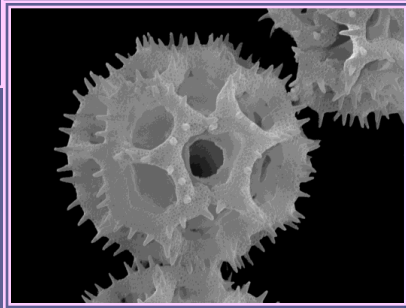


Cytogenetic and palynological studies in species of *Chrysolaena* (Vernonieae, Asteraceae)



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Geographical Distribution



Vernoniae Cass.: pantropical tribe with ca. 1.800 species distributed in America, Asia and Africa. In the New World: 15 subtribes, Old World: 6.

Subtribe Lepidaploinae S.C. Keeley & H. Rob.: newly created, comprises 12 genera.

Chrysolaena H. Rob. includes 18 species concentrated in southern Brazil and northeast Argentina.

Brazil: 15 ssp.

Paraguay: 11 ssp.

Argentina: 6 ssp.

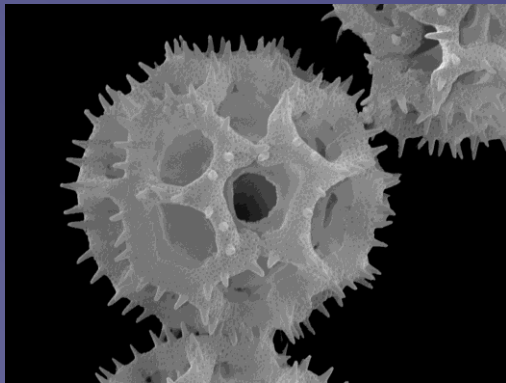
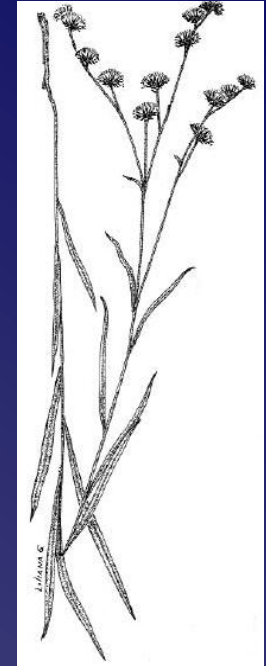
Bolivia: 4 ssp.

Uruguay: 3 ssp.

Perú: 1 ssp.

CHRYSOLAENA H. Rob.

Sericeous or velutine indumentum
Anthers with apical glandular appendage
Style without basal node
Glandular cypselas



Pollen morphology



Basic chromosome number $x=10$

The main purpose was to analyze the meiotic and mitotic chromosomes and the morphology of pollen grains in *Chrysoleaena* species in order to broaden the cytological and palynological information of the genus. We also performed the first study of nuclear DNA content in species of the genus.

MATERIAL Y METHODS

Species analyzed: *C. cognata*, *C. flexuosa*, *C. propinqua*, *C. lithospermifolia*, *C. verbascifolia*, *C. platensis*, *C. obovata*.

Mitosis

From roots pretreated with 8-hydroxyquinoline solution 0.002M for 4-5 hours. Feulgen staining technique. Karyotypes made from 10 metaphases. Morphology of chromosomes determined using the Centromeric Index ($ci = \text{short arm} \times 100 / \text{total length of the chromosome}$). Was calculated: total length of the karyotype (LTC), mean chromosome length (LM), average centromeric index (CI) and Intrachromosomal (A1) and intercromosómica (A2) asymmetry indexes.

Pollen Morphology

The pollen was acetolysed according to Erdtman (1960) and the grains were mounted in glycerol jelly on glass slides. Pollen measurement data were calculated from at least 30 grains per sample: polar axis (P), equatorial diameter (E), exine thickness, etc. The pollen grains were observed with optical microscope (OM) and scanning electron microscope (SEM). The terminology used to describe the pollen grains is suggested by Keeley & Jones (1979) and Punt & al. (1994).

Meiosis and pollen fertility

Anthers squashed and stained with orcein lactopropionic (2%). The pollen fertility, indicated by pollen stainability, was estimated using the technique of carmine-glycerin (1:1).

DNA content by flow cytometry

We estimated the nuclear DNA content using flow cytometry. Three patterns were used as reference or standard: *Paspalum intermedium* Sch 28 857 (diploid, $2C = 1.417 \text{ pg}$), *P. dilatatum* ssp. *flavescens* (tetraploid, $2C = 2.43 \text{ pg}$) and *P. dilatatum* Chirú (hexaploid, $2C = 3.57 \text{ pg}$). The nuclear DNA content ($2C$) was calculated: (average peak of the sample / standard peak average) $\times 2C$ value of the standard (pg).

Species	Voucher	Locations	Ploidy	Mitosis	Meiosis	Pollen	DNA content
<i>C. cognata</i>	3029	Arg. Chaco-Colonia Benitez	6x		x		
<i>C. propinqua</i>	3038	Arg. Misiones-Posadas-A Zaiman	2x		x	x	
<i>C. obovata</i>	3475	Bolivia-Santa Cruz	4x		x	x	
<i>C. platensis</i>	3083	Arg. Misiones-GMB-Dos hermanas	2x		x	x	
<i>C. flexuosa</i>	3035	Arg. Misiones-Posadas-A Zaiman	2x		x		
<i>C. lithospermifolia</i>	2469	Arg. Corrientes	4x		x	x	
<i>C. lithospermifolia</i>	4	Arg. San Roque-Ctes	2x	x	x		x
<i>C. flexuosa</i>	45	Arg. Corrientes-Itá Ibaté	2x	x	x		x
<i>C. verbascifolia</i>	34	Arg. Misiones-G. M. Belgrano	2x		x		x
<i>C. propinqua</i>	43	Arg. Corrientes.Ituzaingó	2x		x		
<i>C. flexuosa</i>	18	Arg. Corrientes-Alvear	4x		x		
<i>C. propinqua</i>	25	Arg. Misiones-San Javier	2x		x		
<i>C. cognata</i>	31	Arg. Misiones-San Ignacio	6x		x		x
<i>C. verbascifolia</i>	3080	Arg. Misiones GMB.campina americo	2x	x			
<i>C. cognata</i>	3053	Arg. Misiones-GMB-Campina	8x	x			x
<i>C. cognata</i>	3040	Arg. Misiones-San Ignacio	4x	x		x	
<i>C. propinqua</i>	3078	Arg. Misiones-GMB-Campina	4x	x			x
<i>C. cognata</i>	3761	Uruguay-Tacuarembó	4x	x			x
<i>C. flexuosa</i>	3703	Uruguay-Artigas	4x	x			x
<i>C. flexuosa</i>	3750	Uruguay-Tacuarembó	4x	x			x
<i>C. flexuosa</i>	5	Arg. Corrientes-San Roque	-			x	x
<i>C. cognata</i>	6	Arg. Corrientes-San Roque	-			x	x
<i>C. propinqua</i>	20	Arg. Corrientes-Virasoro	-			x	
<i>C. lithospermifolia</i>	3	Arg. Corrientes-Saladas	-			x	x
<i>C. verbascifolia</i>	44	Arg. Corrientes-Ituzaingó	-			x	

RESULTS

MEIOSIS

Phase	<i>C. flexuosa</i> 45		<i>C. flexuosa</i> 3035		<i>C. propinqua</i> 25		<i>C. cognata</i> 3029		<i>C. lithospermifolia</i> 4	
MI	N	%	N	%	N	%	N	%	N	%
Regular	184	90,2	177	94,65	145	91,78	78	91,76	112	96,55
Precocious migration	2	0,98	1	0,54	0	0	3	3,53	0	0
Off-plate	18	8,82	9	4,81	13	8,22	4	4,71	4	3,45
Total	204	100	187	100	158	100	85	100	116	100
AI/II										
Regular	137	97,9	79	77,45	164	97,62	67	87,02	85	86,73
Laggards	3	2,14	4	3,92	2	1,19	4	5,19	0	0
Bridges	0	0	19	18,63	2	1,19	6	7,79	13	13,27
Total	140	100	102	100	168	100	77	100	98	100
MII										
Regular	28	96,6	-	-	78	100	-	-	16	100
Off-plate	0	0	-	-	0	0	-	-	0	0
Precocious migration	1	3,45	-	-	0	0	-	-	0	0
Total	29	100	-	-	78	100	-	-	16	100
AII/III										
Regular	60	98,4	15	83,33	43	97,73	13	86,67	26	96,3
Laggards	1	1,64	-	-	1	2,27	-	-	0	0
Bridges	0	0	3	16,67	0	0	2	13,33	1	3,7
Total	61	100	18	100	44	100	15	100	27	100
Fertility										
Fertile	595	98	315	92,1	539	93,9	341	89,27	600	98,52
Infertile	12	1,98	24	7,9	35	6,1	41	10,73	9	1,48
Total	607	100	342	100	574	100	382	100	609	100

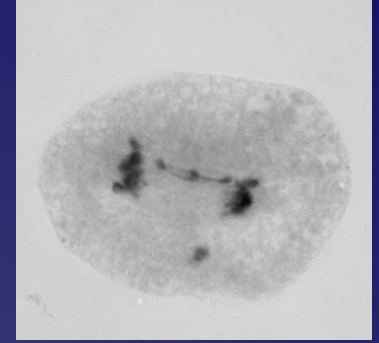
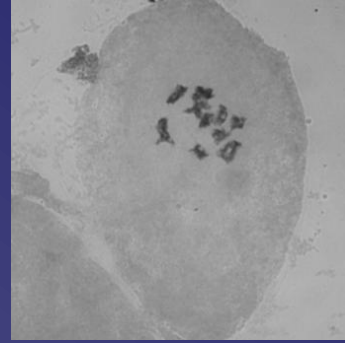
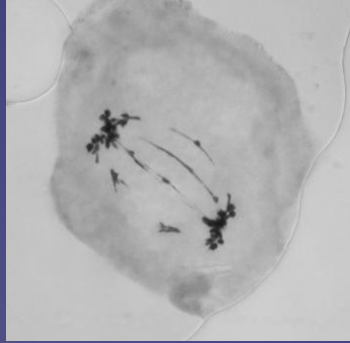
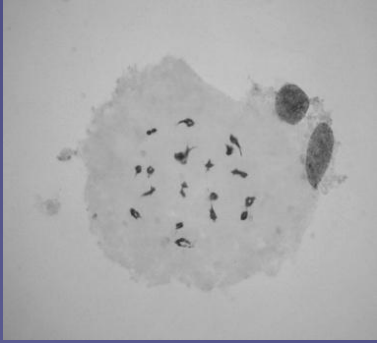
Phase	<i>C. lithospermifolia</i> 2469		<i>C. platensis</i> 3083		<i>C. obovata</i> 3475		<i>C. verbascifolia</i> 34	
MI	N	%	N	%	N	%	N	%
Regular	219	89,75	108	82,44	84	71,79	31	17,72
Precocious migration	2	0,82	1	0,76	2	1,71	0	0
Off-plate	23	9,43	22	16,8	31	26,5	144	82,28
Total	244	100	131	100	117	100	175	100
AI/II								
Regular	164	96,48	69	93,24	75	72,11	92	72,44
Laggards	3	1,76	2	2,7	6	5,77	35	27,56
Bridges	3	1,76	3	4,06	23	22,12	0	0
Total	170	100	74	100	104	100	127	100
MII								
Regular	42	91,3	-	-	35	79,55	-	-
Off-plate	4	8,7	-	-	9	20,45	-	-
Precocious migration	-	-	-	-	-	-	-	-
Total	46	100	-	-	44	100	-	-
AII/III								
Regular	85	74,56	-	-	-	-	-	-
Laggards	24	21,05	-	-	-	-	-	-
Bridges	5	4,39	-	-	-	-	-	-
Total	114	100	-	-	-	-	-	-
Fertility								
Fertile	534	90,66	350	98,04	463	97,47	565	94,8
Infertile	55	9,34	7	1,96	12	2,53	31	5,2
Total	589	100	357	100	475	100	596	100

C. obovata $2n=4x=40$

20 II
18 II + 1 IV
16 II + 2 IV

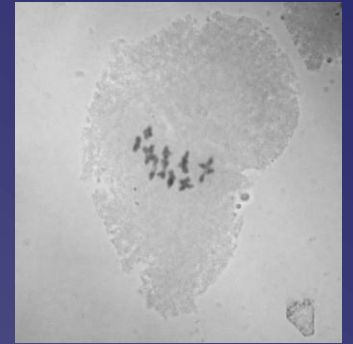
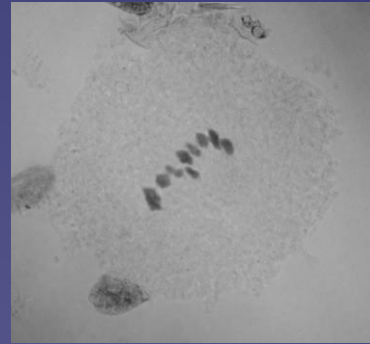
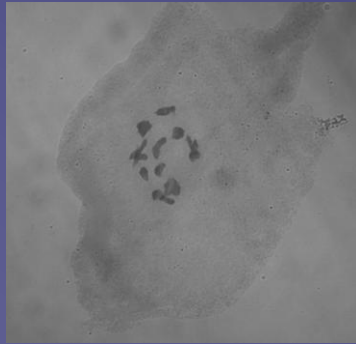
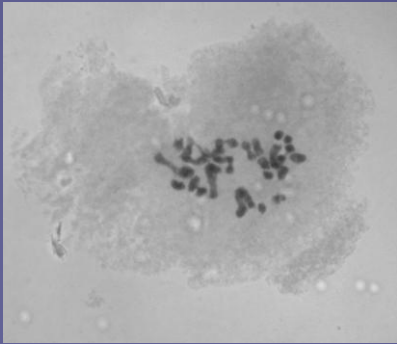
C. platensis $2n=2x=20$

10 II
9 II + 2 I

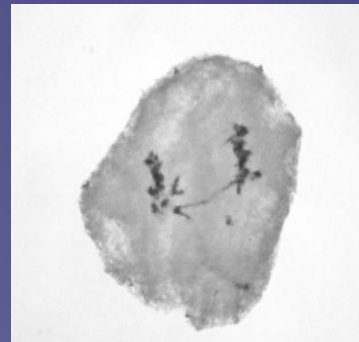


C. lithospermifolia

C. propinqua

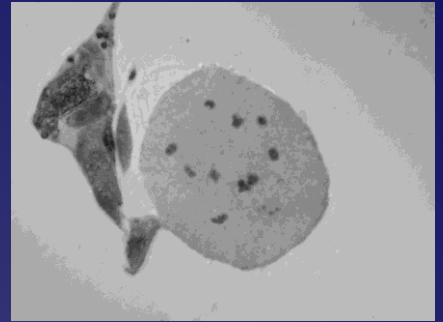
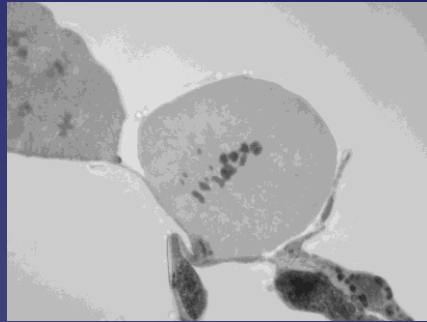
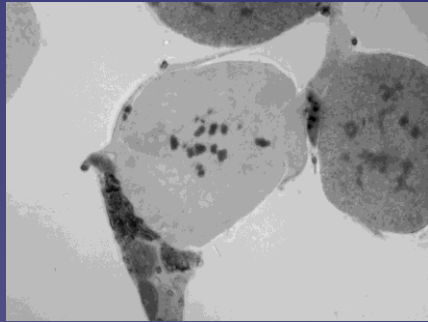
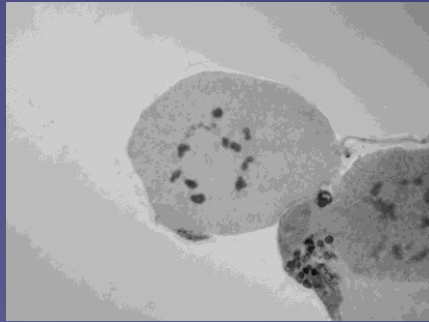


$2n=2x=20$ $2n=4x=40$
10 II 20 II
9 II + 2 I 5 IV + 1 III + 2 II + 13 I

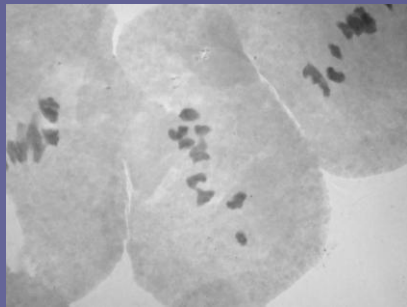
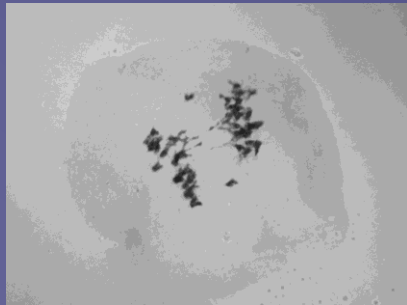


$2n=2x=20$
10 II
9 II + 2 I

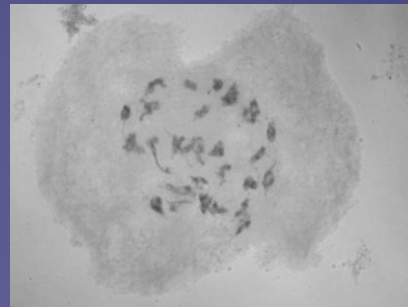
C. verbascifolia 2n=2x=20 10II
10II + 0-6B



C. flexuosa 2n=2x=20 2n=4x=40
20II 10II
18II+1IV 9II+2I



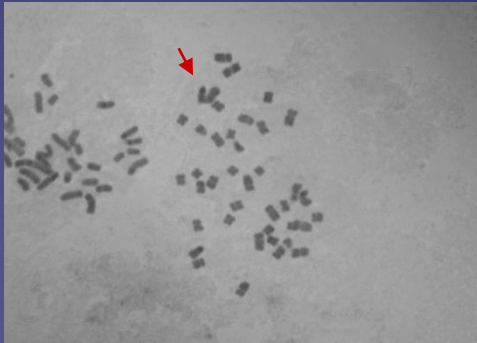
C. cognata 2n=6x=60
30 II
26 II + 1 VI + 2 I
25 II + 2 IV + 2 I



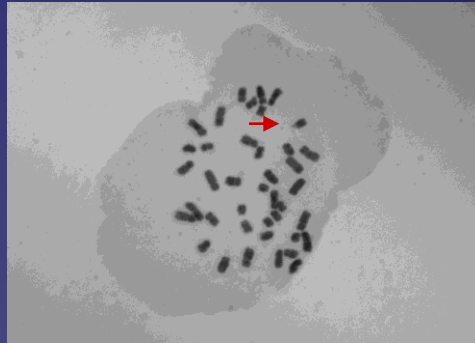
MITOSIS

Somatic chromosome number, ploidy level, karyotype formula, mean chromosome length (LM), total length of karyotype (TKL), average centromeric index (CI) and asymmetry indexes (A₁ and A₂) of the *Chrysoleaena* specimens analyzed.

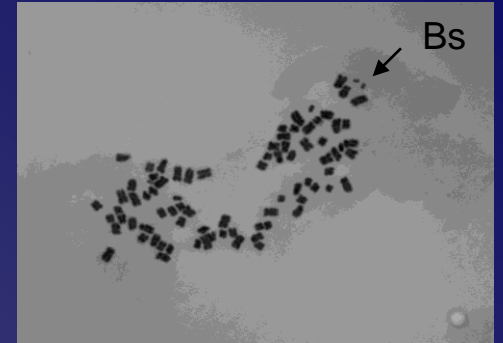
Species	2n	Ploidy	Karyotype Formula	TKL SE (µm)	LM (µm)	CI SE	A1	A2
<i>C. verbascifolia</i> 3080	20	2x	2n=12m + 8sm + B	29,71 0,18	2,70	39,31 1,55	0,27	0,23
<i>C. flexuosa</i> 3703	40	4x	2n=22m + 18sm + B	50,80 0,11	2,54	39,55 1,15	0,33	0,20
<i>C. flexuosa</i> 3750	40	4x	2n=20m + 20sm	44,52 0,12	2,23	38,24 1,08	0,37	0,24
<i>C. flexuosa</i> 45	20	2x	2n=10m + 10sm	24,39 0,19	2,44	38,06 1,41	0,38	0,25
<i>C. propinqua</i> 3078	40	4x	2n=20m + 20sm + B	48,86 0,11	2,44	38,88 1,19	0,35	0,19
<i>C. cognata</i> 3040	40	4x	2n=18m + 22sm	52,96 0,12	2,65	39,06 1,20	0,35	0,21
<i>C. cognata</i> 3761	40	4x	2n=26m + 14sm	48,05 0,12	2,40	39,53 1,06	0,34	0,22
<i>C. cognata</i> 3053	80	8x	2n=54m + 26sm + B	80,55 0,08	2,01	40,42 0,91	0,31	0,24
<i>C. lithospermifolia</i> 4	20	2x	2n=10m + 10sm	18,65 0,14	1,86	39,41 1,29	0,34	0,23



C. cognata 3761 ($2n=4x=40$)



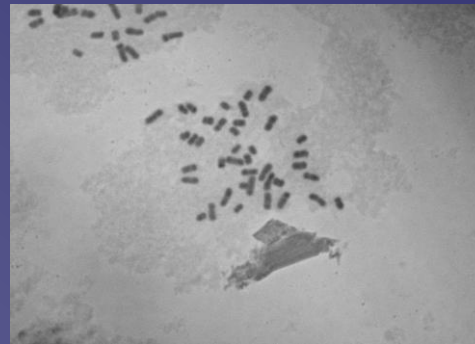
C. cognata 3040 ($2n=4x=40$)



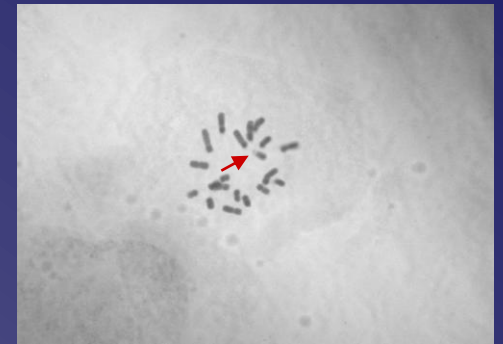
C. cognata 3053 ($2n=8x=80+2B$)



C. flexuosa 3703 ($2n=4x=40$)



C. flexuosa 3750 ($2n=4x=40$)



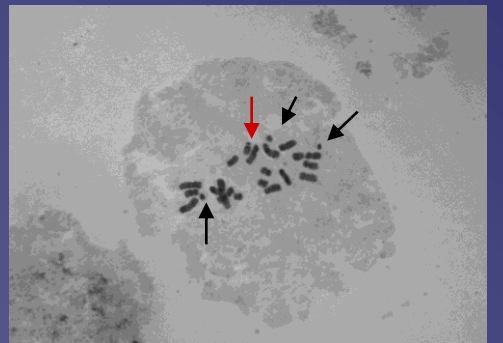
C. flexuosa 45 ($2n=2x=20$)



C. propinqua 3078 ($2n=4x=40$)

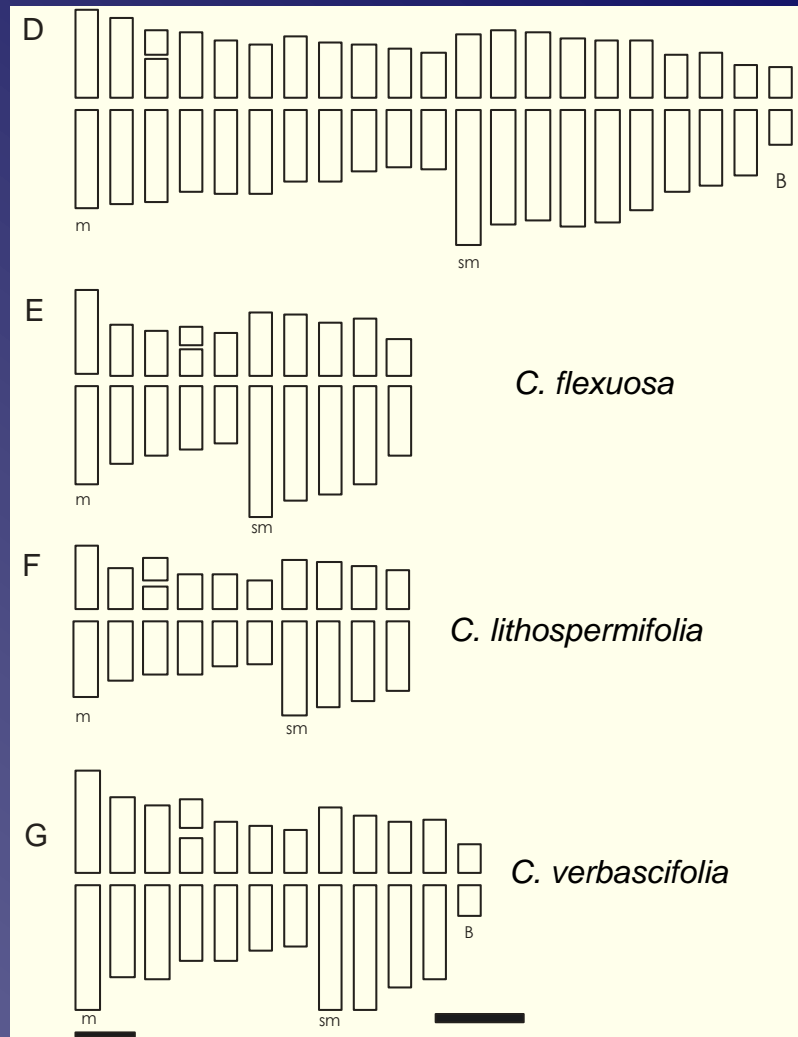
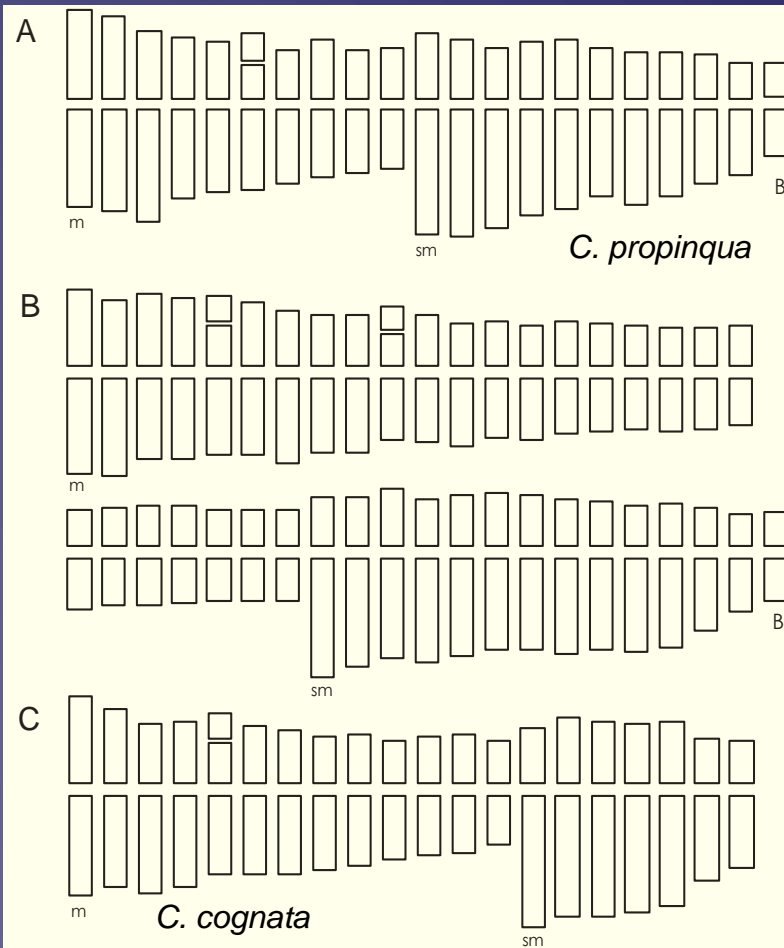


C. lithospermifolia 4 ($2n=2x=20$)



C. verbascifolia 3080
($2n=2x=20+3B$)

IDIODIAGRAMS

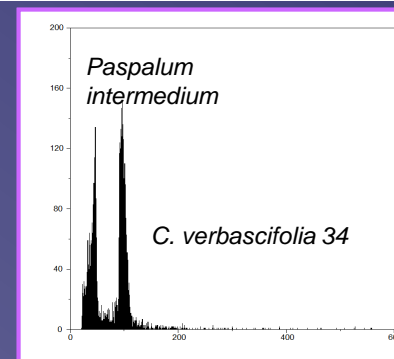
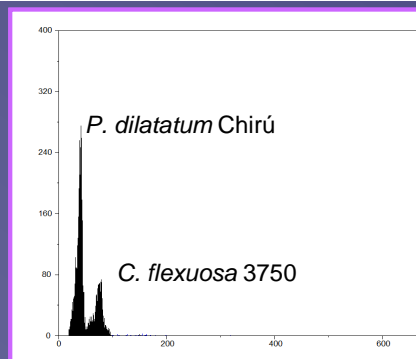
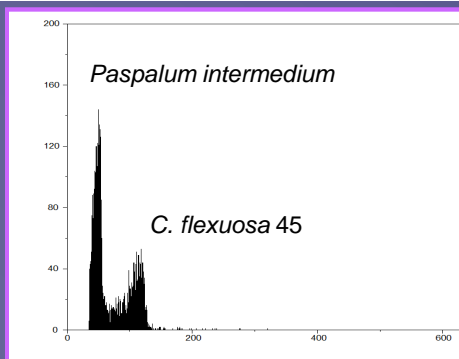


Heteromorphic pair

Scale=1,5 μ m

DNA CONTENT

Species	2n	Ploidy	2C (pg) SE	Cx (pg)
<i>C. cognata</i> 3761	40	4x	6,42 0,08	1,605
<i>C. cognata</i> 3053	80	8x	12,27 0,12	1,53
<i>C. flexuosa</i> 3750	40	4x	7,03 0,11	1,75
<i>C. flexuosa</i> 45	20	2x	3,26 0,03	1,63
<i>C. lithospermifolia</i> 4	20	2x	3,61 0,06	1,805
<i>C. propinqua</i> 3078	40	4x	12,98 0,19	3,24
<i>C. verbascifolia</i> 34	20	2x	3,39 0,03	1,69
<i>C. cognata</i> 31	60	6x	8,74 0,22	1,46
<i>C. lithospermifolia</i> 3	-	-	3,45 0,05	-
<i>C. flexuosa</i> 5	-	-	3,24 0,04	-
<i>C. cognata</i> 6	-	-	1,82 0,02	-
<i>C. flexuosa</i> 26	-	-	3,01 0,03	-
<i>C. cognata</i> 27	-	-	10,43 0,19	-
<i>C. propinqua</i> 29	-	-	3,32 0,03	-

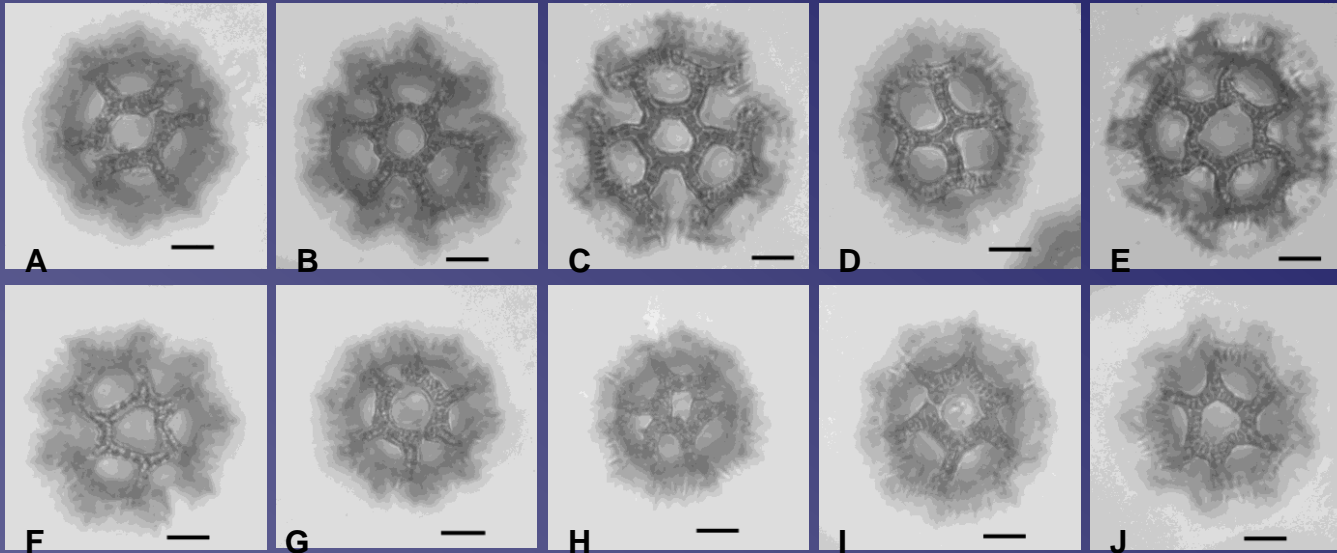


POLLEN MORPHOLOGY

Pollen "type C". Tricolporate, echinolophate, with long germinal furrows, divided into 3 lacunae, 1 oral and 2 aboral. Tectum discontinuous. Outline of lacunae more or less regular, 12 paraperturales and 6 circumpolar, with polar lacuna. Bridges microperforated with spines pointed apex.

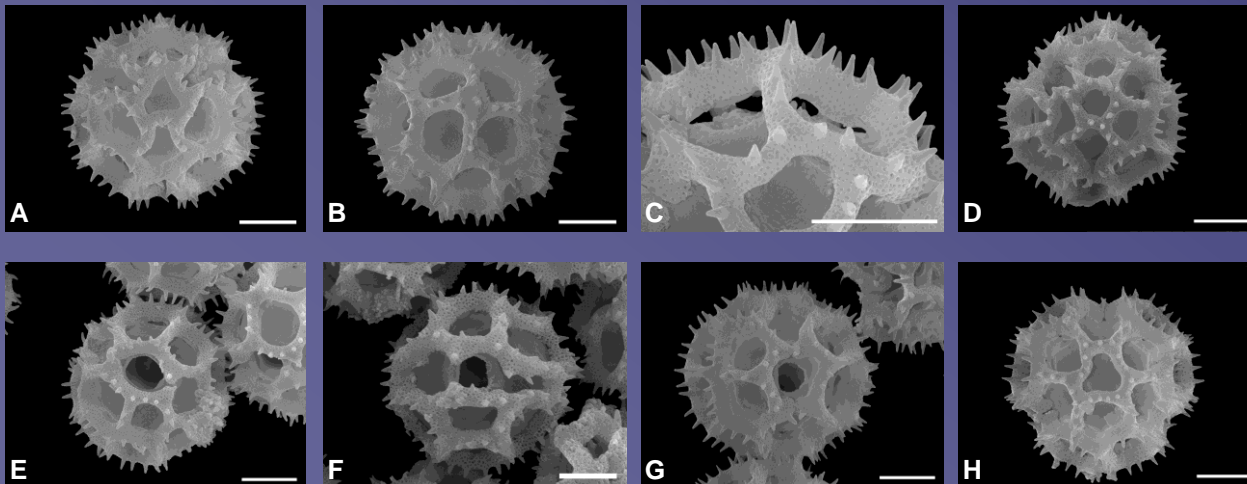
Species	Polar axis (P)	Ecuatorial diameter (E)	P/E	Shape	Colpus length	Pore diameter	Exine thickness	Spine length	Lacuna diameter
<i>C. verbascifolia</i>	42,16(45,51) 8,96	46,24(48,91) 54,4	0,93	Oblate-spheroidal	36,72-42,16	6,80-12,20 x 8,16-10,90	5,44 (6,71) 8,16	2,72 (3,22) 4,08	5,44 (8,57)9,52
<i>C. cognata</i>	40,8(44,56)4 7,6	46,24(51,5)5 5,76	0,86	Oblate-spheroidal	32,64-38,08	5,44-10,88 x 8,16-12,24	5,44 (6,71) 8,16	2,72 (2,83) 3,4	9,52(11,06) 14,96
<i>C. flexuosa</i>	39,44(45,83) 51,68	46,24(51,54) 55,76	0,89	Oblate-spheroidal	32,64-39,44	6,80-10,88 x 6,80-13,6	6,8 (7,43) 9,52	2,72 (3,10) 4,08	8,16(11,42) 14,96
<i>C. lithospermifolia</i>	38,08(41,43) 46,24	35,36(43,75) 46,24	0,95	Oblate-spheroidal	29,90-36,70	5,44-9,52 x 5,44-10,90	5,44 (5,67) 6,8	1,36 (2,68) 2,85	6,8(10,11)1 3,6
<i>C. propinqua</i>	39,44(43,57) 47,6	42,16(47,15) 51,68	0,92	Oblate-spheroidal	34,00-40,80	6,80-9,52 x 6,80-9,52	6,8 (8,29) 9,52	2,04 (2,85) 3,4	8,16(10,79) 13,6
<i>C. platensis</i>	36,72(37,4)3 8,08	40,8(42,84)4 4,88	0,87	Oblate-spheroidal	-	8,16-9,52 x 6,80-8,16	5,44 (6,12) 6,8	2,72 (3,4) 4,08	8,16(8,84)9, 52

Pollen grains of *Chrysolaena* (MO)



A-B. *C. verbascifolia*; C-D. *C. cognata*; E. *C. flexuosa*; F-G. *C. lithospermifolia*; H. *C. platensis*; I-J. *C. propinqua*.

MEB photographs



A-C. *C. cognata*; D-E. *C. flexuosa*; F. *C. lithospermifolia*; G-H. *C. propinqua*.

CONCLUSIONS

MEIOSIS AND POLLEN FERTILITY

- All species showed basic number $x=10$, characteristic of the genus. Chromosome numbers found ranged from $2n=20$ to $2n=60$. The diploid cytotypes was the most common.
- It was observed a high percentage of pollen viability (in all species was over 90%), which corresponds approximately with the meiotic behavior.

MITOSIS – KARYOTYPES – DNA CONTENT

- All spp. analyzed showed basic number $x=10$ and different ploidy levels (diploid to octoploid).
- All taxa showed relatively symmetrical karyotypes, bimodals, consist of metacentric and submetacentric chromosomes, varying only in proportion between species and/or populations.
- Due to prevalence of metacentric chromosomes in the karyotype and the absence of a marked difference between the larger chromosomes and the smallest was observed a high degree of karyotype symmetry (A_1 , A_2).
- In the species examined different cytotypes was observed that there is a positive correlation between the level of ploidy and nuclear DNA content.

POLLEN GRAINS

- All entities examined had pollen (type C), typical of the genus, and in general with very similar characteristics. The slight difference can be observed are at the length of the spines, diameter of lacunae, etc.. Generally the type of pollen has been used in the Vernoniaeae to differentiate genera or sections of some genus, because related species tend to have the same pollen morphology.

● **Chromosome number** is one of the most important features to distinguish *Chrysoleaena* of the remaining genera of the tribe, as no other American genera with basic chromosome number $x = 10$.

● The results confirm that **pollen morphology** is an important character for identification and delimitation of the genus *Chrysoleaena*, but does not allow the identification of species within the same, since all pollen grains have similar characteristics.

THANK YOU FOR YOUR ATTENTION
