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Chromosome studies on plants from Paraguay II

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ABSTRACT

MOLERO, J., J. R. DAVIÑA, A. I. HONFI, D. FRANCO & A. ROVIRA (2006). Chromosome studies on plants from Paraguay II. *Candollea* 61: 373-392. In English, English, French and Spanish abstracts.

Chromosome numbers have been obtained for 39 populations of vascular plants from Paraguay belonging to 33 species grouped in 23 families. Chromosome studies for 13 species is reported for the first time. The data obtained about five other species differ from previous chromosome counts. Some of the numbers are of interest because they relate to endemic or rare species or because they provide data of special evolutionary significance. Noteworthy findings include *Angelonia gardneri* (n = 10), *Angelonia integerrima* ($2n$ = 20), *Asclepias mellodora* ($2n$ = 22), *Aspilia montevidensis* var. *setosa* (n = 34), *Calea uniflora* (n = 16), *Dolichopsis paraguariensis* ($2n$ = 22, n = 11), *Heteropteris glabra* ($2n$ = 31), *Hibiscus striatus* ($2n$ = 52), *Lupinus paraguariensis* ($2n$ = 18), *Microlobius foetidus* ($2n$ = 26), *Myrsine guianensis* ($2n$ = 48), *Thevetia bicornuta* (n = 10), *Tibouchina gracilis* (n = 12) and *Vitex cymosa* (n = 16).

RÉSUMÉ

MOLERO, J., J. R. DAVIÑA, A. I. HONFI, D. FRANCO & A. ROVIRA (2006). Etudes chromosomiques sur des plantes du Paraguay II. *Candollea* 61: 373-392. En anglais, résumés anglais, français et espagnol.

Des nombres chromosomiques ont été obtenus pour 39 populations de plantes vasculaires du Paraguay appartenant à 33 espèces groupées en 23 familles. Pour 13 espèces, il s'agit des premiers comptages. Les données obtenues pour 5 autres espèces diffèrent des comptages chromosomiques précédents. Quelques nombres sont intéressants parce qu'ils se rapportent à des espèces endémiques ou rares ou parce qu'ils fournissent des données sur le plan évolutif. Les résultats remarquables incluent *Angelonia gardneri* (n = 10), *Angelonia integerrima* ($2n$ = 20), *Asclepias mellodora* ($2n$ = 22), *Aspilia montevidensis* var. *setosa* (n = 34), *Calea uniflora* (n = 16), *Dolichopsis paraguariensis* ($2n$ = 22, n = 11), *Heteropteris glabra* ($2n$ = 31), *Hibiscus striatus* ($2n$ = 52), *Lupinus paraguariensis* ($2n$ = 18), *Microlobius foetidus* ($2n$ = 26), *Myrsine guianensis* ($2n$ = 48), *Thevetia bicornuta* (n = 10), *Tibouchina gracilis* (n = 12) et *Vitex cymosa* (n = 16).

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RESUMEN

MOLERO, J., J. R. DAVIÑA, A. I. HONFI, D. FRANCO & A. ROVIRA (2006). Estudios cromosómicos en plantas de Paraguay II. *Candollea* 61: 373-392. En Inglés, resúmenes en Inglés, Francés y Español.

Se han obtenido los números cromosómicos de 39 poblaciones de plantas vasculares que pertenecen a 33 especies, agrupadas en 23 familias, procedentes del Paraguay. Datos cromosómicos sobre 13 especies son por la primera vez indicados De otras cinco especies, los datos aportados discrepan de los anteriores. Algunos de los números obtenidos son interesantes por corresponder a especies endémicas o raras, o porque aportan datos de especial interés evolutivo. Destacamos *Angelonia gardneri* (n = 10), *Angelonia integerrima* ($2n$ = 20), *Asclepias mellodora* ($2n$ = 22), *Aspilia montevidensis* var. *setosa* (n = 34), *Calea uniflora* (n = 16), *Dolichopsis paraguariensis* ($2n$ = 22, n = 11), *Heteropteris glabra* ($2n$ = 31), *Hibiscus striatus* ($2n$ = 52), *Lupinus paraguariensis* ($2n$ = 18), *Microlobius foetidus* ($2n$ = 26), *Myrsine guianensis* ($2n$ = 48), *Thevetia bicornuta* (n = 10), *Tibouchina gracilis* (n = 12) y *Vitex cymosa* (n = 16), entre otras.

KEY-WORDS: Chromosome numbers – CROMOPAR database – Vascular plants – Paraguay

Introduction

The CROMOPAR database, which contains the chromosome numbers of Paraguayan vascular plants, is a project sponsored by the «Agencia Española de Cooperación Internacional» (AECI). It compiles the chromosome numbers of families and species which are published periodically in Flora del Paraguay. There are plans to publish its contents through the Flora del Paraguay checklist (Ramella & al., 2004), which will provide a more complete list of references. Input to the database is currently in the form of contributions from specialists of different taxonomic groups, mainly from Argentina and Brazil, and joint studies, such as the present one, which allow institutional cooperation. According to CROMOPAR's updated estimates, the chromosome studies of Paraguayan vascular plants barely exceed 3% of the approximately 6000 species which consitututes the flora of Paraguay. This percentage is far below overall estimates for the world flora, which is around 35%. It is of the utmost urgency to call for greater attention from the international scientific community and competent organizations in Paraguay so as to facilitate this task. It is especially important to undertake the study of endemic plants. Many of which are seriously threatened or on the verge of extinction due to anthropic activities such as over-intensive land use, deforestation and aggressive farming specially prevalent in Paraguay. In studying them, the evaluation of the wealth and genetic diversity of this unique Paraguayan heritage may be done, and this is beneficial to the production and improvement of useful edible, aromatic and medicinal plants.

Information from studies of the chromosome numbers of vascular plants from Paraguay is fragmentary and widely scattered. Joint work to remedy this was initiated some years ago by teams from the Universidad de Misiones and Universidad Nacional del Nordeste (Argentina), Universidad Nacional de Asunción (Paraguay) and Universidad de Barcelona (Spain). These efforts have given rise to a number of publications on the chromosome numbers of plants from Paraguay (Daviña & al., 2001; Molero & al., 2002). The aim of the present study is to supply further karyological information about vascular plants from Paraguay.

Material & Methods

The materials analysed are from natural populations and were collected in several departments in Paraguay (Table 1). The vouchers of each taxon are listed in Table 1.

Mitotic studies were carried out on root tips obtained from seedlings or cultivated individuals. Two protocols were performed.

Protocol 1 (Argentina-Paraguay): root tips were pretreated in 0.002M 8-hydroxiquinoline for 3-4 h at room temperature and fixed in absolute ethanol/lactic acid solution (5:1) at 4° C for at least 24 h. The procedure followed for staining was the Feulgen technique.

Protocol 2 (Spain): root tips were pretreated in 0.05 colchicine for 2 h, fixed in (3:1) absolute ethanol/glacial acetic acid and stained overnight in 2% aceto-orcein.

Meiotic studies were conducted on young flower buds fixed in absolute ethanol/lactic acid (5:1) and stained with 2% aceto-orcein. Metaphasic plates were photographed on ISO 9 Kodak film and later digitized. The chromosome numbers were determined by analysis of 10 cells in metaphase.

Results and Discussion

Table 1 sets out the results, location and voucher. Selected squashes are presented in Figures 1 and 2.

The results for the different species are discussed below in the context of the family to which each belongs.

Apocynaceae

In *Thevetia bicornuta*, pollen mother cells (PMCs) was observed with 10 II at metaphase I. This is the first chromosome count for the species and the first meiotic study of the genus. The chromosome number reported here is the same as reported for other species in the genus. The number $2n = 20$ has been reported for *T. peruviana* (HARDAS & JOSHI, 1954; CHAUHAN & RAGHUVANSHI, 1977; GADELLA, 1977; VAN DER LAAN & ARENDS, 1985) and *T. neriifolia* (TAPADAR, 1964; GADELLA 1977). In *T. peruviana*, the count of $2n = 18$ reported by PATAK & al. (1949) requires confirmation.

Aristolochiaceae

In *Aristolochia gibertii* ($2n = 2x = 14$; Fig. 2A), the somatic number found by GREGORY (1956) for Brazilian materials of *A. gibertii* var. *paulistana* ($2n = 14$) and the result ($n = 7$) of the meiotic study carried out by COLEMAN (1982) are both confirmed.

Asclepiadaceae

This is the first count for the species *Asclepias mellodora* ($2n = 22$). This number is common to the great majority of species in the genus which have been studied (TROPICOS, 2006).

Asteraceae

In *Acmella decumbens* var. *affinis* ($2n = 2x = 26$), the somatic number is coherent with the gametic number ($n = 13$) reported by TURNER & al. (1979), JANSEN (1985) and HUNZIKER & al. (1985), who studied Argentinian populations. Two varieties of the species are present, var. *affinis* and var. *decumbens*. They are distinguished by slight morphological differences and by their ploidy levels. The var. *affinis* is diploid while var. *decumbens* is tetraploid ($n = 26$, JANSEN 1985). The specimens used were highly typical of var. *affinis*, which might warrant sub-specific taxonomic status in view of its constant chromosome number. The study reported here is the first chromosome count on populations from Paraguay.

Aspilia montevidensis var. *setosa* showed 34 II in PMCs at diakinesis and metaphase I. At prophase II, $n = 34$ chromosomes were observed (Fig. 1A). The collection *Honfi & Daviña 1160* displayed regular meiotic behaviour whereas in *Honfi & Daviña 1128*, the meiosis was irregular with laggard chromosomes at anaphase I and telophase I. PMCs at telophase I presents up to 9 chromosomes not contained in the nuclei. Our observations show that both populations have $2n = 68$ chromosomes. For the same taxon, ROZENBLUM & al. (1985), who used materials from Río Grande do Sul (Brazil), reported a gametic number of $n = 23$ and regular meiotic behaviour. WULFF & al. (1996) regularly observed 11 bivalents in plants from Entre Ríos, Argentina. Our results, obtained with materials from Paraguay, are evidence of a new cytotype of the taxon. Furthermore, this is the first time that $2n = 68$ (the number observed in African populations of *A. africana* (TURNER & LEWIS 1965)) has been reported for species of *Aspilia* from America.

In the genus *Aspilia*, species with $n = 11, 13, 14, 17, 34$ and *B*-chromosomes have been found (MANGENOT & MANGENOT, 1962; GADELLA, 1972; PILZ, 1980; ROZENBLUM & al., 1985; WULFF & al., 1996). The base numbers proposed for *Aspilia* are $x = 14$ and 17 (STUESSY, 1977) and $x = 23$ (ROZENBLUM & al., 1985). Our results show that the populations of *A. montevidensis* var. *setosa* studied is hexaploid with a base number of $x = 11$. This makes it possible to formulate the hypothesis that this polyploid originally evolved through amphiploidy between diploids ($n = 11$) and tetraploids ($n = 23$). The polyploid complex *A. montevidensis* var. *setosa* is made up of diploids ($2n = 2x = 22$) (WULFF & al., 1996), tetraploids ($2n = 4x = 46$; ROZENBLUM & al., 1985) and hexaploids ($2n = 6x = 68$) in this study with a base number of $x = 11$. Polyploid cytotypes posses an additional pair of chromosomes which occur as a bivalent at diakinesis and metaphase I. At the cytobiogeographical level, this polyploid complex is distributed in Argentina, between Ríos ($2x$), southern Brazil ($4x$) and southern Paraguay ($6x$).

Calea uniflora showed regular meiotic behaviour in both populations studied with 16 II at diakinesis and metaphase I (Fig. 1B). This is the first chromosome count on this species. In *Calea,* the chromosome numbers for 32 species have been recorded, mainly from Central America. There are few studies on South American species: *C. clematidea* ($n = 19$; WULFF & al., 1996) from Argentina, *C. jelskii* ($n = 19$; TURNER & al., 1967), *C. angusta* ($n = 13$), *C. pilosa* ($n = 13$) (CARR & al., 1999), *C. serrata* ($n = 19$; COLEMAN, 1970) and *C. pinnatifida* ($n = 19$; COLEMAN, 1968) from Brazil. The most frequently occurring chromosome number among the species studied is $n = 19$, but $n = 9, 10, 13, 15, 16$, ca.17, 18, 19 + Bs, 21 + Bs, 24, ca.27, 32 and 38 also occur, revealing that polyploidy and aneuploidy have played an important role in the evolution of the genus.

Wedelia kerrii showed irregular meiotic behaviour with univalents, bivalents and one quadrivalent. The most widespread meiotic configurations at diakinesis and metaphase I were (6 I + 21 II) and (2 I + 21 II + 1 IV). Other chromosome configurations were also (8 I + 18 II + 1 IV), (4 I + 20 II + 1 IV; Fig. 1C) and (2 I + 23 II). Moreover, laggard chromosomes were observed at anaphase I and telophase I. WAISMAN & al. (1986) found a gametic number of $n = 23$ and regular meiotic behaviour in this species. However, these authors reported that up to three bivalents presented secondary associations, which suggests a polyploid origin for $n = 23$. Our results may be interpreted as aneuploid variation of $n = 23$ with two additional chromosomes ($2n = 46+2$). The presence of a quadrivalent points to translocation or residual homology of polyploid origin. The base number of *Wedelia* is uncertain ($x = 11, 12, 13, 23?$) (STROTHER, 1991). The base number $x = 23$ has been proposed as secondary, or of amphiploid origin, from $x = 11 + 12 = 23$ (WAISMAN & al., 1986). Like STROTHER (1991), *W. kerrii* records $2n = 44$ (22 II) in materials from Mexico (KEIL & al., 1988; see *Wedelia sp. nov.*). This number suggests a tetraploid from a base of $x = 11$. To sum up, *W. kerrii* is a polyploid taxon (in all likelihood tetraploid) displaying aneuploid numerical variations in its geographical distribution area: $2n = 44$ is found in Mexican populations (STROTHER, 1991), $2n = 46$ in populations from north-eastern Argentina (WAISMAN & al., 1986), and $2n = 48$ chromosomes in Paraguayan populations.

Brassicaceae

In *Lepidium bonariense* ($2n = 32$), the chromosome number observed is consistent with a previous report by HURKA & al. (1992) based on Spanish materials (Tenerife island). In a previous count, JONSELL (1975), working with African materials, observed $2n = 64$ chromosomes. The first count using material is reported here from the South American Cone where the species originated, though nowadays it is an introduced plant in anthropic environments in all continents. The base number for *Lepidium* is $x = 8$ (LEE & al., 2002). *Lepidium bonariense* displays tetraploid cytotypes, such as the one found in Paraguay, and octoploid cytotypes, such as the one found in Africa. LEE & al. (2002) suggest that the differentiated species found in America and Australia are the result of allopolyploid hybridization.

Cochlospermaceae

Cochlospermum regium displayed regular meiosis with 18 II in diakinesis and metaphase I. This is the first chromosome study on material from Paraguay. Meiotic study of our materials confirms data obtained in meiosis by MAGLIO & al. (1984), MORAWETZ (1986) and FORNI-MARTINS & al. (1995) with Brazilian materials. Known chromosome numbers for this genus are $2n = 12$ in *C. planchoni* and *C. tinctorium* (MIEGE, 1960; MANGENOT & MANGENOT, 1962), *C. gillivraei*, *C. religiosum* (MORAWETZ, 1986; GILL & al., 1990), and *C. gossypium* (GILL & al., 1979). There are also reports of $2n = 14$ in *C. religiosum* (KRISHNAN, 1977), $2n = 18$ in *C. orinocensis*, $2n = 24$ in *C. vitifolium* (MORAWETZ, 1986), and $2n = 36$ in *C. regium* in this study, MORAWETZ (1986) and FORNI-MARTINS & al. (1995). The base number suggested for this genus is $x = 6$ (FORNI-MARTINS & al., 1995). In the light of available data, a polyploid complex is defined for this genus with diploid, triploid, tetraploid and hexaploid species. FORNI-MARTINS & al. (1995) consider that the hexaploid *C. regium* is a species which arose by triploidy. However, our analysis of meiotic behaviour did not reveal multivalents, univalents or secondary associations of bivalents to support this hypothesis. The results obtained here suggest that this species originated by allopolyploidy.

Commelinaceae

Commelina erecta was found to have $2n = 60$ chromosomes, the first count on natural populations from Paraguay. The same number was found in populations from Brazil (ROMEU PITREZ & al., 2001) and Argentina (JONES & JOPLING, 1972; CRISTOBAL DE HINOJO & al., 1998), and this is the only cytotype found so far in South America.

Cucurbitaceae

In *Cucurbitella asperata* ($n = 13$; Fig. 1D), we regularly observed 13 II in PMCs at diakinesis and metaphase I. This is the first study of meiotic behaviour in the species and the result tallies with the somatic number $2n = 26$ found by COVAS & SCHNACK (1946).

Euphorbiaceae

Cnidoscolus hasslerianus presented $2n = 36$ chromosomes. We know no previous counts for this species, which originated in Paraguay, eastern Bolivia and northern Argentina. The first references for the genus are to be found in BOLKHOSKIKH & al. (1969), who reported $2n = 36$ for *C. basiacanthus, C. stimulosus* and *C. texanus*. More recently, FULVIO (1973) found a haploid count of $n = 18$ in *Cnidoscolus tubulosus* var. *triloba* using materials from Argentina.

Fabaceae

In *Crotalaria incana* subsp. *incana* ($n = 7$; $2n = 14$), the four populations studied gave uniform results, i.e. diploid cytotypes with $2n = 14$ somatic chromosomes which behave in meiosis by forming 7 II. These observations are coherent with several counts to be found in the usual literature, from BOLKHOVSKIKH & al. (1969) to TROPICOS (2006). The somatic number ($2n = 16$) found by MANGOTRA & KOUL (1991) in plants from Brazil is at variance with these results.

The genus *Dolichopsis* (*Phaseoleae*) comprises only three species (LEWIS, 1991; DELGADO, 2004). In *D. paraguariensis*, we observed $2n = 2x = 22$ chromosomes. Meiotic behaviour was regular with the formation of 11 II in PMCs at diakinesis and metaphase I. (Fig. 1E, 1F). This is the first chromosome number reported for the species and the genus. *Dolichopsis paraguariensis* is a diploid species with a base number of $x = 11$, which is proposed as the base number for the genus.

Lupinus paraguarensis ($2n = 18$; Fig. 2B) presents a surprising somatic number, the lowest ever for the genus, which was obtained from a few germinated seeds and requires confirmation. Our result differs from the findings of CONTERATO & SCHIFINO-WITTMANN (2006), who reported $2n = 36$ chromosomes for a Brazilian population of the same species from Rio Grande do Sul (Cruz Alta). The species is at the origin of various groups of *Lupinus* which are endemic to the east-central region of South America, or has served as transition between these groups. On one hand, there are the perennial *Lupinus* with simple leaves from the group *simplicifoliae*, and on the other, the perennial species with compound leaves which make up the complex *L. multiflorus*, to which *L. paraguarensis* (PLANCHUELO RAVELO & DUNN, 1984) belongs. Karyological knowledge of these complexes has recently been enriched by the studies of South American *Lupinus* carried out by SCHMATZ MACIEL & SCHIFFINO-WHITTMANN (2002) and CONTERATO & SCHIFINO-WITTMANN (2006). With regard to species from the above mentioned complexes from south-eastern Brazil, they report $2n = 36$ as the number common to both plurifoliate and unifoliate perennial species. Annual species can present $2n = 36$, or more infrequently 34 or 32 chromosomes, in what appears to be a descending disploid series which originated from $2n = 36$. They conclude that *Lupinus* from eastern South America (usually $2n = 6x = 36$) are cytologically distinct from the North American and Andean taxa (usually $2n = 8x = 48$) and have their own speciation centre in the evolution of which recent polyploidy has not played an important role, contrary to what has occurred in North American *Lupinus*. These results also support the suggestion made by DUNN (1971) and GLADSTONES (1998) that $x = 6$ is the base number of the American species of *Lupinus*. Our result gives rise to a new hypothesis, which we will refrain from putting forward, however, until our findings are confirmed

Previous counts of *Macroptilium lathyroides* var. *lathyroides* ($2n = 22$; Fig. 1F) by MARECHAL (1970) and SHANMUGHASUNDARAM & SUBRAMANIAM (1992) are confirmed by this study, which yielded the same somatic number. DAVIÑA & al. (2001) have announced a gametic number of $n = 11$ for this species.

In *Microlobius foetidus* subsp. *paraguensis*, $2n = 2x = 26$ chromosomes was founded (Fig. 2C). *Microlobius* consists of two species, *M. mimosoides* and *M. foetidus*, the latter with two subspecies, *M. foetidus* subsp. *foetidus* and the one studied here (SOUSA SANCHEZ & ANDRADE, 1992). This is the first chromosome study of the genus and the subspecies analysed is a diploid taxon with $x = 13$ as the most likely base number.

For *Senna occidentalis* ($2n = 26$), this is the first chromosome count, at least for Paraguay. But if we take synonymy into account, numerous chromosome studies have been carried out under the name *Cassia occidentalis*. These have yielded a somatic number of $2n = 26$ (MUTO, 1929; FRAHM-LELIVELD, 1960; SINHA & al., 1972; MA & al., 1984; HUANG & al., 1989) and a gametic number of $n = 13$ (MEHRA, 1972; SINHA & PRASAD, 1973; BIR & SIDHU, 1979; COLEMAN & DEMENEZES, 1980). Under the name *C. occidentalis*, there are many equally long-standing references to two somatic numbers: $2n = 28$ (SENN, 1938; PANTULU, 1940; TURNER, 1956; MIEGE, 1962; SHARMA, 1970; BIR & KUMARI, 1985; YEH & al., 1986) and $n = 14$ (LARSEN, 1971; MEHRA,

1972; CHOUDHARY & CHOUDHARY, 1989; KUMARI & al., 1989; JAHAN & al., 1994). Some exceptions occur, such as in ZHANG (1992), who found $2n = 56$ in plants from China, and SINGHAL & al. (1990), who referred to $n = 12$ (sub *C. occidentalis*) for an Indian population. The two most common cytotypes ($2n = 26, 28$) are present, and irregularly spread, throughout the tropical and subtropical distribution area of America, Asia and Africa. Other cytotypes reported require confirmation.

For *Senna pendula* ($2n = 26$; Fig. 2D), no previous counts are available under this name, but under the synonym *Cassia bicapsularis*, there are records of the somatic number $2n = 28$ (D'AMATO-AVANZI, 1956; IRWIN & TURNER, 1960; DATTA & DATTA, 1973; PANDEY & PAL, 1980) and the gametic number $n = 14$ (COLEMAN & DEMENEZES, 1980; SANJAPPA & DASGUPTA, 1981). The gametic number $n = 9$ has also been reported (GILL & HUSAINI, 1981, 1985). For the number founded in this study, $2n = 26$, it is a new cytotype for this species.

Labiatae

In *Vitex cymosa*, we observed regular meiotic behaviour with 16 II at diakinesis and metaphase I (Fig. 1G). This is the first chromosome number for the species. *Vitex* is considered the basal genus within the *Labiatae* family (PATON & al., 2000) and comprises approximately 380 species (DASSANAYAKE, M. D. & F. R. FOSBERG, 1983). In those species which have been studied cytogenetically, several chromosome numbers have been found. DIJKGRAAF & al., (1995) reported $2n = 32$ as the commonest chromosome number in the genus and considered $x = 8$ as the base number of *Vitex*, because they found the octoploid cytotype in *V. lucens* ($2n = 8x = 64$ chromosomes). However, $n = 12$ has been found in *V. altissima* and *V. arborea* (CHATHA & BIR, 1988) and $2n = 24$ in *V. agnus-castus* (DARLINGTON & WYLIE, 1955), which suggests a derived base number of $x = 6$. *Vitex negundo* displays great cytogenetic instability and a wide diversity of chromosome numbers; the gametic numbers $n = 13$ (BIR & SAHNI, 1983; CHATHA & BIR, 1988), $n = 16$ (SANDHU & MANN, 1988), and $n = 17$ (KATHOON & ALI, 1993), and the somatic number $2n = 34$ (MA & al., 1990). For *V. negundo* var. *heterophylla*, $2n = 34$ has also been reported (ZHANG & SHANGGUAN, 1990).

Our findings suggest that *V. cimosa* is tetraploid with a base number of $x = 8$.

Lythraceae

For *Cuphea racemosa* ($2n = 44$), the somatic number found is the same as the gametic number ($n = 22$) reported by GRAHAM (1989) for materials from Brazil. However, it differs from other reports of $n = 16, 20$, also got from Brazilian materials, and $n = 24$ obtained from Mexican materials (GRAHAM, 1982). It is also at variance with the haploid number of $n = 20$ reported by GRAHAM & CAVALCANTI (2001) for materials from Bolivia. Intraspecific aneuploidy also occurs in this species, as it does in several other species of *Cuphea*, a genus of great citological complexity. We report the first somatic count on the species based on Paraguayan materials.

Malpighiaceae

For *Heteropterys glabra* ($2n = 31$; Fig. 2E), this chromosome number, which is reported for the first time in the genus, may be interpreted as belonging to an aneuploid that derives from a hexaploid with a base number of $x = 5$ ($2n = 6x + 1$). Previous counts for this species are found under the name *H. angustifolia* ($2n = 34$; SEMPLE, 1970). Cytological data are available for only 16% of the species of *Heteropterys*. From these, 49.9% have $2n = 20$, 21.4% have $2n = 30$ and the rest have $2n = 34$, $2n = 42$, $2n = 56$ or $2n = 58$ (LOMBELLO & FORNI-MARTINS, 2002, 2003). This is a cytologically unstable genus which displays aneuploidy, diploidy and poliploidy. LOMBELLO & FORNI-MARTINS (2003) explain these chromosome numbers in *Heteropterys* and other genera of *Malpighioideae* by proposing a base number of $x = 5$ for the sub-family.

Malvaceae

For *Hibiscus striatus* ($2n = 52$; Fig. 2F), this is the first chromosome record for this species. It is also a new chromosome number for the genus, which shows a wide range of numbers, from $2n = 18$ for *H. sabdarifa* to $2n = 144$ for *H. altissimus* and $2n = 180$ for *H. birendranath* (SHARMA & SHARMA, 1962).

Melastomataceae

For *Tibouchina gracilis,* showing $n = 12$, this is the first chromosome number for the species. *Tibouchina* includes some 250 species, but very few have been studied karyologically. The chromosome number $n = 9$ has been reported for the following taxa: *T. rufipilis*, *T. galeottiana*, *T. scabriuscula*, *T. longifolia*, *T. longisepala* var. *spathulata*, *T. fraterna*, *T. hintonii*, *T. sebastianopolitana* and *T. versicolor* (SOLT & WURDACK, 1980; ALMEDA & CHUANG, 1992; ALMEDA, 1997). Several species are polyploids and intraspecific polyploidy occurs. The following taxa have been reported with $n = 18$: *T. breedlovei*, *T. geitneriana*, *T. laxa*, *T. naudininana*, *T. candolleana*, *T. chironioides*, *T. ciliaris*, *T. clavata*, *T. glomerata*, *T. grandulosa*, *T. lindeniana*, *T. organensis* and *T. simplicicaulis* (SOLT & WURDACK, 1980; ALMEDA & CHUANG, 1992; ALMEDA, 1997). Very high levels of polyploidy ($14x$) are observed in *T. aristeguietae*, for instance, and in *T. lepidota* ($n =$ ca. 62 and $n = 63$) (HUNZIKER & al., 1985; ALMEDA & CHUANG, 1992; ALMEDA 1997). The above-mentioned species constitute a group with a base number of $x = 9$. In an other small group of species, $x = 7$ is the most likely base number. This group comprises *T. pumila* ($n = 7$), *T. semidecandra* ($n = 28$) and *T. urvilleana* ($2n = 56$) (SOLT & WURDACK, 1980; MEENAKUMARI & KURIACHAN, 1990). The discovery of $n = 12$ in *T. gracilis* is new for the genus and points to the existence of a new base number of $x = 12$.

Myrsinaceae

The first chromosome count on this species yielded $2n = 48$ chromosomes for *Myrsine guianensis*. Different chromosome numbers have been recorded for the genus; *M. seguini*, *M. africana*, *M. australis*, *M. coxii*, *M. divaricata*, *M. kermadecensis*, *M. nummularifolia*, *M. salicina*, *M. chathamica*, *M. capitellata* and *M. oliveri* have $2n = 46$ chromosomes (CHUANG & al., 1963; FAURE, 1968; BEDI & al., 1980; OGINUMA & al., 1994; DAWSON, 1995). However, two cytotypes, $2n = 46$ and $n = 22$, have been reported for *M. semiserrata* (MEHRA, 1976; BEDI & al., 1980). All the mentioned species were studied using materials of non-American origin. Furthermore, BORGMANN (1964) found $2n = 24$ chromosomes in an unidentified species of *Rapanea* from New Guinea. The only species analysed on the basis of American populations are *Myrsine ferruginea*, with $2n = 48$ (GADELLA & al. 1969) for Brazilian populations, and *M. matensis* with $2n = 46$ (MOLERO & al., 2002) for Paraguayan populations. It would appear that the two most widespread cytotypes are also present in South America, notably in Paraguay.

Passifloraceae

For *Passiflora misera*, $2n = 2x = 12$ was recorded. The species of the genus *Passiflora* can be divided into four karyological groups represented by the base numbers $x = 6$, $x = 9$, $x = 10$ and $x = 12$ (MELO & GUERRA, 2003). The chromosome number reported for *P. misera,* a diploid, matches those reported by MELO & al. (2001) and MELO & GUERRA (2003) for materials from south-eastern Brazil. However these authors also found the tetraploid $2n = 36$ in plants from north-eastern Brazil. In *P. misera* from north-eastern Argentina, 9 II in PMCs at diakinesis has also been observed (DEGINANI & ESCOBAR, 2002). Our report is the first chromosome record for *P. misera* in Paraguay and extends the geographical distribution of diploids of this species to include that country.

Poaceae

For *Paspalum simplex* ($2n = 2x = 40$), previous counts for this taxon are confirmed (Saura, 1941; Espinoza & Quarín, 1998; Urbani & al., 2002). However, Paraguayan populations of this species are studied here for the first time.

Polygonaceae

For *Ruprechtia laxiflora* ($2n = 4x? = 28$; Fig. 2G), a previous count yielded $2n = 28$ chromosomes in Argentinian populations (under the name of *R. polystachya* in Covas & Schnack (1947) and Cocucci (1957)). In *R. apetala*, $2n = 28$ has also been observed (sub. *R. corylifolia;* Cocucci, 1957). However, $2n = 30$ chromosomes has been found in *R. salicifolia* and $2n = 112$ in *R. triflora* (Cocucci, 1957). Darlington & Wylie (1955) proposed $x = 7$ as the base number for the genus and Cocucci (1957) proposed $x = 14$, but further studies are needed to confirm this. This is the first count for Paraguay.

Portulacaceae

For *Talinum paniculatum* ($n = 12$), twelve bivalents were observed in meiosis, confirming the somatic counts ($2n = 24$ chromosomes) published by Steiner (1944), Tjio (1948) and Xu & al. (1992).

Ranunculaceae

For *Clematis montevidensis* ($n = 8$), the gametic number found confirms a previous report by Hunziker & al. (1985) for Argentinian material. The chromosome number found is the most widespread in the genus.

Scrophulariaceae

The meiotic behaviour of *Angelonia gardneri* was regular, with 10 bivalents at diakinesis and metaphase I (Fig. 1H). Occasionally, a quadrivalent was observed. Chromosome segregation was generally regular at anaphase I. Sometimes an unfragmented bridge was observed. In other instances, a laggard chromosome was observed near the nucleus at telophase I. The presence of a quadrivalent and an unfragmented bridge is indicative of a translocation. In root somatic cells of *A. integerrima,* $2n = 20$ chromosomes were found. These are the first chromosome studies for both species. Both taxa are diploid. Only three species in the genus *Angelonia*, except the species studied here, have been subjected to chromosome studies to date: *A. cubensis*, *A. grandiflora* and *A. salicariaefolia* (Raghavan & Srinivasan, 1940; Verma & Dhillon, 1967; Sharma, 1970; Chandran & Bhavanandan, 1983; Subramanian & Pondmudi, 1987). All have the same chromosome number and ploidy level: $2n = 2x = 20$. A base number of $x = 10$ is proposed for the genus.

Solanaceae

For *Solanum pilcomayense* var. *pilcomayense* ($2n = 24$; Fig. 2H), the somatic number found by Ratera (1943) under the synonym *S. basilobum* is confirmed. It also fits the gametic number ($n = 12$) reported by Moscone (1992) for materials from Argentina. Our results are the first report for Paraguay.

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Table 1. – Specimen seen and results of the chromosome counting (*: first chromosome number for this taxon; **: chromosome numbers differing from previous reports).

Taxon	*n*	2*n*	Location and Voucher
Apocynaceae *****Thevetia bicornuta*** Müll. Arg.	10		**Pte Hayes:** Puerto Falcón, *J. Daviña & A. Honfi 542* (MNES).
Aristolochiaceae ***Aristolochia gibertii*** Hook.	14		**Cordillera:** Atyrá, *J. Molero & B. Benitez 12.99* (PY).
Asclepiadaceae *****Asclepias mellodora*** A. St.-Hil.	22		**Cordillera:** km 627 on the road from Coronel Oviedo to Mbutý, 25°15'36"S 56°23'51"W, *J. Molero & R. Duré 31.99* (BCN).
Asteraceae ***Acmella decumbens*** var. ***affinis*** (Hook. & Arn.) R. K. Jansen	26		**Pte Hayes:** Pto Militar (by the river Paraguay), 25 km on the West route to Pozo Colorado, 23°31'25"S 57°46'01"W, *A. Schinini, J. Molero, R. Duré & M. Quintana 35589* (CTES, PY).
Asteraceae ******Aspilia montevidensis*** var. ***setosa*** (Griseb.) Cabrera	34		**Central:** on route 3, between Loma Grande and Altos, *A. Honfi & J. Daviña 1160* (LP, MNES); San Lorenzo, Campus UNA, arboretum, *A. Honfi & J. Daviña 1128* (LP, MNES).
Asteraceae *****Calea uniflora*** Less.	16		**Paraguarí:** Tebicuary river, 2 km from Villa Florida, *J. Daviña & A. Honfi 547* (LP, MNES). **Central:** on route 3, between Nueva Colombia and Loma Grande, *A. Honfi & J. Daviña 1159* (LP, MNES).
Asteraceae ******Wedelia kerrii*** N. E. Br.	21II+ 6I		**Itapúa:** on route 1, 30 km from Encarnación, *J. Daviña & A. Honfi 551* (LP, MNES).
Brassicaceae ***Lepidium bonariense*** L. var. ***bonariense***	32		**Pte Hayes:** Pto Militar, 10 km W to Pozo Colorado, 23°26'31"S 57°19'42"W, *A. Schinini, J. Molero, R. Duré & M. Quintana 35578* (BCN, CTES, PY).
Cochlospermaceae ***Cochlospermum regium*** (Schrank) Pilg.	8		**Asunción:** cultivated in the "Jardín Botánico de la Facultad de Ciencias Químicas (UNA)".

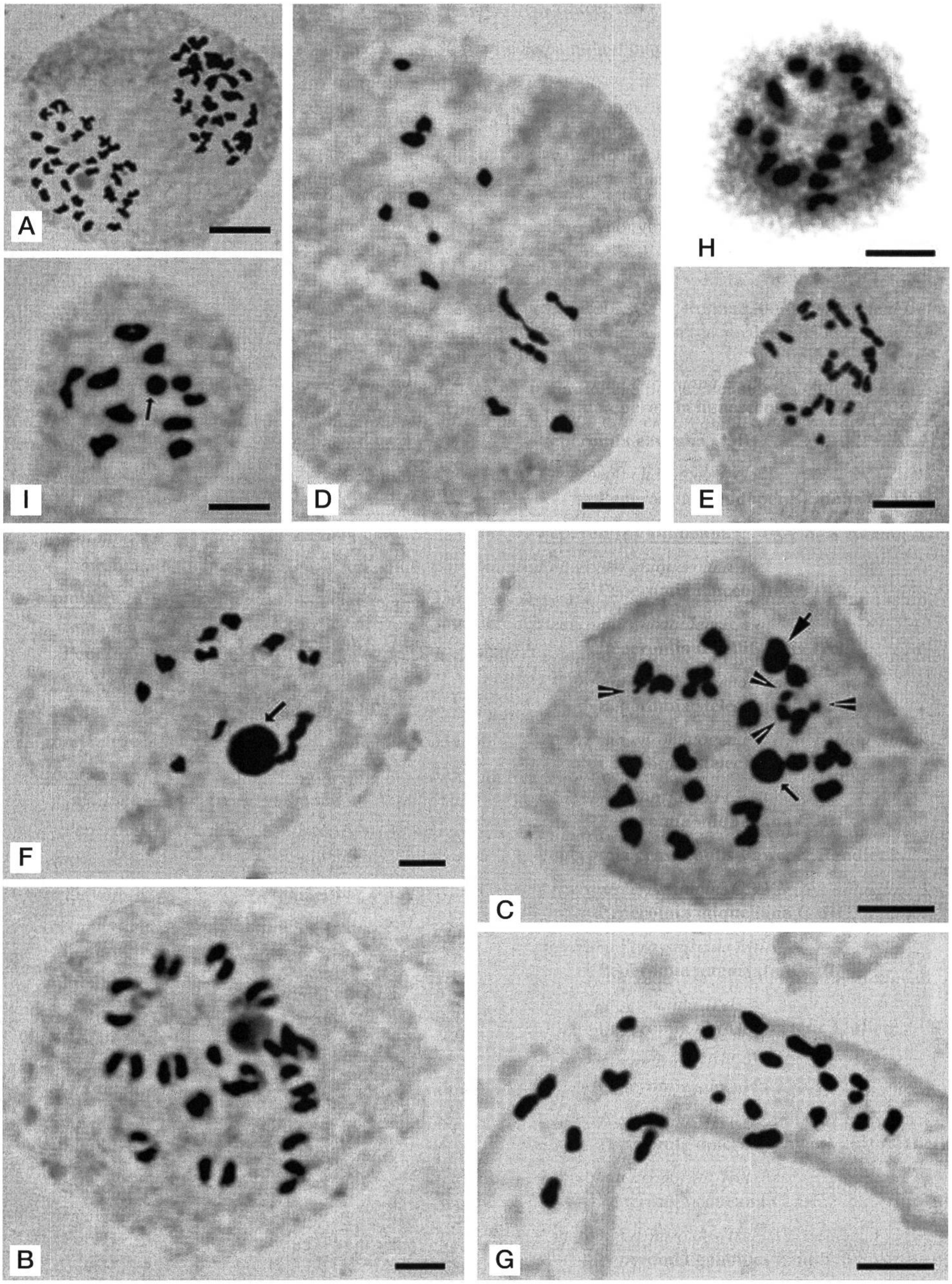
Taxon	n	2n	Location and Voucher
Commelinaceae ***Commelina erecta*** L.	60		**Cordillera:** Altos, *M. Grabiele, J. Daviña & A. Honfi 45* (MNES).
Cucurbitaceae ***Cucurbitella asperata*** (Hook.) Walp.	13		**Pte Hayes:** Trans-Chaco Route, 5 km from the crossroad to Pto Falcón, *J. Daviña & A. Honfi 515* (MNES).
Euphorbiaceae ****Cnidoscolus hasslerianus*** (Pax) Pax	36		**Cordillera:** Atyrá, *B. Benitez & A. Elizeche P-1.99* (BCN, MA 709783); Saltos de Piraretá, *B. Benitez 12/98, D. Franco, J. Molero, A. Rovira* (BCN, MA 709784).
Fabaceae ***Crotalaria incana*** L.	7	14	**Cordillera:** Atyrá, surroundings of Casa del Monte, *M. Quintana, J. Molero & R. Duré 924* (PY). **Pte Hayes:** Puerto Falcón road, *J. Daviña & A. Honfi 538* (MNES); Trans-Chaco Route, 5 km Crossroad to Pto Falcón, *J. Daviña & A. Honfi 517* (MNES). **Central:** San Lorenzo, Campus UNA, *A. Honfi & J. Daviña 1129* (MNES).
Fabaceae ****Dolichopsis paraguariensis*** Hassl.	11	22	**Pte Hayes:** Trans-Chaco route, 5 km crossroad Pto Falcón, *J. Daviña & A. Honfi 516* (PY).
Fabaceae *****Lupinus paraguariensis*** Chodat & Hassl.	18		**Amambay:** Cordillera Amambay, estancia Pitui, *A. Schinini, J. Molero, R. Duré & M. Quintana 35476* (BCN, PY).
Fabaceae ***Macroptilium lathyroides*** (L.) Urb.	22		**Central:** San Lorenzo, Vivero Forestal Nacional, *A. Honfi & J. Daviña 1140* (MNES).
Fabaceae ****Microlobius foetidus*** subsp. ***paraguensis*** (Benth.) M. Sousa & G. Andrade	26		**Pte Hayes:** Pto Militar (by the river Paraguay), 25 km west route to Pozo Colorado, 23°31'25"S 57°46'01"W, *A. Schinini, J. Molero, R. Duré & M. Quintana 35589* (CTES, PY).
Fabaceae ***Senna occidentalis*** (L.) Link	26		**San Pedro:** 5 km along the road from Itacuruby del Rosario to Villa Rosario, *J. Molero & R. Duré 48/99* (BCN, PY).

Taxon	*n*	2*n*	Location and Voucher
Fabaceae *****Senna pendula*** (Willd.) H. S. Irwin & Barneby	26		**San Pedro:** 5 km along the road from Itacuruby del Rosario to Villa Rosario, *J. Molero & R. Duré 50/99* (BCN, PY).
Labiatae ****Vitex cymosa*** Spreng.	16		**Central:** Capiatá, *A. Honfi & J. Daviña 1030* (MNES).
Lythraceae ***Cuphea racemosa*** (L. f.) Spreng. var. ***racemosa***	44		**Guairá:** Melgarejo, 25°41'62'52"S 56°14'52"W, *J. Molero & R. Duré 77/99* (BCN).
Malpighiaceae *****Heteropterys glabra*** Hook.	31		**Paraguarí:** Caañabé, in secondary forest, *J. Simón & B. Benitez 52/98* (PY, BCN).
Malvaceae ****Hibiscus striatus*** Cav.	52		**Pte Hayes:** 56 km E of Pozo Colorado, *A. Schinini & J. Molero, R. Duré & M. Quintana 35600* (BCN, PY).
Melastomataceae ****Tibouchina gracilis*** (Bonpl.) Cogn.	12		**Central:** on road from Aregua to Ypacarai, *J. Daviña & A. Honfi 529* (MNES).
Myrsinaceae ****Myrsine guianensis*** (Aubl.) Kuntze	48		**Cordillera:** Arroyo Ytú, *M. Quintana, J. Molero & R. Duré 917* (PY).
Passifloraceae ****Passiflora misera*** Kunth	12		**San Pedro:** between Itacuruby and Villa del Rosario, *J. Molero & R. Duré 57/99* (BCN).
Poaceae ***Paspalum simplex*** Morong	40		**Pte Hayes:** Pto Falcón, *J. Daviña & A. Honfi 540* (MNES); Pto Falcón, *J. Daviña & A. Honfi 541* (MNES).
Polygonaceae ***Ruprechtia laxiflora*** Meisn.	28		**Cordillera:** Atyrá, surroundings of Casa del Monte, *J. Molero 18/99* (BCN).
Portulacaceae ***Talinum paniculatum*** (Jacq.) Gaertn.	12		**Alto Paraguay:** 9 km from Pozo Colorado, 23°30'47"S 58°41'57"W, *A. Schininii 35609, J. Molero, R. Duré & M. Quintana* (CTES).

Taxon	*n*	*2n*	Location and Voucher
Ranunculaceae ***Clematis montevidensis*** Spreng.	8		**Central:** Puente Guggiari, *J. Daviña & A. Honfi 1110* (MNES).
Scrophulariaceae ****Angelonia gardneri*** Hook.	10		**Misiones:** on route 1, Villa Florida, *J. Daviña & A. Honfi 566* (MNES).
Scrophulariaceae ****A. integerrima*** Spreng.	20		**San Pedro:** 1 km along the road from General Aquino to Villa Rosario, 24°27'3"S 56°55'3"W, *R. Duré & J. Molero* (BCN, CTES).
Solanaceae ***Solanum pilcomayense*** Morong	24		**Pte Hayes:** 56 km E. of Pozo Colorado, 23°30'46"S 58°13'55"W, *A. Schinini & J. Molero, R. Duré & M. Quintana 35594* (BCN).

Fig. 1.– **A:** *Aspilia montevidensis* var. *setosa (Daviña & Honfi 1160),* prophase II in meiosis, $n = 34$; **B:** *Calea uniflora (Daviña & Honfi 547),* diakinesis with 16 II and presence of a nucleolus; **C:** *Wedelia kerrii*, diakinesis with 4 I + 20 II + 1 IV an a nucleolus [the bold arrow (▲) points the univalents; the large arrow (^) points the quadrivalent; the arrow (↑) points to the nucleolus]; **D:** *Cucurbitella asperata*, metaphase I in meiosis with 13 II; **E:** *Dolichopsis paraguariensis*, somatic mitosis with $2n = 22$; **F:** *Dolichopsis paraguariensis*, diakinesis in meiosis with 11 II and a nucleolus [the arrow (↑) points to the nucleolus]; **G:** *Macroptilium lathyroides*, somatic mitosis with $2n = 22$; **H:** *Vitex cymosa*, prometaphase in meiosis with 16 II; **I:** *Angelonia gardneri,* CMP in meiosis with 10 II and a nucleolus [the arrow (↑) points to the nucleolus].

Scale bar: 5 µm.


A
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D
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G

Fig. 2. – Metaphasic plates of somatic mitosis. **A:** *Aristolochia gibertii*, $2n = 14$; **B:** *Lupinus paraguariensis*, $2n = 18$; **C:** *Microlobius foetidus* subsp. *paraguensis*, $2n = 26$; **D:** *Senna pendula*, $2n = 26$; **E:** *Heteropteris glabra*, $2n = 31$; **F:** *Hibiscus striatus*, $2n = 52$; **G:** *Ruprechtia laxiflora*, $2n = 28$; **H:** *Solanum pilcomayensis*, $2n = 24$.

Scale bar: 5µm.