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Karyological studies in *Jaborosa* (Solanaceae)

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Sixteen species of *Jaborosa* (Solanaceae), including eight endemic to Argentina, were studied karyologically. The numbers $n = 12$ and/or $2n = 24$ were found in all species, the majority of the cases being new reports. Mitotic chromosomes were small- to medium-sized, the average length varying from 2.95 to 4.93 μm. All species had one to three chromosome pairs with satellites. The karyotypes, obtained for 13 species, were slightly asymmetrical: A_1 ranged from 0.228 to 0.483, A_2 ranged from 0.095 to 0.182, and Paszko's asymmetry index ranged from 0.84 to 3.47. In all species, metacentric chromosomes were the most common, followed by submetacentrics, but subtelocentrics were rare. Morphological similarities and sectional arrangements were not reflected in either a principal components analysis plot or asymmetry index plot, but the species could be singled out by their karyotype formulae and the different karyotype parameters taken. In *Jaborosa*, a notably diversified genus, exo-morphological evolution has taken place, together with evident chromosome rearrangements, whose disposition is different and not as clear as in related genera. *Botanical Journal of the Linnean Society*, 2008, **156**, 467–478.

ADDITIONAL KEYWORDS: Argentina – chromosomes – cytotaxonomy – meiotic numbers – mitotic numbers.

INTRODUCTION

The family Solanaceae, with 92 genera (Hunziker, 2001), is one of the most important amongst the Angiosperms, as it includes several species of great economic, ethnobotanic, scientific, and ornamental value, such as tobacco (*Nicotiana tabacum* L.), chilli peppers (*Capsicum* spp.), tomato (*Solanum lycopersicum* L.), eggplant (*S. melongena* L.), and potato (*S. tuberosum* L.). Other members of the family have been useful to botanists in elucidating evolutionary processes, such as chromosome speciation (Moscone, 1992; Badr *et al*., 1997), reproductive biology (Bowers, 1975; Whalen, 1978), cattle toxicology (McLennan & Kelly, 1984), and plant virology (Kim & Fulton, 1984). In addition, many groups within Solanaceae have been the subject of phylogenetic studies based on either molecular or morphological data (Olmstead & Palmer, 1992; Hala & Olmstead, 1996; Hoare & Knapp, 1997; Olmstead *et al*., 1999), in which different clades (subfamilies with their tribes, in the traditional sense) have been recognized.

The focus of this work is centred on *Jaborosa* Juss. (subfamily Solanoideae Schltdl., tribe Jaboroseae Miers), a South American genus that extends from southern Peru to southernmost Patagonia. It comprises 23 species that inhabit the western Andean zone of the continent, with the exception of *J. integrifolia* Lam. and *J. runcinata* Lam., which grow in the basins of the Paraná and Uruguay rivers (Barboza & Hunziker, 1987). Ten of these 23 species are endemic to Argentina and are present especially in the central and western dry areas of the country. The species of *Jaborosa* are frequently rhizomatous perennial herbs (except *J. bergii* and *J. sativa*, which are annual or biennial), hemicryptophytes, or geophytes. Chemical screenings of several of them have revealed the presence of withanolides, which are promising steroid compounds with antitumoral activity (Mísico *et al*., 1997, 2002; Anjaneyulu, Rao & Lequerne, 1998; Nicotra *et al*., 2003).

From the phylogenetic point of view, *Jaborosa* is related closely to *Lycium* L. and *Nolana* L.f. and more distantly to the tribe Hyoscyameae (Olmstead &

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Palmer, 1992; Hoare & Knapp, 1997; Olmstead *et al*., 1999). The species of *Jaborosa*, formerly divided into different genera (*Himeranthus* Endl., *Lonchestigma* Dunal, and *Trechonaetes* Miers), have been grouped by Barboza (1986) and Barboza & Hunziker (1987) into a single genus on the basis of many morpho-anatomical and palynological traits. Considering mainly pollen features, these authors divided *Jaborosa* into two sections: *Jaborosa* sect. *Lonchestigma* (Dunal) Wettstein, with colporate pollen grains having reticulate exines, and *Jaborosa* sect. *Jaborosa*, with porate grains having smooth exines.

Although several chromosome studies have been made in Solanaceae with profitable results (Bernardello & Anderson, 1990; Moscone, 1992; Moscone *et al*., 1995, 2003; Sheidai, Mosallanejad & Khatamsaz, 1999; Acosta & Moscone, 2000; Acosta *et al*., 2005; Chiarini & Bernardello, 2006), available information on these aspects in *Jaborosa* is relatively scarce. Until now, the chromosome numbers of only five species were known (Ratera, 1943; Vignoli, 1945; Rahn, 1960; Moore, 1981) and karyotypes were lacking for all members of the genus. This is unfortunate as chromosome numbers and karyotype information are important in the study of evolutionary patterns (Raven, 1975) and can be as systematically useful as morphological features (Stebbins, 1958, 1971). Indeed, the karyotypes of several species of *Atropa* and *Lycium* (Stiefkens & Bernardello, 1996, 2000, 2002; Badr *et al*., 1997) and of species of the less closely related genera *Anisodus*, *Przewalskia*, *Atropanthe*, and *Hyoscyamus* (Sheidai *et al*., 1999; Tu *et al*., 2005) are available and reveal different patterns of chromosome evolution. This type of analysis is bound to become more important when more molecular data on chromosome organization and size have been collected. Indeed, these studies are preliminary to any attempt to produce a phylogeny for a specific plant group.

Given this situation, the aims of this work were as follows: (1) to make meiotic and mitotic counts in species of *Jaborosa*, as well as karyotypes, when possible; and (2) to reach a better understanding of the intergeneric and interspecific systematic relationships of the genus.

MATERIAL AND METHODS

All species analysed were collected in the field in their natural habitats in Argentina. Vouchers are kept at the Museo Botánico de Córdoba herbarium (CORD). Table 1 includes the specimens studied and their collection data. Meiotic chromosomes were observed in pollen mother cells from squashed young anthers, fixed in a 3 : 1 ethanol–acetic acid mixture for 12 h and stained with acetocarmine. Mitotic chromosomes were examined in squashes of root tips that were obtained from germinating seeds. Seeds were soaked for 24 h in running water, and then placed on moist filter paper in Petri dishes and incubated at 30 °C. Root tips, collected after 1 day, were pretreated in saturated *p*-dichlorobenzene in water for 2 h at room temperature, fixed in 3 : 1 ethanol–acetic acid, and stained with alcoholic hydrochloric acid carmine (Snow, 1963). Permanent mounts were prepared following the methods of Bradley (1948) or Bowen (1956). To make the different mitotic chromosome measurements, at least ten metaphases of each accession were photographed with a Leica DFC 300 FX camera mounted on an Axiophot microscope with phase contrast illumination. The measurements taken for each chromosome pair were as follows: *s* (short arm), *l* (long arm), and *c* (total chromosome length). The arm ratio ($r = l/s$) was then calculated and used to classify the chromosomes as recognized by Levan, Fredga & Sandberg (1964). In addition, the total haploid chromosome length of the karyotype (*tl*), based on the mean chromosome length for each accession, average chromosome length, and average arm ratio were calculated. When necessary, data comparisons between accessions were made by means of analysis of variance (ANOVA) tests. To look for associations between pairs of karyotype variables, linear regression tests were performed. The length measurements were used to construct the karyotype of each accession, when the data were sufficient, the chromosomes being organized according to Bernardello & Anderson (1990). Karyotype asymmetry was estimated using the indices A_1 (intrachromosomal asymmetry index) and A_2 (interchromosomal asymmetry index) of Romero Zarco (1986), and the coefficients CV_{CL} (coefficient of variation of chromosome length), CV_{CI} (coefficient of variation of centromeric index), and AI (karyotype asymmetry index) of Paszko (2006). Principal components analysis (PCA) was used to reveal karyotype similarities between the taxa [considered as operational taxonomic units (OTUs)]. For this purpose, the following variables, which do not implicate chromosome homologies, were employed: tl, c, r, A_1, A_2 and the number of satellited pairs (sat). INFOSTAT version 1.1 (Infostat Group, 2002) was employed in all the analyses involving numerical data.

RESULTS

The gametic numbers of 18 accessions of 12 species are given in Table 1. Figures 1–5 show some of the meiotic chromosomes found. All species have $n = 12$. The length of bivalents averages from 1.5 μm in *J. odonelliana* to 3.75 μm in *J. araucana* (Fig. 3).

The somatic numbers of 17 accessions of 13 species were obtained. Figures 6–19 illustrate the

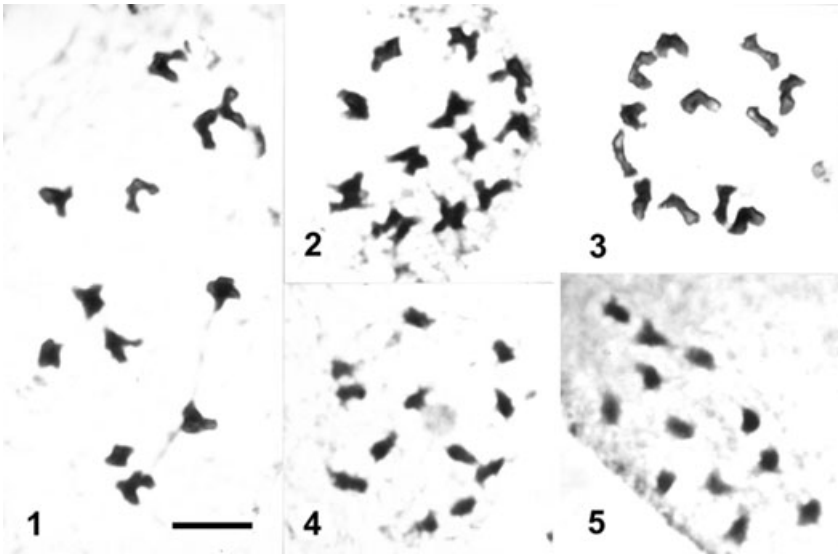
Table 1. Collection data of the material studied, and mitotic and meiotic counts of *Jaborosa* species

Species	Origin, collector, and number	Meiosis	Mitosis
Section *Lonchestigma* (Dunal) Wettstein			
J. araucana Phil.	Mendoza prov., Ambrosetti *et al*. 1448	12II	–
J. bergii Hieron.*	San Luis prov., Hunziker *et al*. 24806	12II	–
	La Rioja prov., Subils 3761	12II	–
	San Luis prov., Chiarini 585	–	24
	Córdoba prov., Chairini 584	–	24
J. caulescens Gill. et Hook. var. *caulescens*	San Juan prov., Barboza 70	12II	–
	Mendoza prov., Barboza 211	–	24
J. caulescens var. *bipinnatifida* (Dunal) Reiche	La Rioja prov., Biurrun & Molina 5126	–	24
J. kurtzii Hunz. et Barboza*	Neuquén prov., Barboza *et al*. 1182	–	24
	Mendoza prov., Hunziker 24818	12II	–
J. laciniata (Miers) Hunz. et Barboza	Mendoza prov., Hunziker *et al*. 24818	12II	–
	Mendoza prov., Hunziker 24820	12II	–
J. lanigera (Phil.) Hunz. et Barboza*	Salta prov., Barboza 179	–	24
J. leucotricha (Speg.) Hunz.*	Mendoza prov., Hunziker 24808	12II	–
	Mendoza prov., Hunziker 24812	12II	–
	Mendoza prov., Barboza *et al*. 1250	–	24
J. odonelliana Hunz.*	Salta prov., Subils *et al*. 3594	12II	–
J. parviflora (Phil.) Hunz. et Barboza	Salta prov., Barboza *et al*. 1445	–	24
J. reflexa Phil.	Chubut prov., Subils 4075	12II	–
	Chubut prov., Balzaretti & Sánchez, CORD 883	–	24
J. riojana Hunz. et Barboza	La Rioja prov., Barboza 249	–	24
J. rotacea (Lillo) Hunz. et Barboza	Tucumán prov., Subils 3271	12II	–
	Tucumán prov., Subils *et al*. 3549	12II	–
	Tucumán prov., Hunziker 24874	–	24
	Catamarca prov, Barboza *et al*. 1152	–	24
J. sativa (Miers) Hunz. et Barboza*	Catamarca prov., Hunziker 24714	12II	–
	Catamarca prov., Barboza & Oberti 843	–	24
Section *Jaborosa*			
J. integrifolia Lam.	Santa Fe prov., Subils *et al*. 4209	12II	24
	Chaco prov., Di Fulvio 804	12II	–
	Corrientes prov., Barboza *et al*. 359	–	24
J. oxipetala Speg.*	Tucumán prov., Barboza *et al*. 773	–	24
J. runcinata Lam.	Entre Ríos prov., Hunziker 24861	12II	24

*Endemic to Argentina.

metaphases observed. Mitotic chromosomes of *Jaborosa* are small- to medium-sized (Table 2; Figs 6–20). The average chromosome length varies from 2.95 to 4.93 μm. The shortest (2.90 μm) was pair 12 in a cell of *J. reflexa* and the longest (5.73 μm) was pair 1 in a cell of *J. rotacea* (Hunziker 24874). All accessions present small spherical satellites whose diameter is equal to, or less than one-half of, the chromosome diameter. Six species show only one satellited pair Figs 6–14, 15, 16, 18), six have two pairs (Figs 8, 9, 13, 17, 19), and *J. kurtzii* shows three pairs (Fig. 12). Satellites are always attached to the short arms, generally on metacentric chromosomes, but three species show satellites on submetacentrics as well (Fig. 20). At the intraindividual level, the presence of satellites on both members of a chromosome pair at the same time fluctuated from 30 to 100% of the cells analysed, depending on the species. In *J. kurtzii*, satellites were visible on both members of the two metacentric pairs, whereas a satellite on a submetacentric pair was seen always on only one of the homologues.

Karyotype formulae were established for 13 species (Table 2), and the karyotypes obtained are represented by idiograms calculated from the mean values (Fig. 20). In *J. integrifolia* and *J. rotacea*, it was possible to compare the karyotypes of different populations. According to statistical tests, no significant differences were detected between the accessions of *J. integrifolia*. The two accessions of *J. rotacea* have the same karyotype formula, but differ in the length of some chromosome pairs, especially the satellited ones (Table 2; Fig. 20). Overall, karyotypes are



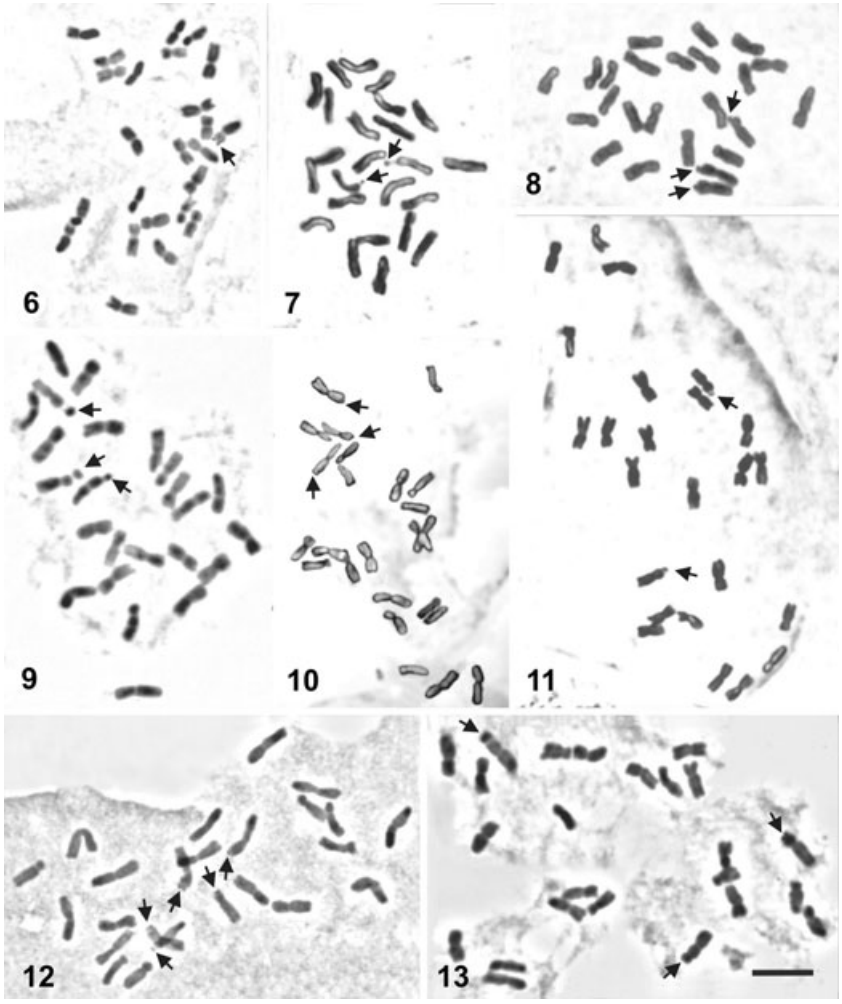
Figures 1–5. Photomicrographs of meiotic chromosomes in diakinesis/metaphase of some *Jaborosa* species. Fig. 1. *J. laciniata* (Hunziker *et al*. 24818). Fig. 2. *J. rotacea* (Subils *et al*. 3549). Fig. 3. *J. araucana* (Ambrosetti *et al*. 1448). Fig. 4. *J. bergii* (Hunziker *et al*. 24806). Fig. 5. *J. odonelliana* (Subils *et al*. 3594). Scale bar, 5 μm.

Table 2. *Jaborosa* taxa studied, karyotype formulae, total haploid genome length in μm (*tl*), average chromosome length in μm ± standard deviation (*c*), average arm ratio in μm ± standard deviation (*r*), intrachromosomal asymmetry index (A_1), interchromosomal asymmetry index (A_2), coefficient of variation of centromeric index (CV_{CI}), coefficient of variation of chromosome length (CV_{CL}), and karyotype asymmetry index (AI)

Species	Karyotype formula	*tl*	*c*	*r*	A_1	A_2	CV_{CI}	CV_{CL}	AI
J. bergii	10m† + 2sm	54.17	4.52 ± 0.46	1.36 ± 0.03	0.228	0.100	10.02	10.02	1.00
J. caulescens var. *binnitafida*	9m* + 3sm	42.96	3.56 ± 0.48	1.38 ± 0.05	0.305	0.095	8.83	9.52	0.84
J. caulescens var. *caulescens*	9m* + 3sm	36.42	3.03 ± 0.28	1.53 ± 0.04	0.309	0.112	11.23	11.18	1.26
J. integrifolia	8m* + 4sm	50.70	4.22 ± 0.11	1.50 ± 0.09	0.309	0.092	11.45	9.22	1.06
J. kurtzii	8m† + 3sm* + 1st	56.27	4.69 ± 0.98	1.79 ± 0.05	0.337	0.148	21.65	14.83	3.21
J. lanigera	7m* + 5sm*	43.32	3.61 ± 0.72	1.64 ± 0.42	0.351	0.109	13.08	10.94	1.43
J. leucotricha	9m* + 3sm	43.00	3.58 ± 0.26	1.53 ± 0.09	0.294	0.218	14.36	21.81	3.13
J. oxipetala	8m* + 3sm + 1st	38.70	4.02 ± 0.49	1.63 ± 0.59	0.348	0.182	8.09	18.17	1.47
J. parviflora	6m* + 6st*	43.61	3.63 ± 0.46	1.65 ± 0.07	0.354	0.088	12.11	8.81	1.07
J. reflexa	7m† + 4sm + 1st	43.59	3.63 ± 0.62	1.76 ± 0.71	0.345	0.160	21.74	15.97	3.47
J. riojana	5m* + 7sm*	41.34	3.44 ± 0.25	1.59 ± 0.10	0.334	0.096	11.37	9.59	1.09
J. rotacea (Hunziker 24874)	8m† + 3sm + 1st	59.22	4.93 ± 0.17	1.60 ± 0.02	0.483	0.117	21.28	11.7	2.49
J. rotacea (Barboza *et al*. 1152)	8m† + 3sm + 1st	52.32	4.36 ± 0.78	1.64 ± 0.06	0.322	0.134	16.12	13.39	2.16
J. runcinata	10m* + 2sm	36.15	3.01 ± 0.39	1.41 ± 0.07	0.260	0.091	11.67	9.13	1.07
J. sativa	7m* + 5sm	50.06	4.17 ± 0.69	1.70 ± 0.34	0.379	0.088	11.64	8.84	1.03

*One or †two chromosome pairs bearing satellites.

slightly asymmetrical: the asymmetry index ranges of Romero Zarco (1986) are $A_1 = 0.228–0.483$ and $A_2 = 0.095–0.182$, and AI of Paszko (2006) ranges from 0.84 to 3.47 (Table 2). In all species, metacentric chromosomes are the most common (67% of all the chromosomes), followed by submetacentric chromosomes (30%), but subtelocentric chromosomes are rare (3%) and telocentric chromosomes are absent.

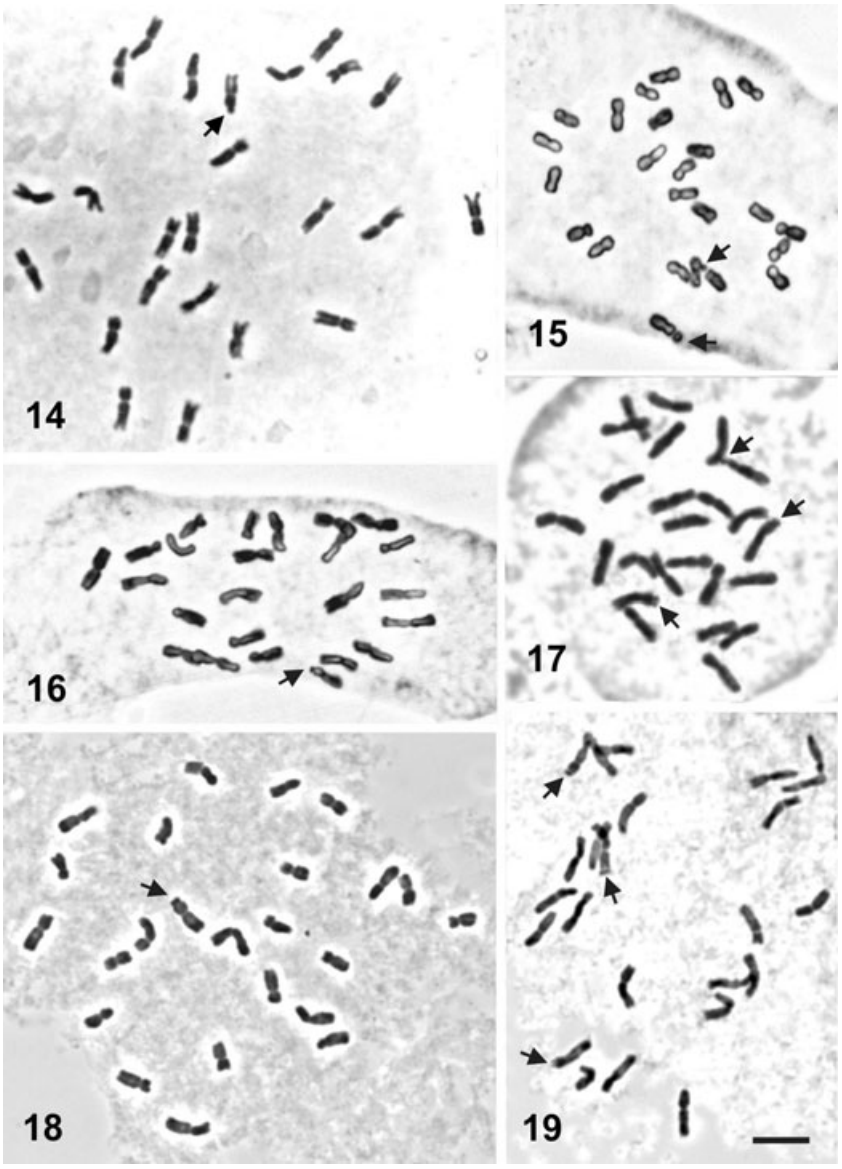


Figures 6–13. Photomicrographs of mitotic metaphases of *Jaborosa* species. Fig. 6. *J. runcinata* (Barboza *et al*. 1479). Fig. 7. *J. sativa* (Barboza and Oberti 843). Fig. 8. *J. lanigera* (Barboza 179). Fig. 9. *J. bergii* (Chiarini 584). Fig. 10. *J. reflexa* (CORD 883). Fig. 11. *J. caulescens* var. *bipinnatifida* (Biurrun and Molina 5126). Fig. 12. *J. kurtzii* (Barboza *et al*. 1182). Fig. 13. *J. rotacea* (Barboza *et al*. 1152). Arrows indicate satellites. Scale bar, 5 μm.

The karyotypes of *J. runcinata* and *J. bergii* are the most symmetrical, and those of *J. rotacea*, *J. sativa*, and *J. leucotricha* are comparatively asymmetrical.

No association between karyotype length and asymmetry, or any other association with biological meaning, could be drawn from regression tests (data not shown). Nevertheless, species can be distinguished by a combination of karyotype formulae, karyotype length, asymmetry indices, and position of satellites on a particular chromosome pair (Table 2).

As a result of the PCA based on karyotype features, the first and second principal components explain 70% of the variability, although such variability is scattered between the five variables. The eigenvalues (Table 3) indicate that *c* is the most important variable in the first axis, whereas *r* is the most important in the second. OTUs are quite scattered in the space formed by the first and second principal components. The separation of species in the PCA plot and in the scatter plots of asymmetry indices (Fig. 21) does not strictly reflect their morphological similarities, sectional arrangement, or geographical distribution. Each species can be singled out in all diagrams, but the three species of sect. *Jaborosa* are separated between them, especially *J. oxipetala*. The species of sect. *Lonchestigma* with entire or nearly entire leaves (*J. kurtzii*, *J. riojana*, *J. sativa*, *J. rotacea*) and the short-lived (*J. sativa* and *J. bergii*) species are also separated. In the diagrams of asymmetry indices (Fig. 21A, B), five species (*J. reflexa*, *J. leucotricha*, *J. oxipetala*, *J. kurtzii*, and two different accessions of *J. rotacea*) are separated from the rest. The separation is more evident in Figure 21B, in which the remaining eight species are grouped together. The



Figures 14–19. Photomicrographs of mitotic metaphases of *Jaborosa* species. Fig. 14. *J. integrifolia* (Subils *et al*. 4209). Fig. 15. *J. caulescens* var. *caulescens* (Barboza 211). Fig. 16. *J. oxipetala* (Barboza *et al*. 773). Fig. 17. *J. riojana* (Barboza 249). Fig. 18. *J. leucotricha* (Barboza *et al*. 1250). Fig. 19. *J. parviflora* (Barboza *et al*. 1445). Arrows indicate satellites. Scale bar, 5 μm.

accessions of *J. rotacea* always drift apart because of the large chromosomes, with *J. runcinata* on the opposite side because of its small chromosomes.

DISCUSSION

Chromosome numbers

The meiotic counts of *J. integrifolia* and *J. runcinata* confirm previous data (Ratera, 1943; Vignoli, 1945), and those of the other ten species are new reports. Meiosis is regular, always with 12 bivalents in diakinesis/metaphase I. The gametic number $x = 12$ was established for all the accessions studied.

The mitotic counts of *J. integrifolia* and *J. leucotricha* confirm previous reports (Vignoli, 1945; Rahn, 1960), and the numbers of the remaining species and varieties are reported here for the first time. All of the species are diploids and the basic number $x = 12$ is thus confirmed for the genus. It is also the number typical of the subfamily Solanoideae, in which the genus *Jaborosa* is placed (Olmstead *et al*., 1999; Hunziker, 2001), and is the most common in the

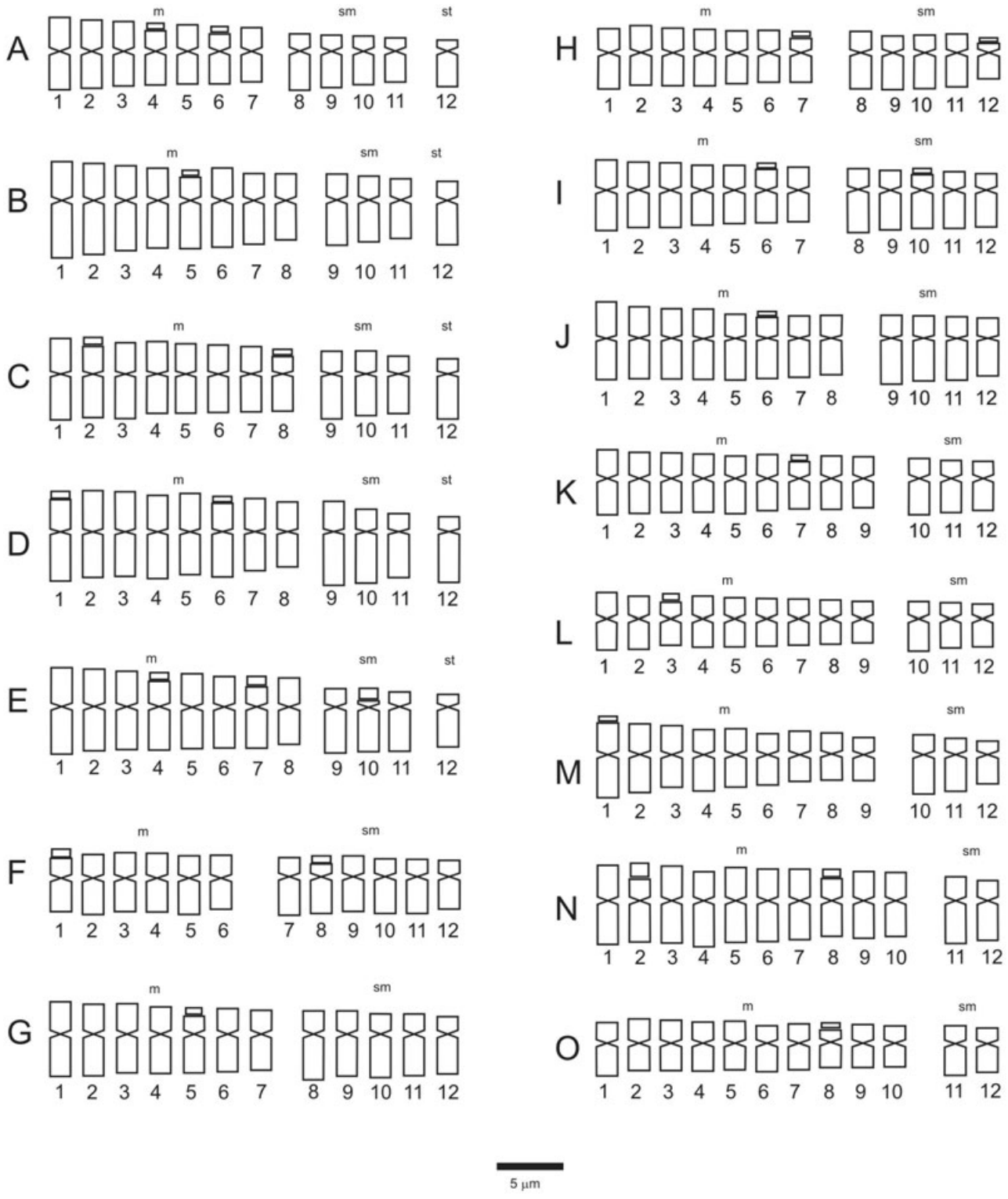


Figure 20. Idiograms for *Jaborosa* accessions, based on mean values: A, *J. reflexa*; B, *J. oxipetala*; C, *J. rotacea* (Hunziker 24874); D, *J. rotacea* (Barboza *et al*. 1152); E, *J. kurtzii*; F, *J. parviflora*; G, *J. sativa*; H, *J. riojana*; I, *J. lanigera*; J, *J. integrifolia*; K, *J. caulescens* var. *bipinnatifida*; L, *J. caulescens* var. *caulescens*; M, *J. leucotricha*; N, *J. bergii* (Chiarini 584); O, *J. runcinata*.

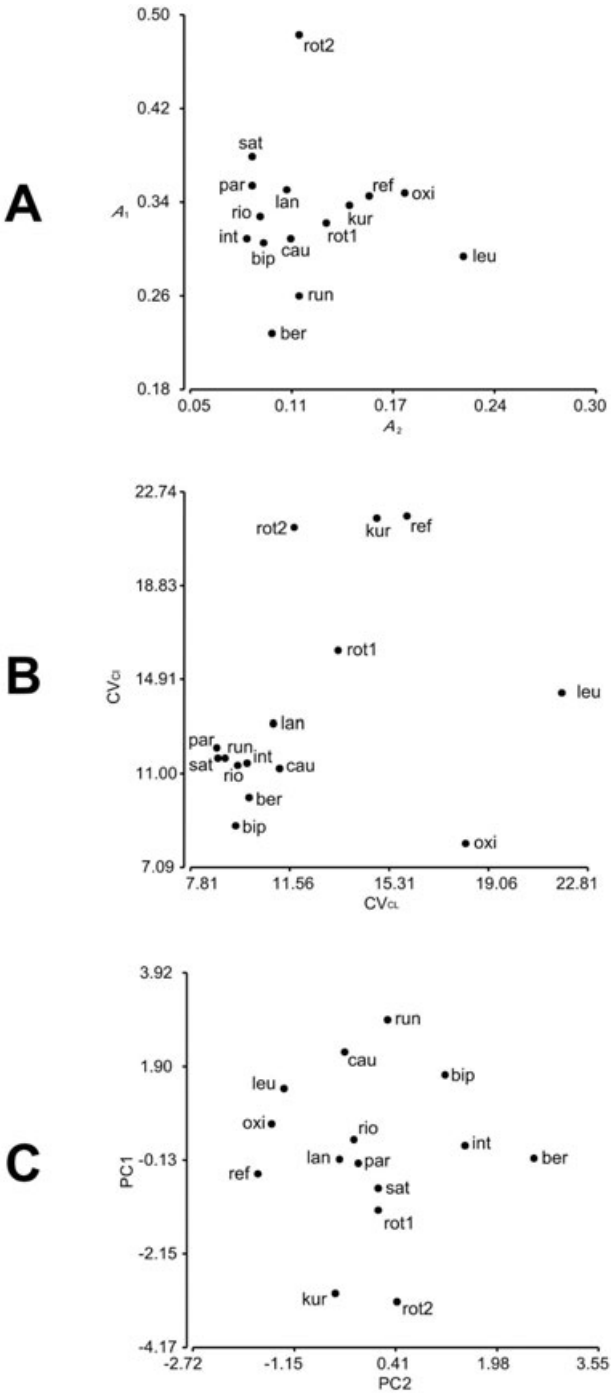


Figure 21. Scatter diagrams of karyotype parameters of *Jaborosa* species: A, A_1 vs. A_2; B, CV_{CI} vs. CV_{CL}; C, result of the principal components analysis, based on six karyotype characters, showing projections of operational taxonomic units onto the first two principal components (PC). ber, *J. bergii*; bip, *J. caulescens* var. *bipinnatifida*; cau, *J. caulescens* var. *caulescens*; int, *J. integrifolia*; kur, *J. kurtzii*; lan, *J. lanigera*; leu, *J. leucotricha*; oxi, *J. oxipetala*; par, *J. parviflora*; ref, *J. reflexa*; rio, *J. riojana*; rot1, *J. rotacea* (Hunziker 24874); rot2, *J. rotacea* (Barboza *et al*. 1152); run, *J. runcinata*; sat, *J. sativa*.

Table 3. Eigenvector coefficients for the first two axes for principal components analysis of *Jaborosa* species

Variable*	Principal component 1	Principal component 2
lt	– 0.50	0.39
c	– 0.52	0.30
r	– 0.37	– 0.60
A_1	– 0.39	– 0.29
A_2	– 0.01	– 0.56
sat	– 0.44	– 0.04

*See text for meaning of symbols.

family (*c*. 50% of the studied species; cf. Bolkhovskikh *et al*., 1969; Hunziker, 2001). In this study, more than one accession was examined in several taxa, revealing an intraspecific stability of chromosome number. The stable number of chromosomes in all *Jaborosa* species analysed to date (17 out of 23, i.e. 74% of the species of the genus) confirms that neither polyploidy nor aneuploidy/dysploidy has played a significant role in the speciation of the genus. By contrast, these mechanisms have undoubtedly been very important in the evolution of the allied Hyoscyameae, which presents the numbers $x = 6, 7, 11, 14, 17$ and high ploidy levels (Badr *et al*., 1997; Sheidai *et al*., 1999; Tu *et al*., 2005).

Chromosome size

In the context of Angiosperms, the mitotic chromosomes of *Jaborosa* are small (Guerra, 2000), but relative to other genera of Solanaceae they are of intermediate size (Badr *et al*., 1997). The lengths found in *Jaborosa* are between the records for *Cestrum* (*c*. 6–10 μm: Badr *et al*., 1997; Sykorova *et al*., 2003) and *Solanum* (1–3.5 μm: Bernardello & Anderson, 1990; Acosta *et al*., 2005; Chiarini & Bernardello, 2006). The chromosome sizes of *Jaborosa* are similar to those of other less closely related genera, such as *Capsicum* (Moscone, 1990, 1999) and *Cyphomandra* (Pringle & Murray, 1991, 1993) of subfamily Solanoideae, *Dyssochroma* of subfamily Juanulloideae (Piovano, 1989; Acosta & Moscone, 2000), or *Browallia* of subfamily Cestroideae (Badr *et al*., 1997). They are much larger than those of the species of more closely related genera, such as *Atropa* (0.81–1.14 μm: Badr *et al*., 1997), *Hyoscyamus* (1–2 μm: Badr *et al*., 1997; Tu *et al*., 2005), and *Lycium* (1.6–2.5 μm: Stiefkens & Bernardello, 1996, 2000, 2002, 2006). The differences between *Jaborosa* and the Hyoscyameae may be a result of ploidy level,

amongst other reasons, as an increase in chromosome number is usually associated with a decrease in chromosome size. By contrast, the differences between *Jaborosa* and *Lycium*, a genus comprising shrubby species, may be attributed to habit, as woody species usually have small chromosomes (Stebbins, 1971; Stiefkens & Bernardello, 1996, 2000, 2002, 2006).

Satellites

Within the many genera of Solanaceae studied to date, the number of satellited chromosome pairs is variable between the species of a single genus. For instance, in the subfamily Solanoideae, species of *Capsicum* may show one to four satellited chromosome pairs (Moscone, 1990, 1999; Moscone *et al*., 1995) and species of *Hyoscyamus* may have one to three pairs (Sheidai *et al*., 1999), whereas many *Solanum* species show just one pair (Bernardello, Heiser & Piazzano, 1994; Acosta *et al*., 2005). By contrast, species of *Lycium* always show one satellited chromosome pair (Stiefkens & Bernardello, 1996, 2000, 2002, 2006). The presence of satellites is not constant in the species studied here. On the contrary, they were missing in many of the cells analysed, and sometimes were visible in only one of the homologues. Variation in the number of satellited pairs within a single species may be determined by the level of transcription, the number of ribosomal genes, or the state of condensation of the chromatin (Warburton & Henderson, 1979; Von Kalm & Smyth, 1984; Medina *et al*., 1986). At the interspecific level, a reason for the variation in satellite number may be that satellited chromosomes are composed of heterochromatin, which is highly variable. Moreover, translocations may be responsible for changing the position of the satellites, whereas duplications and deletions can cause differences in number. The entities whose satellites were seen in one of the homologues could be considered to be heterozygous for these features. From the evolutionary point of view, it is possible that one satellited pair was the ancestral condition and that additional pairs arose by duplications in more advanced taxa. A similar proposal has already been made to explain numerical and structural satellited pair variation in *Capsicum* (Moscone *et al*., 1995).

Karyotype features

In several species of Solanaceae, the shorter the chromosome length, the more terminal the position of the centromere (Pringle & Murray, 1991), a feature which the studied species of *Jaborosa* also share. By contrast, *Jaborosa* species present subtelocentric chromosomes, which are relatively unusual in the Solanaceae (for example, Bernardello & Anderson, 1990; Acosta & Moscone, 2000). Indeed, some *Nicotiana* species, from the subfamily Cestroideae, are unique in having a karyotype composed mostly of subtelocentric chromosomes (Goodspeed, 1954; Villa, 1984), and, in the subfamily Solanoideae, karyotypes with at least one subtelocentric pair have been described for only a few species of *Hyoscyamus*, *Capsicum*, and *Solanum* (Moscone, 1990, 1999; Bernardello *et al*., 1994; Sheidai *et al*., 1999; Acosta *et al*., 2005). In a general survey of the family Solanaceae, Badr *et al*. (1997) reported values of r ranging from 1.17 to 2.78, whereas, in the species studied here, they ranged from 1.36 to 1.79. Thus, the karyotype asymmetry of *Jaborosa* is intermediate relative to the whole family, but relatively high with respect to the subfamily Solanoideae. Indeed, karyotypes of *Jaborosa* are neither as asymmetrical as those of *Solanum* subgenus *Leptostemonum* species (Acosta *et al*., 2005; Chiarini & Bernardello, 2006), nor as symmetrical as those of *Dyssochroma* or most *Solanum* species (Bernardello & Anderson, 1990; Acosta & Moscone, 2000). Amongst the closest relatives of *Jaborosa* which have been studied cytologically, *Lycium* presents remarkable differences. Although sharing the same chromosome number, *Lycium* differs from *Jaborosa* in having constant karyotypes (the karyotype formula is the same for all the species: 11m + 1sm, with a satellite on pair 1) that are very symmetrical ($A_1 = 0.12–0.18$ and $A_2 = 0.12–0.16$) according to Stiefkens & Bernardello (1996, 2000, 2002, 2006). Karyotypes with high levels of symmetry have been considered to be primitive (Stebbins, 1971), but, at the same time, karyotype orthoselection has been proposed for the maintenance of complements formed by chromosomes of approximately the same length, with median or submedian centromeres (Moscone *et al*., 2003). Several genera of Solanaceae, such as *Hyoscyamus*, *Lycium*, *Dyssochroma*, *Cestrum*, and *Capsicum*, could have undergone this phenomenon, but *Jaborosa* departs from this uniform karyotype pattern. Indeed, with its increased karyotype asymmetry, *Jaborosa* appears to be an advanced genus with respect to other members of the clade or the subfamily. In addition, it comprises annual or perennial herbs (hemicryptophytes or geophytes), which constitutes another derived condition. In contrast, woody perennial species, such as those of *Lycium*, which are regarded as primitive, have constant and less diversified karyotypes (Stebbins, 1958, 1971; Brandham, 1983; Ehrendorfer, 1983).

Intraspecific variability

Intraspecific karyotype variability is a well-known phenomenon in many vascular plants, and can sometimes be even greater than interspecific variation,

depending on the group in question (Chennaveraiah & Habib, 1966; Datta, 1968; Pickersgill, 1971; Kuriachan, 1981). Within Solanaceae, the case of *Capsicum* has been studied thoroughly (Datta, 1968; Moscone, 1990, 1999), with the conclusion reached that intraspecific karyotype variation is not rare in this genus. A similar situation has taken place in *Jaborosa*: although the two samples of *J. integrifolia* did not show any significant differences, the two samples of *J. rotacea* (geographically well separated) showed the same karyotype formula but different chromosome sizes and satellite positions. Nevertheless, the techniques employed do not allow us to be certain about the intraspecific variation between the two samples of *J. rotacea*: statistical differences between them concern purely chromosome size, but do not necessarily involve homology relationships. Further analyses (such as chromosome banding) are needed to investigate whether there are differences (apart from size) between samples in determined chromosome pairs. If any differences were found, those of size could be attributed either to the degree of chromatin condensation or to heterochromatin accumulation, whereas different satellite positions could be the result of translocations, as mentioned above. The same kinds of differences were detected here amongst the varieties of *J. caulescens*, but were accompanied by exo-morphological variability (leaf division, flower size).

Karyotypes and Systematics

Jaborosa shows a large diversification over its entire distribution area. This diversification is evident in many aspects, but mainly in floral characteristics, as the flowers are adapted to several kinds of pollinator (Cocucci, 1999). Cytologically, there is also a noticeable diversification, which suggests that exo-morphological evolution within the genus has taken place in conjunction with evident chromosome rearrangements. In fact, each species can be distinguished by its karyotype features; therefore, evolution in *Jaborosa* has occurred regardless of the conservation of a particular karyotype formula.

However, the arrangement of species in the A_1 vs. A_2 diagram (Fig. 21A) is similar to that in the CV_{CI} vs. CV_{CL} diagram (Fig. 21B), but the separation of the species is more remarkable in the latter. According to Paszko (2006), the CV_{CI} vs. CV_{CL} diagram is preferable because it is better suited to demonstrate relationships between closely related taxa. Nevertheless, none of the diagrams reflects infrageneric divisions: the three species of section *Jaborosa* (*J. integrifolia*, *J. runcinata*, and *J. oxipetala*) do not form a group according to their karyotypic features, but are mixed with the species of section *Lonchestigma* (Fig. 21A, B). Neither the short-lived species (*J. bergii* and *J. sativa*) nor the species with entire leaves (*J. kurtzii*, *J. riojana*, *J. rotacea*) appear together in any of the plots. Interestingly, *J. parviflora* and *J. lanigera* appear in the same group in the diagrams, and they are indeed sympatric and morphologically similar. In addition, *J. kurtzii* and *J. reflexa*, allied species that share the same habit, corolla type, and flower indumentum, appear close to each other and distant from the rest in Figure 21B. The species separation in the plots does not reflect any geographical association either. In the PCA plot (Fig. 21C), the species are more scattered. *Jaborosa bergii*, one of the annuals, is placed on the right, as its chromosomes are all about the same size, whereas *J. reflexa*, a perennial which has notable differences in size amongst its chromosome pairs, is on the left. The accessions of *J. rotacea*, a perennial adapted to fly pollination, are separated because of the large chromosomes, with *J. runcinata* (a perennial adapted to moth pollination) on the opposite side, because of its small chromosomes. The accessions distribution in Figure 21 does not reflect exo-morphological similarities amongst species or sectional arrangements. An explanatory pattern of the disposition of chromosome rearrangements within the genus cannot be inferred from the data, which creates the need for further cytological investigations.

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