Dept. Santa Cruz. Prov. Ñuflo de Chávez. 3 km NW from San Javier, road to the tannery. 16° 16' 38" S–62° 32' 01" W. 518 m.a.s.l. *Dematteis* et al. *3475* (CTES).	Via do Pico & Dematteis, 2012
C. platensis (Spreng.) H. Rob.	20	-	4*x*	Argentina. Buenos Aires. Pdo. Ensenada, Boca Cerrada, 150 m from Río de La Plata. 34° 47' 36" S–58° 0' 7" W. 4 road to the tannery. *Hunziker* et al. *10228* (SI).	Galiano & Hunziker, 1987
	10	-	2*x*	Argentina. Corrientes. Dept. Capital. 15 km S of Corrientes city. 27° 33' 49" S–58° 44' 50" W. 68 m.a.s.l. *Hunziker* et al. *11068* (SI).	Galiano & Hunziker, 1987
	20	-	4*x*	Argentina. Misiones. Dept. Capital, Arroyo Zaimán. 27° 25' 15" S–55° 54' 43" W. 94 m.a.s.l. *Dematteis* et al. *442* (MNES, CTES, SI).	Dematteis, 1996
	-	80	8*x*	Brazil. Paraná. Campos Mourão. 24° 5' 48" S–52° 25' 30" W. 638 m.a.s.l. *Krapovickas & Cristóbal 46948* (CTES, MBM).	Dematteis, 1997a
	-	60	6*x*	Brazil. Indaiatuba, SP DA. 23° 9' 43" S–47° 7' 18" W. 694 m.a.s.l. *Oliveira V.M. 85* (UEC).	Oliveira et al., 2012
	10	-	2*x*	Argentina. Misiones. Dept. General Manuel Belgrano. 2.2 km S from Dos Hermanas. 25° 18' 33" S–53° 45' 33" W. 768 m.a.s.l. *Dematteis* et al. *3083* (CTES).	Via do Pico & Dematteis, 2012
C. propinqua (Spreng.) H. Rob.	10	20	2*x*	Argentina. Misiones, Posadas. 27° 27' 30" S–55° 57' 7" W. 121 m.a.s.l. *Dematteis 12* (MNES, CTES, SI).	Dematteis, 1996
	-	20	2*x*	Argentina. Misiones. Dpto. Capital, Ea. El Porvenir. 27° 29' 4.65" S–55° 57' 10.06" W. 161 m.a.s.l. *Dematteis & Solís Neffa 505* (CTES).	Dematteis, 1997a
	10	20	2*x*	Argentina. Misiones. Dept. Capital. Arroyo Zaimán. 27° 25' 29" S–55° 54' 14" W. 90 m.a.s.l. *Dematteis 371* (MNES).	Dematteis, 2002
	-	20	2*x*	Paraguay. Dept. Amambay. Estancia San Luis. Sierra de Amambay, near the limit between Brazil and Paraguay. 22° 35' 50" S–55° 40' 22" W. 647 m.a.s.l. *Schinini* et al. *35810* (CTES, PY).	Dematteis et al., 2007

Continued

Table 1 Continued

Species	*n*	2*n*	Ploidy	Voucher	References
	10	-	2*x*	Argentina. Misiones. Dept. Capital. Field near A° Zaimán. 27° 26’ 51” S−55° 54’ 16” W. 59 m.a.s.l. *Dematteis* et al. *3038* (CTES).	Via do Pico & Dematteis, 2012
	-	40+0-2Bs	4*x*	Argentina. Misiones. Dpto. General Manuel Belgrano. Campina de Americo, graveyard. 26° 16’ 27” S−53° 42’ 00” W. 809 m.a.s.l. *Dematteis* et al. *3078* (CTES).	Via do Pico & Dematteis, 2013
C. propinqua (Hieron) H. Rob. var. *canescens* (Chodat) Dematt.	10	20	2*x*	Paraguay. Dept. Amambay. 25 km N of P. J. Caballero, way to Colonia Estrella. 22° 31’ 47” S- 55° 47’ 28” W. 539 m.a.s.l. *Dematteis & Schinini 869* (CTES, FCQ).	Dematteis, 2002
C. sceptrum (Chodat) Dematt.	-	80	8*x*	Paraguay. Dept. Amambay. 41 km S from Bella Vista, way to río Aquidaban. 22° 32’ 29” S−55° 54’ 39” W. 343 m.a.s.l. *Dematteis* et al. *909* (CTES, FCQ).	Dematteis, 2002
	-	40 + 0-2Bs	4*x*	Paraguay. Amambay. Pedro Juan Caballero. Near neighborhood commune. 22° 35’ 8” S−55° 43’ 17” W. 920 m.a.s.l. *Dematteis & Vega 4289* (CTES).	Via do Pico & Dematteis, 2014
C. simplex (Less.) Dematt.	-	40	4*x*	Brazil. Paraná. 50 km de Curitiba, near route to Ponta Grossa. s. d. 25° 10’ 29.86” S−49° 38’ 28.66” W. 848 m.a.s.l. *Ruas 5726* (FUEL).	Ruas et al., 1991
	-	32*	-	Brazil. Joaquim Felício, MG RA. 17° 46’ 2” S − 44° 10’ 26” W. 658 m.a.s.l. *Costa 570* (UEC).	Oliveira et al., 2012
C. verbascifolia (Less.) H. Rob.	-	20	2*x*	Argentina. Misiones. Dpto. Capital, entrance to El Porvenir. 27° 29’ 4.65” S−55° 57’ 10.06” W.160 m.a.s.l. *Dematteis & Solís Neffa 507* (CTES).	Dematteis, 1997b
	-	20	2*x*	Argentina. Misiones. Dept. General Manuel Belgrano. Campina de Américo. 26° 16’ 28” S−53° 41’ 59” W. 720 m.a.s.l. *Dematteis* et al. *2608* (CTES).	Angulo & Dematteis, 2009a
	-	20 + 0-7Bs	2*x*	Argentina. Misiones. Dpto. General Manuel Belgrano. Campina de Americo, graveyard. 26° 16’ 35” S−53° 41’ 58” W. 808 m.a.s.l. *Dematteis* et al. *3080* (CTES).	Via do Pico & Dematteis, 2013
	10 + 0-1B	-	2*x*	Argentina. Misiones. Dept. Capital. Way to A° Zaimán. 27° 26’ 47” S−55° 54’ 11” W. 93 m.a.s.l. *Via do Pico* et al. *28* (CTES).	Via do Pico & Dematteis, 2017
	10 + 0-6Bs	-	2*x*	Argentina. Misiones. Dept. General Manuel Belgrano. Campina de Americo. Graveyard. 26° 16’ 28” S−53° 41’ 59” W. 813 m.a.s.l. *Via do Pico* et al. *34* (CTES).	Via do Pico & Dematteis, 2017
	10 + 0-3Bs	-	2*x*	Argentina. Misiones. Dept. Concepción. 2 km NW from Concepción de la Sierra. Way to Apóstoles. 27° 58’ 11” S−55° 31’ 46” W. 159 m.a.s.l. *Via do Pico* et al. *41* (CTES).	Via do Pico & Dematteis, 2017
	10	-	2*x*	Argentina. Misiones. Dept. San Javier. 20.4 km de San Javier. Way to Azara. 27° 52’ 43” S-55° 17’ 05” W. 94 m.a.s.l. *Via do Pico* et al. *58* (CTES).	Via do Pico & Dematteis, 2017

(Herbarium Codes: ASU: Arizona State University, U.S.A. Arizona, Tempe; BAL: UNMdP (Universidad Nacional de Mar del Plata)-INTA (Instituto Nacional de Tecnología Agropecuaria), Argentina, Buenos Aires, Balcarce; BCN: University of Barcelona, Spain; CTES: *Instituto de Botánica del Nordeste, Argentina, Corrientes;* FCQ: *Universidad Nacional de Asunción, Paraguay, San Lorenzo;* FUEL: Universidade Estadual de Londrina, Brazil, Paraná, Londrina; GA: *University of Georgia, U.S.A. Georgia, Athen.;* LP: Museo de La Plata, Argentina, Buenos Aires, La Plata; LPB: Herbario Nacional de Bolivia, Universidad Mayor de San Andrés, Bolivia, La Paz; MA: Real Jardín Botánico, Spain, Madrid; MBM: Museu Botânico Municipal, Brazil, Paraná, Curitiba; MEXU: Universidad Nacional Autónoma de México, Mexico, Distrito Federal, Ciudad Universitaria, Ciudad de Mexico; MNES: Universidad Nacional de Misiones, Argentina, Misiones, Posadas; PY: Centro de Estudios y Colecciones Biológicas para la Conservación, Paraguay, Asunción; SI: Instituto de Botánica Darwinion, Argentina, Buenos Aires, San Isidro; UEC: Universidade Estadual de Campinas, Brazil, São Paulo, Campinas). *Populations not included in the statistical and cytogeographic analyses.

Table 2 Chromosome counts (meiotic and/or mitotic), ploidy level, locality details, and voucher information of the 43 populations of *Chrysolaena* species analyzed in this study

Species	*n*	2*n*	Ploidy	Voucher
C. cognata (Less.) Dematt.	-	40	4*x*	Uruguay. Dept. Tacuarembó. Gruta de los Cuernos, 20 km NW from Tacuarembó. 31° 37' 17" S−56° 02' 26" W. 236 m.a.s.l. *Dematteis* et al. *3779* (CTES).
	10	-	2*x*	Argentina. Corrientes. Dept. San Roque. 12 km N from San Roque. Route 12. 28° 27' 51" S−58° 43' 16" W. 72 m.a.s.l. *Via do Pico* et al. *6* (CTES).
	30	-	6*x*	Argentina. Misiones. Dept. Capital. Way to the Zaimán stream. 27° 26' 40" S−55° 53' 59" W. 93 m.a.s.l. *Via do Pico* et al. *27* (CTES).
	30	-	6*x*	Argentina. Misiones. Dept. San Ignacio. 5.5 km W de General Urquiza. Way to Puerto Helena. 27° 04' 56" S−55° 25' 06" W. 156 m.a.s.l. *Via do Pico* et al. *31* (CTES).
	30	-	6*x*	Argentina. Misiones. Dept. General Manuel Belgrano. 4 km E of Dos Hermanas. Way to Bernardo de Irigoyen. Route 17. 26° 17' 15" S−53° 43' 42" W. 771 m.a.s.l. *Via do Pico* et al. *32* (CTES).
	10	-	2*x*	Argentina. Corrientes. Dept. Ituzaingó. Paraje Libertad. Route 41. 27° 42' 23" S−56° 28' 32" W. 86 m.a.s.l. *Via do Pico* et al. *50* (CTES).
	-	60	6*x*	Argentina. Corrientes. Dept. Santo Tomé. Pcial. Route 40. 40 km W of Santo Tomé. Way to Galarza. 28° 25' 18" S−56° 14' 46" W. 95 m.a.s.l. *Via do Pico* et al. *53* (CTES).
	-	80	8*x*	Argentina. Misiones. Dept. General Manuel Belgrano. Campina de Americo. Graveyard. 26° 16' 29" S−53° 41' 59" W. 812 m.a.s.l. *Via do Pico* et al. *95* (CTES).
	-	60	6*x*	Argentina. Misiones. Dept. Apóstoles. 1.6 km E of Azara. Way to Río Uruguay. 28° 04' 24" S- 55° 39' 49" W. 103 m.a.s.l. *Via do Pico* et al. *102* (CTES).
	-	20 + 0-3Bs	2*x*	Argentina. Formosa. Dept. Patiño, RN 81, 1374 km. Way to Ibarreta. 25° 14' 25" S−59° 47' 53" W. 108 m.a.s.l. *Pérez* et al. *20* (CTES).
	10	-	2*x*	Argentina. Corrientes Capital. Riachuelo. 27° 33' 45.7" S−58° 44' 35.9" W. 57 m.a.s.l. *Pérez* et al. *23* (CTES).
	10	20	2*x*	Argentina. Corrientes. Dept. San Martín. Reserva Privada Tres Cerros. Cerro Nazareno. Forest path. 29° 06' 29.6" S−56° 56' 06.6" W. 112 m.a.s.l. *Pérez Y. 30* (CTES).
	-	40	4*x*	Argentina. Misiones. Dept. San Ignacio, way to Club del Río. 27° 16' 38.9" S−55° 33' 26" W. 152 m.a.s.l. *Pérez* et al. *49* (CTES).
	-	20	2*x*	Paraguay. Guairá. Dept. General Garay. Cerro Tres Kandú (Cerro Peró). 25° 56' 9.3" S−56° 09' 15.2" W. *De Madrignac B.* et al. *658* (CTES).
	-	80	8*x*	Paraguay. Amambay. Parque Nacional Cerro Corá, trail to the Cerro Muralla. 22° 39' 35.6" S−55° 59' 32" W. *De Madrignac B.* et al. *797* (CTES).
C. cordifolia Dematt.	-	70	7*x*	Argentina. Misiones. Dept. General Manuel Belgrano. Campina de Americo. Graveyard entrance. 26° 16' 32.48" S−53° 41' 56.84" W. 812 m.a.s.l. *Via do Pico* et al. *98* (CTES).
C. flexuosa (Sims) H. Rob.	20	-	4*x*	Argentina. Corrientes. Dept. Alvear. 5.5 km. N from Alvear. Way to Santo Tomé. 29° 03' 14" S−53° 31'15" W. 55 m.a.s.l. *Via do Pico* et al. *18* (CTES).
	-	20	2*x*	Argentina. Corrientes. Dept. Santo Tomé. 7 km S from Santo Tomé. Way to Virasoro. 28° 31' 40" S−56° 06' 14" W. 65 m.a.s.l. *Via do Pico* et al. *19* (CTES).

Continued

Table 2 Continued

Species	*n*	2*n*	Ploidy	Voucher
	10	-	2x	Argentina. Corrientes. Dept. Empedrado. El Sombrero. 27° 42' 12.2" S–58° 45' 52.6" W. 64 m.a.s.l. *Pérez 9* (CTES).
	-	40	4x	Argentina. Corrientes. Dept. San Martín. La Cruz. Tres Cerros. Cerro Nazareno. 29° 06' 18.5" S–56° 56' 08.4" W. *Pérez* et al. *35* (CTES).
	20	-	4x	Argentina. Corrientes. Mercedes. Route 123, 183 km. 29° 31' 52.8" S–57°32' 53.7" W. 71 m.a.s.l. *Pérez* et al. *37* (CTES).
C. lithospermifolia (Hieron.) H. Rob.	-	20	2x	Argentina, Corrientes: Dept. Saladas. A° San Lorenzo. 28° 06' 45" S–58° 46' 12" W. 55 m.a.s.l. *Via do Pico* et al. *3* (CTES).
	10	-	2x	Argentina. Corrientes. Dept. San Roque. 13.4°km. S from San Roque. 28° 41' 52" S–58° 42' 41" W. 76 m.a.s.l. *Via do Pico* et al. *9* (CTES).
	20	-	4x	Argentina. Corrientes. Mercedes. Pcial. Route 213. 29° 33' 44" S–57° 30' 40" W. 54 m.a.s.l. *Pérez* et al. *38* (CTES).
	20	-	4x	Argentina. Corrientes. Mercedes. Route 123. 29° 00' 45.2" S–58° 30' 19.4" W. 50 masl. *Pérez* et al. *41* (CTES).
	-	20	2x	Paraguay. Paraguarí. Dept. Paraguarí. San José street, Route Paraguarí-Piribebuy. 25° 34' 07.5" S–57° 06' 34.2" W. *De Madrignac B.* et al. *646* (CTES).
C. platensis (Spreng.) H. Rob.	10 + 0-4Bs	-	2x	Argentina, Misiones. Dept. General Manuel Belgrano. Campina de Americo. 26° 16' 36" S–53° 50' 09" W. 594 m.a.s.l. *Vega* et al. *155* (CTES).
	-	60	6x	Paraguay. Caaguazú. Dept. Caaguazú. Route 13, Colonia Mbocayá, A° Johyby. 25° 23' 25.4" S–56° 00' 41.4" W. *De Madrignac B.* et al. *666* (CTES).
C. propinqua (Spreng.) H. Rob.	10	-	2x	Argentina. Corrientes. Dept. Santo Tomé. Virasoro. 12.7 km S from Virasoro. Way to Santo Tomé. 28° 08' 42" S–56° 03' 57" W. 127 m.a.s.l. *Via do Pico* et al. *20* (CTES).
	10	-	2x	Argentina. Misiones. Dept. San Javier. 20.4 km W from San Javier. Way to Azara. 27° 52' 43" S–55° 17' 05" W. 94 m.a.s.l. *Via do Pico* et al. *25* (CTES).
	20	-	4x	Argentina. Misiones. Dept. General Manuel Belgrano. Campina de Américo. Graveyard. 26° 16' 28" S–53° 41' 59" W. 813 m.a.s.l. *Via do Pico* et al. *33* (CTES).
	10	-	2x	Argentina. Corrientes. Dept. Ituzaingó. 1.8 km S from San Carlos. 27° 45' 28" S–55° 54' 24" W. 187 m.a.s.l. *Via do Pico* et al. *43* (CTES).
	-	20	2x	Argentina. Misiones. Dept. San Javier. Loc. Itacaruaré. 400 m from Route 2. 27° 52' 41" S–55° 17' 05" W. *Via do Pico* et al. *100* (CTES).
	-	20	2x	Argentina. Misiones. Dept. Apóstoles. 1.6 km E from Azara. Way to Río Uruguay. 28° 04' 24" S–55° 39' 49" W. 103 m.a.s.l. *Via do Pico* et al. *101* (CTES).
C. propinqua (Hieron) H. Rob. var. *canescens* (Chodat) Dematt.	-	40	4x	Argentina. Misiones. Dept. General Manuel Belgrano. Campina de Americo. Graveyard. 26° 16' 29" S–53° 41' 59" W. 812 m.a.s.l. *Via do Pico* et al. *94* (CTES).
C. sceptrum (Chodat) Dematt.	-	40	4x	Paraguay. Amambay. Dept. Amambay. Route 5, Parque Nacional Cerro Corá, path near route 5. 22° 40' 19.1" S–56° 01' 40" W. *De Madrignac B.* et al. *713* (CTES).
	-	40	4x	Paraguay. Amambay. Route 5, towards Bella Vista Norte, Near guaraní school. 22° 25' 59.9" S–56° 17' 12.8" W. *De Madrignac B.* et al. *783* (CTES).
C. simplex (Less.) Dematt.	-	40	4x	Brazil. Minas Gerais. Uberaba. Fazenda Santa Jualiana. 300 m from BR-050 (Rodovia Federal Radial). 19° 17' 44" S–48° 7' 31" W. 950 m.a.s.l. *Oliveira, GC. 1294* (HUFU, CTES).
C. verbascifolia (Less.) H. Rob.	10	-	2x	Argentina. Misiones. Dept. San Javier. 20.4 km W from San Javier. Way to. 27° 52' 43" S–55° 17' 05" W. 94 m.a.s.l. *Via do Pico* et al. *24* (CTES).

Continued

Table 2 Continued

Species	n	$2n$	Ploidy	Voucher
	10	-	2x	Argentina, Misiones: Concepción de La Sierra. 2 km NW from Concepción de la Sierra. Way to Apóstoles. 27° 58' 11'' S−55° 31' 46'' W. 159 m.a.s.l. *Via do Pico* et al. *60* (CTES).
	-	20	2x	Argentina. Misiones. Dept. San Javier. Loc. Itacaruaré. 400 m from Route 2. 27° 52' 42'' S−55° 17' 05'' W. *Via do Pico* et al. 99 (CTES).
	-	20	2x	Argentina. Misiones. Dept. General Manuel Belgrano. Campina de Americo. 26° 17' 52.92'' S−53° 48' 0.87'' W. 764 m.a.s.l. *Vega* et al. *151* (CTES).
	-	20	2x	Argentina. Misiones. Dpto. General Manuel Belgrano, Route 14. Way to Bernardo de Irigoyen, Graveyard. Campina de Americo. 26° 16' 29'' S−53° 41' 59'' W. 812 m.a.s.l. *Pérez* et al. *53* (CTES).

(Herbarium Codes: CTES: Instituto de Botánica del Nordeste, Argentina, Corrientes; HUFU: Universidade Federal de Uberlândia, Brazil, Minas Gerais, Uberlândia).

references lacking geographical coordinates but having an exact description of collecting sites, coordinates were retrieved from Google Earth Pro 7.1 (Google Inc.).

To relate the distribution of ploidy levels to geographical and ecological parameters, we used elevations as measured in the field with GPS, and the 19 BioClim climate layers at 2.5 arc min. resolution from WorldClim (Hijmans et al., 2005). A general cytogeographic analysis was carried out with all the populations and cytotypes of *Chrysolaena* geo-referenced. The distribution of diploids versus polyploids (tetraploids, hexaploids, heptaploids, octoploids) was compared. To assess differences in intraspecific distributions of cytotypes, we mapped cytotypes of four species for which we had more than ten observations: *C. cognata*, *C. flexuosa*, *C. lithospermifolia* and *C. propinqua*. The BioClim variables highly correlated with each other were determined by a Pearson correlation analysis (coefficient $\geq$0.75). In total, nine BioClim variables representing climatic variability were selected: *BIO1: annual mean temperature, BIO2: mean diurnal range (mean of monthly (max temp-min temp)), BIO3: isothermality (mean diurnal range/annual range in temp x 10x), BIO5: max temperature of warmest month, BIO8: mean temperature of wettest quarter, BIO9: mean temperature of driest quarter, BIO12: annual precipitation, BIO15: precipitation seasonality (coefficient of variation), and BIO18: precipitation of warmest quarter.* A principal component analysis (PCA) of the nine environmental variables and one geographical variable (*altitude*) was carried out to provide insights into the overall distribution pattern of particular cytotypes and to reveal their ecological preferences. Both statistical analyses were carried out with Infostat software version 2018 (Di Rienzo et al., 2018). Full details of the populations of *Chrysolaena* analyzed, including ploidy levels, geographical coordinates and values of bioclimatic variables are available from Dryad (Data S1).

3 Results

3.1 Chromosome numbers and ploidy

Mitotic and/or meiotic chromosome numbers are reported for 43 populations of nine species of *Chrysolaena* (Table 2); 72 previously published cytological records have been included (Table 1). All the entities analyzed for the first time presented a basic chromosome number $x = 10$. The chromosome numbers of the analyzed entities ranged from $2n = 20$ to $2n = 80$. Of the 43 populations studied, 22 were diploids ($2n = 2x = 20$), 12 tetraploids ($2n = 4x = 40$), six hexaploids ($2n = 6x = 60$), two octoploids ($2n = 8x = 80$), and one heptaploid ($2n = 7x = 70$) belonging to *Chrysolaena cordifolia*; this is the first chromosome count for the species (Fig. 1K). In *C. verbascifolia* only a diploid cytotype was found (Figs. 1J, 2J), and in *C. sceptrum* only tetraploids were found (Figs. 2K, 2L). The *C. cognata* populations presented diploid (Figs. 1B, 2A), tetraploid, hexaploid (Figs. 1A, 2B, 2C), and octoploid cytotypes (Fig. 2D), and 0–3 B chromosomes were observed in a diploid population. *Chrysolaena flexuosa* (Figs. 1D, 2E), *C. lithospermifolia* (Figs. 1E, 1F, 2F) and *C. propinqua* (Figs. 1H, 1I, 2H, 2I) presented diploid and tetraploid populations. A new cytotype was found for *C. propinqua* var. *canescens* ($2n = 4x = 40$) (Fig. 2I). The populations of *C. platensis* showed $2n = 6x = 60$ (Fig. 2G), and $2n = 2x = 20$ with 1-4 B chromosomes (Fig. 1G). *Chrysolaena simplex* presented $2n = 4x = 40$. Chromosome numbers from new localities of *C. lithospermifolia* ($2n = 2x = 20$) and *C. platensis* ($2n = 6x = 60$) from Paraguay are reported for the first time.

Of all the populations of *Chrysolaena* analyzed cytogeographically (104 populations with $x = 10$, 43 counts made in this study and 61 previously reported), 46.16% corresponded to populations with diploid cytotype, 31.73% were tetraploids, 13.46% hexaploids, 1.92% heptaploids, 5.77% octoploids, and only 0.96% corresponded to the mixed population of *C. cognata* (5x-6x) (Fig. 3).

3.2 Cytotype distribution of *Chrysolaena*

For the cytogeographic study, we analyzed 104 populations of *Chrysolaena* (see Tables 1, 2). The four most frequent cytotypes of *Chrysolaena* (2x, 4x, 6x and 8x) had largely overlapping distributions (Fig. 4). Moreover, between two and five species, each with a different cytotype, can be found co-ocurring in the same area, as observed in three geographical locations: Campina de Americo (General Manuel

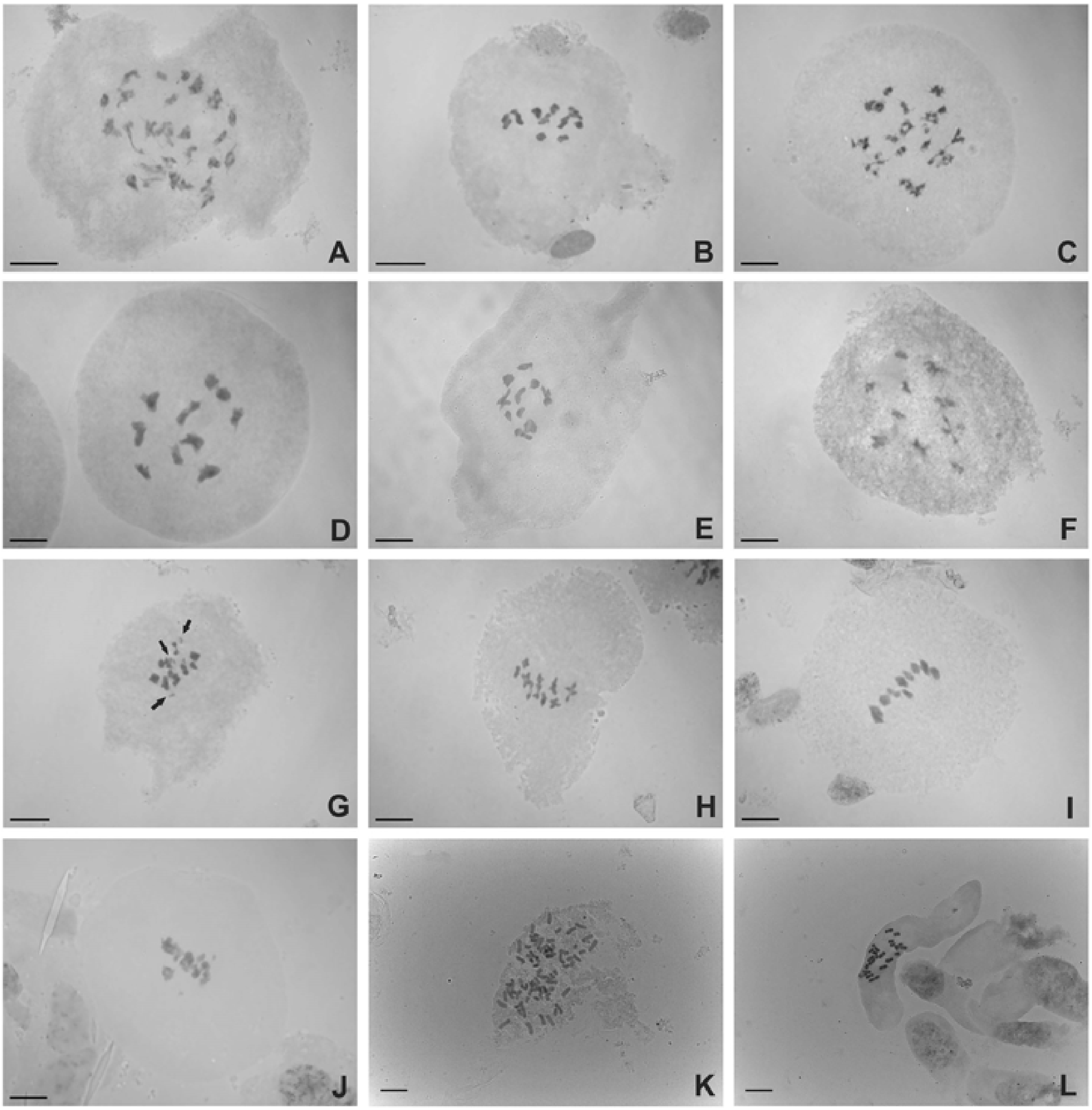

Fig. 1. Meiotic (**A–J**) and mitotic (**K, L**) chromosome numbers of *Chrysolaena* species with herbarium number. **A,** *C. cognata* N°31 ($2n = 6x = 60$). **B,** *C. cognata* N°50 ($2n = 2x = 20$). **C,** *C. flexuosa* N°18 ($2n = 4x = 40$). **D,** *C. flexuosa* N°19 ($2n = 2x = 20$). **E,** *C. lithospermofolia* N°3 ($2n = 2x = 20$). **F,** *C. lithospermifolia* N°9 ($2n = 2x = 20$). **G,** *C. platensis* N°155 ($2n = 2x = 20$). **H,** *C. propinqua* N° 25 ($2n = 2x = 20$). **I,** *C. propinqua* N°43 ($2n = 2x = 20$). **J,** *C. verbascifolia* N°24 ($2n = 2x = 20$). **K,** *C. cordifolia* N°98 ($2n = 7x = 70$). **L,** *C. lithospermifolia* N°646 ($2n = 2x = 20$). Scale Bar = 10 μm.

Belgrano Department, Misiones, Argentina), Zaimán creek, (Misiones, Capital Department, Argentina), and Pedro Juan Caballero (Amambay, Paraguay) (Fig. 4). In Campina de Americo, *C. cognata* (8*x*), *C. platensis* (2*x*), *C. propinqua* (4*x*), *C. cordifolia* (7*x*) and *C. verbascifolia* (2*x*) were found in the same area. *Chrysolaena verbascifolia* (2*x*), *C. propinqua* (2*x*), *C. flexuosa* (2*x*), *C. cognata* (6*x*) were found in Zaimán creek, and *C. cristobaliana* (7*x*), *C. propinqua* var. *canescens* (2*x*), and *C. sceptrum* (4*x*) were detected in Pedro Juan Caballero.

Polyploids occupied a larger area than diploids (Fig. 5). Most of the diploid populations were restricted to the northeastern region of Argentina (Corrientes and Misiones provinces), and a few dispersed populations were found in Brazil, Paraguay and Uruguay. Most of the tetraploids were located in the south of the study area, mainly in Uruguay. However, two populations occurred in Bolivia and Paraguay. Hexaploid populations were concentrated in two separated regions: one group of hexaploids was located in the northeast of Argentina, mainly in the province of Misiones, and the other group in the south of the distribution area, in the province of Buenos Aires, Argentina. Only one hexaploid population was recorded in Brazil (*C. platensis*). The octoploid and odd cytotypes (7*x*) were rare. The octoploid populations were close to each other. One population was recorded in the northeastern region of Paraguay (department of Amambay), one in the south of Brazil (state of Parana) and the others in

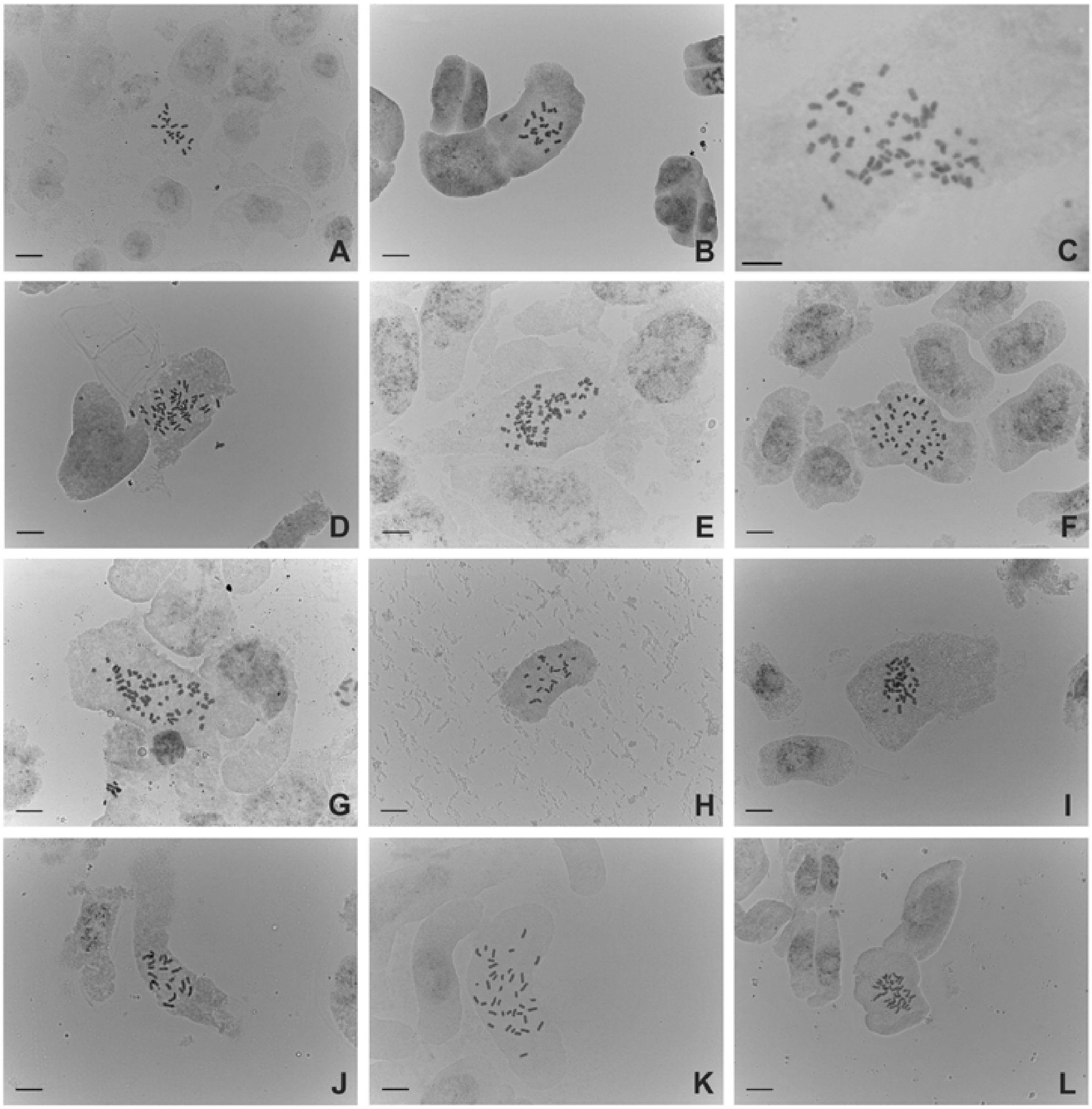

Fig. 2. Mitotic chromosome numbers of *Chrysolaena* species with herbarium number. **A,** *C. cognata* N°658 ($2n = 2x = 20$). **B,** *C. cognata* N°30 ($2n = 2x = 20$). **C,** *C. cognata* N°53 ($2n = 6x = 60$). **D,** *C. cognata* N°102 ($2n = 6x = 60$). **E,** *C. cognata* N°115 ($2n = 8x = 80$). **F,** *C flexuosa* N°35 ($2n = 4x = 40$). **G,** *C. platensis* N°72 ($2n = 6x = 60$). **H,** *C. propinqua* N°100 ($2n = 2x = 20$). **I,** *C. propinqua* var. *canescens* N°94 ($2n = 4x = 40$). **J,** *C. verbascifolia* N°53 ($2n = 2x = 20$). **K,** *C. sceptrum* N°82 ($2n = 4x = 40$). **L,** *C. sceptrum* N°783 ($2n = 4x = 40$). Scale Bar = 10 μm.

the northeast of Argentina (province of Misiones). Finally, there were populations with only one or a few records. *Chrysolaena cordifolia* and *C. cristobaliana* (both heptaploid species) located in the northeast of Argentina (Misiones province) and in the north of Paraguay (Amambay department), respectively; and the mixed population of *C. cognata* found in Argentina (province of Misiones).

Regarding diploids, higher-level polyploids (7*x* and 8*x*) occur in higher altitude areas, above 300 m (Fig. 6). Tetraploids and hexaploids occupied a wider range of altitude, between 2 m to approximately 900 m. The diploids also presented a wide range of altitudes. The greatest number of diploid populations was concentrated between 50 and 150 m.

3.3 Intraspecific distributions

The analysis of the species with the greatest frequency and variation of cytotypes showed that the distribution of *C. cognata* cytotypes did not have a clear pattern; diploids and polyploids of all levels were distributed in a scattered manner (Fig. 7). In *C. lithospermifolia* diploid populations were found to be distributed in the north of the sampling area of the species, whereas tetraploids were distributed in the south (Fig. 7). *Chrysolaena flexuosa* showed a clear cytotype distribution pattern (Fig. 8). Diploids were distributed in the north of the study area; the tetraploid populations were found in the south, and hexaploids were in the southernmost region, in a hilly area in the province of Buenos Aires (Argentina).

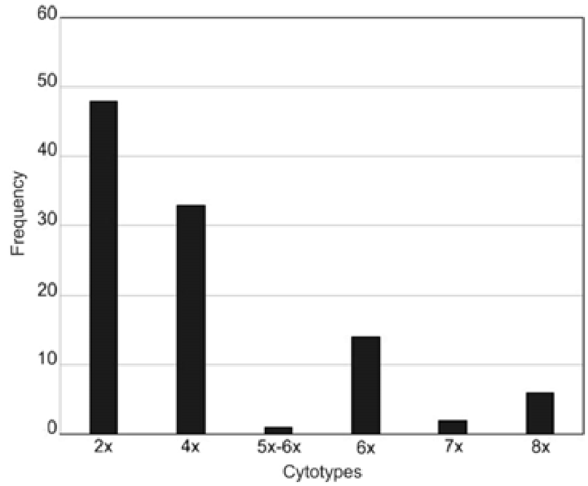

Fig. 3. Histogram of frequencies of cytotypes of 104 populations of *Chrysolaena* cytogeographically analyzed.

Fig. 5. Distribution of diploid and polyploid cytotypes (4*x*, 6*x*, 7*x*, 8*x*) of *Chrysoalena* in South America.

A single diploid population was found close to tetraploids in Uruguay (Fig. 8). Most of diploid populations of *C. propinqua* were distributed in the northeast of Corrientes province and in the west of Misiones, Argentina, and only two populations were located in the north of Paraguay; tetraploids were located in the east of Misiones province (Argentina) (Fig. 8).

3.4 Statistical analyses

The correlation analysis reduced the 19 Bioclim variables to nine less-correlated variables. The PCA produced two main components that explained 68% of the variation observed (PC1 40.7% and PC2 27.3%) (Fig. 9). BioClim variables were mainly correlated with the first axis. Variables indicating precipitation and altitude were positively correlated and variables reflecting temperatures were negatively correlated. In the second axis, the most representative variables were those indicating temperatures. Analyses of the ecological differentiation of cytotypes showed that ecological boundaries among them were not strictly defined; however, some general preferences were evident. The distribution of all the cytotypes was quite dispersed within the four quadrants. The highest polyploids (7*x* and 8*x*) were scattered in the diagram influenced by different variables related to temperature and precipitation. The very distant points in quadrants I and II corresponded to

Fig. 4. Distribution of cytotypes of *Chrysolaena* in South America. Blown-up areas show populations of northeast of Argentina and of Pedro Juan Caballero locality (Paraguay).

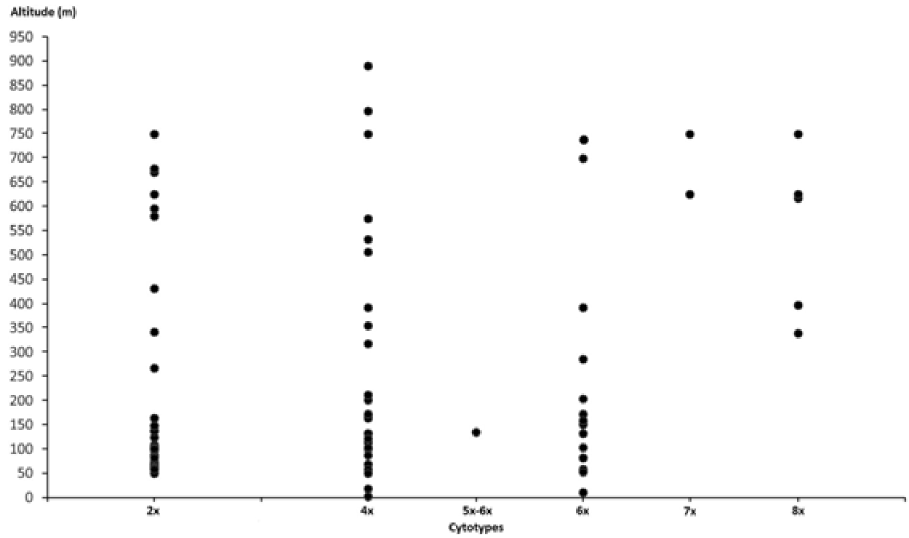

Fig. 6. Diagram representing the cytotypes with its altitude.

the populations with the northernmost and southernmost distributions within the study area, respectively.

4 Discussion

4.1 Cytotaxonomy and ploidy level

This is the first study that compiles all known chromosome numbers for *Chrysolaena*. The 43 populations of the nine cytogenetically analyzed species presented the basic chromosome number $x = 10$, a number proposed as characteristic of the genus (Dematteis, 1997a), and different chromosome numbers or ploidy levels ranging from diploid ($2n = 2x = 20$) to octoploid ($2n = 8x = 80$). The present data document the most extensive polyploid series known for *Chrysolaena* to date, with six ploidy levels (2*x*, 4*x*, 5*x*, 6*x*, 7*x* and 8*x*). These results are consistent with the basic number and some chromosome numbers reported for the genus in previous studies (Dematteis, 1997a, 1997b, 2002, 2009; Dematteis et al., 2007; Angulo & Dematteis, 2009a, 2009b; Via do Pico & Dematteis, 2012, 2013, 2014, 2017). However, the results found in this study broaden the cytological information of the genus, since the first chromosome count of *C. cordifolia* is reported, as well as a new cytotype for *C. propinqua* var. *canescens*, and counts for populations of new geographical localities (*C. lithospermifolia* and *C. platensis* from Paraguay).

The chromosome numbers found in the 15 analyzed populations of *C. cognata* ($2n = 2x = 20$, $2n = 4x = 40$, $2n = 6x = 60$ and $2n = 8x = 80$) agree with previous counts reported for the species (Dematteis, 1997a, 2002; Angulo & Dematteis, 2009a, 2009b; Via do Pico & Dematteis, 2012, 2013). However, they do not agree with those reported by Jones (1979) in Brazilian populations, with $n =$ ca. 17, ca. 33-34 and 34. The chromosome number of the analyzed *C. flexuosa* populations is in agreement with that found in previous studies (Dematteis, 1997a, 2002; Angulo & Dematteis, 2009a, 2009b; Via do Pico & Dematteis, 2012, 2013); however, it does not agree with the count of $n =$ ca. 17 reported by Jones

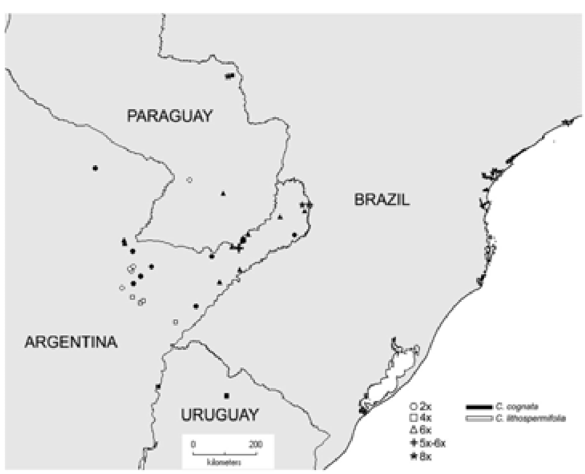

Fig. 7. Distribution of cytotypes of *Chrysoalena cognata* and *C. lithospermifolia.*

Fig. 8. Distribution of cytotypes of *Chrysoalena flexuosa* and *C. propinqua.*

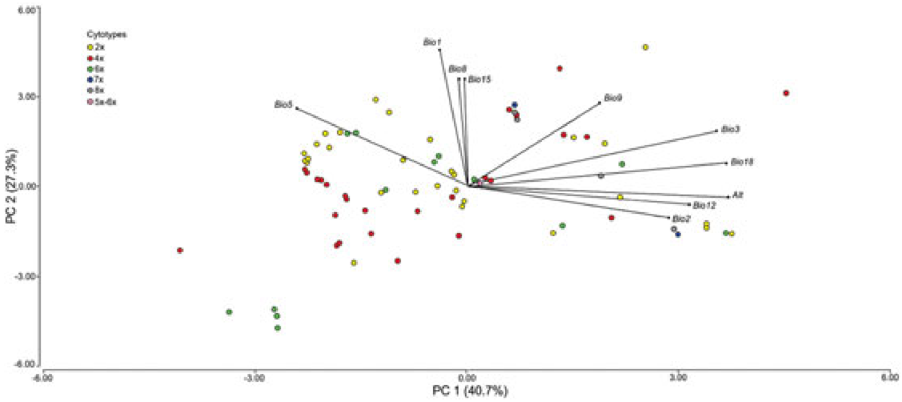

Fig. 9. Principal Components Analysis of nine environmental variables and altitude at sampling points of *Chrysolaena*. Points represent sampling localities.

(1979) in a population from Uruguay and with the $n=$ ca. 30–32 found by Hunziker et al. (1990) in specimens of Argentina. The chromosome number found in *C. simplex* ($2n=4x=40$) agrees with the count made by Ruas et al. (1991) in a population of Paraná state (Brazil); however, it differs from the one found by Oliveira et al. (2012) for a locality of Minas Gerais (Brazil).

Different chromosome numbers are often attributed to the same taxon in the literature, and this is one of the main problems in cytotaxonomic studies (Mansanares et al., 2002). These differences may be attributed to incorrect counts (due to the small size of the chromosomes) or to the misidentification of the species (mainly in taxonomically complex groups). Another cause is the occurrence of polyploidy between species and individuals of the same species (Mansanares et al., 2002; Weiss-Schneeweiss et al., 2003). Some of the chromosome numbers found in the present study do not agree with previous counts made in the same taxa, which may be due to the previously mentioned causes. The Vernonieae tribe not only is one of the most complex within the Asteraceae from a taxonomic point of view, which makes taxon identification difficult, but also includes numerous species for which phenomena of polyploidy have been documented (Keeley et al., 2007; Angulo & Dematteis, 2012).

Preliminary studies considered the basic chromosome number $x=10$ as typical of Old World Vernonieae in genera such as *Aedesia* O.Hoffm., *Erlangea* Sch.Bip., *Ethulia* L., *Gutenbergia* Sch.Bip. and *Vernonia* s.s. (Jones, 1979). However, when the basic number $x=10$ was found in *Chrysolaena* species, it was considered to be the characteristic basic number of the genus (Dematteis, 1998, 2002). Since there are no other American genera with this basic number, it was postulated that this is probably one of the most important characters to separate *Chrysolaena* from the other American Vernonieae (Dematteis, 2009). The results presented here, together with previous chromosomal studies, support the basic number $x=10$ as typical of *Chrysolaena* and show that it allows us to clearly distinguish the genus from other genera of Vernonieae from America.

Polyploidy is a very common phenomenon in the Asteraceae family and occurs in most clades (Semple & Watanabe, 2009). Jones (1979) and Ruas et al. (1991) suggested that the Vernonieae of the New World show a marked diversity in chromosome numbers and high rates of polyploid species with respect to the Old World entities. It is estimated that 25% of the South American entities are polyploid, mostly tetraploid and octoploid (Angulo & Dematteis, 2012). *Lessingianthus* is one of the genera with the highest proportion of polyploids, nearly 82.5% of a total of 39 taxa, and with ploidy levels up to 11x (Ruas et al., 1991; Dematteis, 1996, 1997b, 2002; Dematteis et al., 2007; Oliveira et al., 2007; Angulo & Dematteis, 2009a, 2009b, 2012). The information provided in the present study, together with previously published chromosome numbers, reveals that the South American genus *Chrysolaena* is cytologically complex and that polyploidy has played a very important role in the evolution of the group. Most (91%) of the species studied to date are polyploid. Despite the great diversity of cytotypes found, diploids and tetraploids are the most frequent among populations. The results also reveal the occurrence of intraspecific polyploidy in 82% of the total species studied. The variability of cytotypes observed among populations agrees with previous studies indicating that *C. cognata* and *C. platensis* have the greatest variability of chromosome numbers in the genus (Angulo & Dematteis, 2009a, 2009b; Dematteis, 2009; Via do Pico & Dematteis, 2012, 2013). However, not all species of *Chrysolaena* appear to have variable ploidy levels. *Chrysolaena verbascifolia* is exclusively diploid so far.

Although polyploidization is frequently associated with species diversification, due to the barrier to gene flow resulting from chromosomal multiplication, variations in ploidy also occur at the intraspecific level (Husband et al., 2013). This phenomenon is mainly associated with three causes: (i) duplication of the genome, which is a common mutation in many natural populations; (ii) depending on the frequency and pathways of polyploid formation, diploids and polyploids can be maintained as genetically homogeneous populations; (iii) polymorphism in the ploidy level may be the result of erroneous taxonomic approaches, since morphological divergence is often used as a criterion for species delimitation and polyploid lineages may not be recognized at this level; this occurs especially in autopolyploids, which generally exhibit a strong resemblance to their diploid parents (Soltis et al., 2007; Husband et al., 2013). In many cases, patterns of variation within species with different ploidy levels can provide information on the early stages of polyploid evolution. Taking into account this assumption, the *Chrysolaena* species with variation in ploidy levels would be at the initial stages of evolution; i.e., they would be a group of young or newly formed polyploid taxa.

The highest ploidy levels found in this study are the heptaploid ($2n = 7x = 70$), found in *C. cordifolia*, and the octoploid ($2n = 8x = 80$) found in *C. cognata*. The chromosome number found in *C. cordifolia* is the third report of odd polyploidy in the genus, after records reported for *C. cognata* and *C. cristobaliana* (Dematteis, 2002; Via do Pico & Dematteis, 2014). Other cases of odd polyploidy have been reported in the Vernonieae, such as in *Lessingianthus macrocephalus* (Less.) H. Rob., with $2n = 11x = 176$, one of the highest ploidy levels found within the Asteraceae (Angulo & Dematteis, 2012). The high ploidy levels combined with odd chromosomal complements suggest irregular meiotic behavior and possible sterility of individuals. Apomictic reproduction is very common in plants with these characteristics. Apomixis is a natural process that allows clonal reproduction by means of seeds, avoiding meiosis and fertilization, and which originates offspring genetically identical to the maternal plant (Nogler, 1984). Polyploidy and apomixis can co-occur, since they share common developmental pathways. Both phenomena can be induced after a hybridization event, involve the production of unreduced gametes and can restore fertility after hybridization (Husband et al., 2013). Apomixis is very frequent in the Asteraceae family and has been documented in 2.9% of the genera (Noyes, 2007), such as *Campuloclinium* DC. of the Eupatorieae tribe (Farco et al., 2012), *Crepis* L., *Hieracium* L. and *Taraxacum* F. H. Wigg. of the Lactuceae tribe and *Elephantopus* L. within the Vernonieae (Noyes, 2007). Considering these previous records and the occurrence of very high and odd ploidy levels, the *Chrysolaena* populations analyzed in this study are likely to show apomictic reproduction.

Chrysolaena cordifolia is a species with few collection records (only three known herbarium specimens), with few individuals forming its population (no more than three) and with a very restricted geographical distribution. The species is only known for the north of Corrientes and northeast of Misiones (Argentina) so far (Dematteis, 2009). Morphologically, it resembles *C. cognata* and *C. platensis*, from which it is mainly distinguished by the presence of basally cordate leaves, an infrequent feature in the genus. These three species can be found growing in the same area or locality. All these characteristics, added to the high and odd ploidy level ($7x$) found in this study for *C. cordifolia*, suggest that *C. cordifolia* could be a hybrid, probably between *C. cognata* ($8x$) and *C. platensis* ($6x$).

Chrysolaena cristobaliana grows in fields and Cerrados of southern Mato Grosso and Mato Grosso do Sul (Brazil), and in northeastern Paraguay. There is only one known chromosome count for *C. cristobaliana* ($7x$), with presence of 0 to 7 B chromosomes per cell (Via do Pico & Dematteis, 2017). Morphologically, it is also closely related to *C. cognata*, from which is distinguished by the leaf shape, pubescence color and number of flowers. Both species can occur in the same area together with *C. cognata*, *C. propinqua* and *C. sceptrum*. As it was postulated for *C. cordifolia*, *C. cristobaliana* could be a hybrid species, and its probable parents could be *C. cognata*, *C. platensis* or *C. sceptrum*.

Jones (1975) defined the B chromosomes as extra chromosomes of a basic chromosome set that clearly do not correspond to the category of aneuploidy or polyploidy. One of the possible origins of the B chromosomes would be by spontaneous generation because of genomic rearrangements after interspecific hybridization events (Sapre & Deshpande, 1987). The ability to hybridize between closely related species was demonstrated in *Vernonia* species of North America and Africa (Keeley & Robinson, 2009). The phenomenon of hybridization has been proposed to explain the existence of mixed populations and odd cytotypes in *C. cognata* (Dematteis, 2002, 2009). This phenomenon could explain the occurrence of B chromosomes and odd ploidy levels found in the species of *Chrysolaena*. The possibility that hybridization occurs among some species due to the variability of cytotypes, the high levels of ploidy found and the distribution areas shared among closely related species is a reasonable hypothesis. These assumptions show the need for other types of studies on *Chrysolaena* species. Previous studies have suggested an autopolyploid origin in some polyploid *Chrysolaena* species (Via do Pico & Dematteis, 2012); however, given the cytotype variation observed, it is very likely that polyploids originated not only by autopolyplody but also by other mechanisms.

4.2 Cytotype distribution

This study represents the first cytogeographic study of a genus of the Vernonieae tribe. The results obtained did not allow us to find a clear distribution pattern that depends on climatic factors for *Chrysolaena* populations, since both diploids and polyploids can occupy areas of very similar environmental conditions and even co-exist in the same area. However, we found a geographic pattern in the distribution of ploidy levels. The diploids presented a more restricted distribution, they were mainly concentrated in northeastern Argentina, and the polyploids occupy a wider range covering an area that extends to the limits of the typical distribution area of the genus.

Polyploidy has historically been considered associated with higher tolerance to environmental stress (Grant, 1981). Although numerous taxa display these trends, exceptions have been noted and no general explanation has been proposed (Stebbins, 1950). A tendency of higher-level polyploids ($7x$, $8x$) to occupy areas of higher altitude with respect to diploids was observed in *Chrysolanea*. However, diploids, tetraploids and hexaploids can also occur in these areas. The results obtained agree with the current view that the geographic and ecological distribution of polyploids do not follow simple patterns (Husband et al., 2013; Kessler et al., 2014), and that there may be alternative patterns that could better explain polyploid persistence, rather than shifts to new climatic niches (Glennon et al., 2014).

The Neotropical Region is considered the most diverse and least understood terrestrial area in the world (Ceballos & Ortega-Baes, 2011). Various biodiversity hotspots have been recognized in this area, including the Brazilian Cerrado and the Atlantic Forest of Brazil, which are regarded of priority at a global scale due to their species richness and endemism, as well as the high threat they face (Myers et al., 2000; Ceballos & Ortega-Baes, 2011). However, many of these areas have been selected using only one aspect of diversity, such as species richness, endemism or threatened species (Orme et al., 2005). Today, other aspects of biodiversity should be known and used when selecting diverse areas for conservation. Much of the geographic distribution of *Chrysolaena* covers the Brazilian Cerrado and the Atlantic Forest of Brazil. Environmental heterogeneity, together with polyploidization and hybridization, has been proposed to explain the high species richness and endemism reported in these areas (Echternacht et al., 2011; Gomes et al., 2018). The study of the geographical distribution of cytotypes is a key requirement to understand the role of polyploidy in diversification (Sonnleitner et al., 2010; Gomes et al., 2018). Cytogeographic patterns may reveal significant amounts of diversity by identifying multiple chromosomal races among related species or within a single taxonomic species and, therefore, contribute to the conservation of rare species and ecological restoration (Soltis et al., 2007). The analysis of geographic distribution of *Chrysolaena* revealed three areas with a high diversity of cytotypes and species: Campina de Americo (Misiones, Argentina), Zaimán creek (Misiones, Argentina), and Pedro Juan Caballero (Amambay, Paraguay). Continuous hybridization and polyploidization events could have occurred, which would have given rise to such diversity of cytotypes in geographically reduced areas. However, more research is still in needed to understand how these events would be taking place. The results of this study pave the way to other types of studies in *Chrysolaena* and provide information that could be useful to design possible conservation strategies in certain areas of the Neotropics of central South America.

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Supplementary Material

The following supplementary material is available online for this article at http://onlinelibrary.wiley.com/doi/10.1111/jse.12471/suppinfo:

Data S1. Available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.hk2p481