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Cytogenetic studies of Southern South-American *Ilex*

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Abstract - The dioecious genus *Ilex* (Aquifoliaceae) has been poorly studied from a cytogenetic point of view. Chromosome numbers of only ca. 5% of the species have been reported. In the present work the chromosome number of eight taxa from Argentina and Brazil are presented: *Ilex taubertiana* Loes., *I. integerrima* (Vell.) Reissek and *I. pseudobuxus* Reissek present $2n = 40$, being this the first report for these species. A diploid number of 40 is also confirmed for *I. paraguariensis* A. St. - Hil, *I. brevicuspis* Reissek, *I. dumosa* Reissek and *I. theezans* Reissek, and $2n=80$ for *I. argentina* Lillo. Species with $2n=40$ ($n=20$) show a regular meiotic behaviour with 20 bivalents, while *I. argentina* also shows a regular meiosis with 40 bivalents. Those individuals of *I. paraguariensis* analyzed in the present paper do not show a heteromorphic bivalent as previously reported and ascribed to sex chromosomes.

Key words: *Ilex*, Aquifoliaceae, chromosome number, polyploidy.

INTRODUCTION

The predominantly dioecious genus *Ilex* is not only the largest of the family Aquifoliaceae (as it comprises about 600 cosmopolitan species) but also has great economic importance. More than 200 species grow in South America, being this the center of species diversity (LOESENER 1901, 1908, 1942; LOIZEAU 1994; GIBERTI 1995a; CUÉNOUD *et al*. 2000). However, only 12 taxa are represented in Southern South America and up to now, only 6 native members of the genus have been found in Argentina (GIBERTI 1998).

The "yerba mate" tree, *Ilex paraguariensis,* is native from South eastern Brazil, North eastern Argentina and Paraguay, and isolated localities of Uruguay. It is probably the best known South American *Ilex* species, and it is also the only one with important economic value. Moreover, several aspects of this crop plant are still unknown. From a taxonomic point of view its relationships with another species of the same genus are poorly understood (GIBERTI 1989).

Reports on *Ilex* chromosome numbers are relatively scarce; only 38 species of the whole genus have been analysed and they represent about 5% of the species. Among them, SAURA (1944), ANDRÉS and SAURA (1945), GRONDONA (1954), NIKLAS (1987), BARRAL *et al*. (1995) and DAVIÑA (1998) reported the chromosome numbers for *I. theezans*, *I. dumosa* var. *dumosa*, *I. paraguariensis*, *I. argentina* and *I. brevicuspis.* In the present study we report the chromosome number of 8 taxa from Argentina and Brazil whose geographical distribution is shown in Fig. 1.

MATERIALS AND METHODS

All the materials we examined were collected from living specimens grown at the Plant Germplasm Bank of the Experimental Station of

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Table 1 – Haploid chromosome number of the *Ilex* species here analyzed. * First report.

Species	Number of studied individuals	Gametic number	References
I. breviscuspis	7	20	DAVIÑA, 1998 Present work
I. paraguariensis	10	20	SAURA, 1944 ANDRÉS and SAURA, 1945 NIKLAS, 1987 BARRAL *et al.*, 1995 Present work
*I. integérrima**	7	20	Present work
I. theezans	4	20	ANDRÉS and SAURA, 1945 Present work
I. dumosa	3	20	ANDRÉS and SAURA, 1945 Present work
*I. pseudobuxus**	5	20	Present work
*I. taubertiana**	3	20	Present work
I. argentina	5	40	BARRAL *et al.*, 1995 Present work

INTA Cerro Azul (L. N. Alem Department, Misiones Province, Argentina). List of herbarium specimens and the original geographic location of seeds that match our chromosome sampling collections follows:

Ilex argentina Lillo: ARGENTINA. Tucumán: Acheral-El Nogalar, Prat Kricun *et al.* 187 (BACP). *I. brevicuspis* Reissek ARGENTINA. Misiones: San Vicente, Prat Kricun *et al.* 1 (BACP); Tobuna, Greizerstein & Giberti 3 (BACP); San

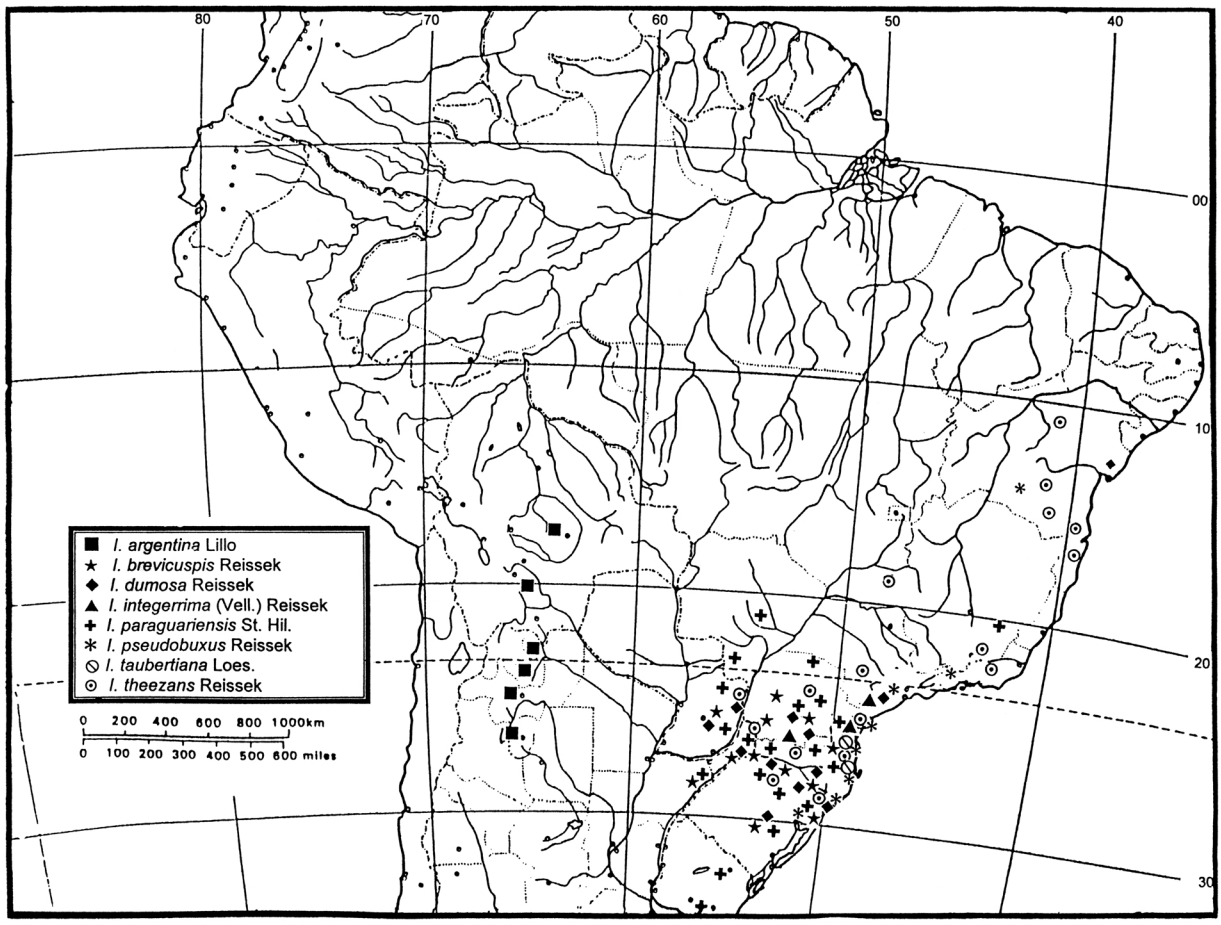

Fig. 1 – Distribution of the *Ilex* species here analyzed.

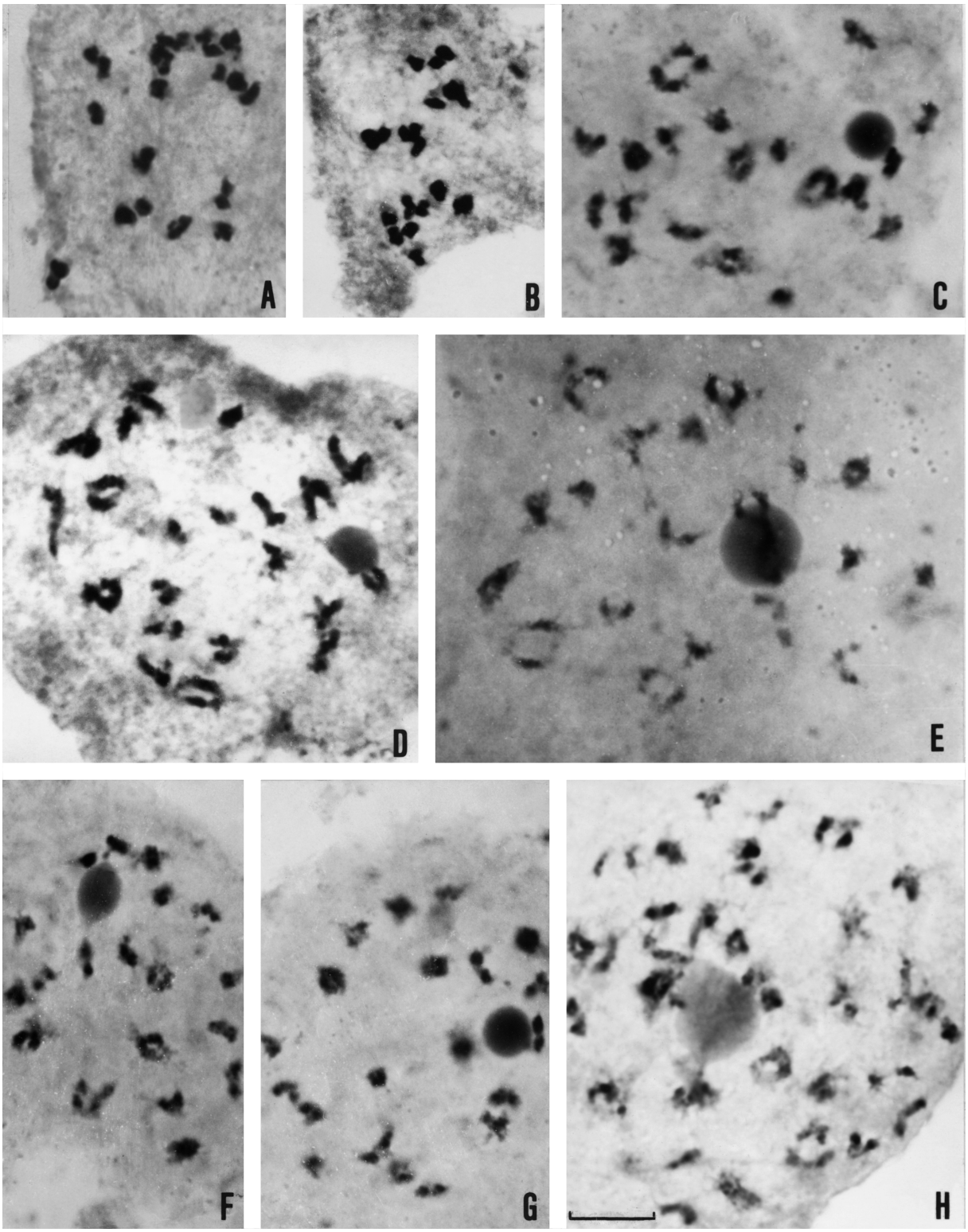

Fig. 2 – (A-B): Prometaphases I of *I. paraguariensis* (A) and *I. taubertiana* (B), both with 20 II (bivalents); (C-H) Diakinesis; of *I. pseudobuxus*, (C), *I. brevicuspis* (D), *I. integerrima* (E), *I. dumosa* (F), *I. theezans* (G) with 20 II and of *I. argentina* (H) with 40 II. Bar 10 µm, all with the same magnification.

Pedro, Greizerstein & Giberti 4 (BACP); Gramado Cruce, Greizerstein & Giberti 5, 6 (BACP); Gobernador López, Prat Kricun *et al.* 49 (BACP); Campo Viera, Prat Kricun *et al.* 33 (BACP). *I. dumosa* Reissek var. *dumosa*: ARGENTINA. Misiones: Campo Viera, Prat Kricun *et al.* 7 (BACP); Oberá, Prat Kricun *et al.* 3 (BACP). BRASIL. Paraná: Paranaguá, Prat Kricun *et al.* 112 (BACP). *I. integerrima* (Vell.) Reissek: BRASIL. Paraná: Quatro Barras, Prat Kricun *et al.* 108 (BACP); São Mateus do Sul, Prat Kricun *et al.* 114 (BACP); Irati, Prat Kricun *et al.* 117 (BACP); Texeira Soares, Prat Kricun *et al.* 118 (BACP). *I. paraguariensis* A. St. - Hil:. ARGENTINA. Misiones: Oberá, Greizerstein & Giberti 1 (BACP); Paraíso, Greizerstein & Giberti 37 (BACP). BRASIL. Santa Catarina: Chapecó, Greizerstein & Giberti 28 (BACP). *I. pseudobuxus* Reissek: BRASIL. Rio Grande do Sul: Torres, Prat Kricun *et al.* 124 (BACP); Campo Bon, Prat Kricun 224 (BACP). *I. taubertiana* Loes.: BRASIL. Rio Grande du Sul: São Francisco de Paula, Prat Kricun 233 (BACP). *I. theezans* Reissek: BRASIL. Santa Catarina, Major Vieira, Prat Kricun *et al.* 19 (BACP); Paraná, Catanduvas, Prat Kricun *et al.* 45 (BACP).

For meiotic preparations young flower buds were fixed in 3:1 (absolute ethanol : glacial acetic acid) and stored at 4ºC. The slides were obtained squashing the anthers in a drop of 2% propionic haematoxylin. The slides were frozen with dry ice, the cover glass removed with a razor blade, air dried and mounted in a synthetic resin (Euparal). The photomicrographs were taken with a Zeiss microscope.

RESULTS

All the species presented a regular meiosis. *Ilex brevicuspis, I. dumosa, I. integerrima, I. paraguariensis*, *I. pseudobuxus, I. taubertiana* and *I. theezans*, all of them presented 20 bivalents (Figs. 1A-G), while *Ilex argentina* showed 40 bivalents (Fig. 1H). All the species presented homomorphic bivalents and in *I. argentina* no multivalents or metaphase I out-of-plate univalents were detected.

No variability in chromosome number or in meiotic behaviour was detected among the individuals belonging to different localities of the same given species.

A summary of our results and previous reports is given In Table 1.

DISCUSSION

Our results are the first chromosome report for *Ilex integerrima*, *I. pseudobuxus* and *I. taubertiana*, and all of them present $2n$=40, n=20 (Table 1). On the other hand, chromosome number for the remaining species agree with previous reports on specimens from different localities (ANDRÉS and SAURA 1945; GRONDONA 1954; NIKLAS 1987; BARRAL *et al.*, 1995; DAVIÑA, 1998).

No heteromorphic bivalent has been detected in any of the species here analyzed. ANDRÉS and SAURA (1945) reported the occurrence of an heteromorphic bivalent in *I. paraguariensis*, suggesting that it could be a sex chromosome pair. However, our results agree with those of BARRAL *et al.* (1995) in *I. paraguariensis*, who did not found any heteromorphic bivalent.

Most reported species possess $2n$=40 (Table 1) but DNA content is only known for three of them: *I. aquifolium* L. with 2C=2.3 pg (BENNETT and SMITH 1991); *I. paraguariensis*, 2C=2.23 ± 0.08 pg, and *I. argentina*, 2C= 4.27 ± 0.07 pg (BARRAL *et al.* 1995). These data, together with the similarity in chromosome size among the species, suggest that the variation in genome size among species within the same ploidy level would have not played a significant role in the evolutionary history of the genus.

Thus far, *Ilex argentina* is the only South American *Ilex* species with $2n$=80. Up to the present, some other species with high chromosome number have been reported, such as the Polynesian species *I. anomala* Hook. f. and Arn. ($2n$=80) (CARR 1978), *I. verticillata* (L.) A. Gray ($2n$=72), native to North America, and the Asiatic entity *I. pedunculosa* Miq. ($2n$=120) (GOLDBLATT 1976, 1981).

Cytogenetic studies have shown that the increase in genome size as consequence of the increase in the ploidy level should be one of the conspicuous cytological differences within the genus. Moreover, differences in the ploidy level may indicate reproductive isolation for *Ilex* species with otherwise few scarce morphological differences. This situation could be applied to *Ilex argentina* and *I. paraguariensis*.

Amongst other taxa that inhabit subtropical rainforests on Eastern Andean slopes *I. argentina* is the southernmost representative for the genus in such mountainous environment.

Ilex argentina grows from the neighbourhoods of Santa Cruz de la Sierra (Bolivia) up to the vicinity of Andagalá (Catamarca Province, Argentina). Because of its coarse external similarities with I*lex paraguariensis*, it has often been mistaken with it, the true "yerba mate" tree (GIBERTI 1995b). Thus, *Ilex paraguariensis* was considered as a native Bolivian species (ANDREWS 1985; EDWIN and REITZ 1967), sharing a similar exomorphology with native Bolivian *Ilex argentina*.

These results may prove useful for a better understanding of the origin and phylogeny within the genus *Ilex*, as well as contribute to plant breeding improvements of the economically important species.

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LITERATURE CITED

ANDRÉS J.M. and SAURA F., 1945 – *Los cromosomas de la yerba mate y otras especies del género Ilex*. Publ. Inst. Genét. Fac. Agron. Vet. Buenos Aires, 2: 161-168.

ANDREWS S., 1985 – *A checklist of the Aquifoliaceae of Bahia*. Rodriguésia, 37: 36-44.

BARRAL G., POGGIO L. and GIBERTI G.C., 1995 – *Chromosome numbers and DNA content from Ilex argentina (Aquifoliaceae)*. Bol. Soc. Argent. Bot., 30: 243-248.

BENNETT M.D. and SMITH J.B., 1991 – *Nuclear DNA amount in Angiosperms*. Philos. Trans., Ser.B., 334: 309-345.

CARR G.D., 1978 – *Chromosome numbers of Hawaian flowering plants and the significance of Cytology in selected taxa*. Amer. J. Bot., 65: 236-242.

CUENOUD P., DEL PERO MARTINEZ M.A., LOIZEAU P.-A., SPICHIGER R., ANDREWS S. and MANEN J.-F., 2000 – *Molecular Phylogeny and Biogeography of the genus Ilex L. (Aquifoliaceae)*. Ann. Bot., 85: 111-122.

DAVIÑA J., 1998 – *The karyotype of Ilex brevicuspis Reissek (Aquifoliaceae)*. Phyton (Buenos Aires), 47: 217-222.

EDWIN G. and REITZ R., 1967 – *Aquifoliáceas*. In: R. Reitz (ed.), "Flora Ilustrada Catarinense", 1. AQUI. Itajaí, Santa Catarina, Brasil.

GIBERTI G.C., 1989 – *Los parientes silvestres de la yerba mate y el problema de su adulteración*. Dominguezia, 7: 3-22.

–, 1995a – *Ilex en Sudamérica, Florística, Sistemática y Potencialidades con relación a un Banco de Germoplasma para la Yerba Mate*. In H. Winge, Ferreira A.G., de Araujo Mariath J.E. and Tarasconi L.C. (eds.), "Erva-Mate: biologia e cultura no Cone Sul", pp. 303-312. Editora da UFRGS, Porto Alegre, Brasil.

–, 1995b – *Aspectos oscuros de la corología de Ilex paraguariensis St. Hil*. In H. Winge, Ferreira A.G., de Araujo Mariath J.E. and Tarasconi L.C. (eds.), "Erva-Mate: biologia e cultura no Cone Sul", pp. 289-300. Editora da UFRGS, Porto Alegre, Brasil.

–, 1998 – *Hallazgo de Ilex brasiliensis (Aquifoliaceae) en la Argentina*. Bol. Soc. Argent. Bot., 33: 137 - 140.

GOLDBLATT P., 1976 – *New or noteworthy chromosome records in the Angiosperms*. Ann. Missouri Bot. Gard., 63: 889-895.

–, 1981 – *Index to plant chromosome numbers 1975-1978*. Missouri Botanical Garden 5. St. Louis, USA.

GRONDONA E.M., 1954 – *Historia de la yerba mate. II. Sinonimia, cariología y distribución geográfica*. Revista Argent. Agron., 21: 9-24.

LOESENER T., 1901 – *Monographia Aquifoliacearum. I*. Nova Acta Acad. Caes. Leop. - Carol. German. Nat. Cur., 78: pp. VIII + 600 + 15 tabs.

–, 1908 – *Monographia Aquifoliacearum. II*. Nova Acta Acad. Caes. Leop. - Carol. German. Nat. Cur., 89: pp. 313 + 3 maps.

–, 1942 – Aquifoliaceae. In H. HARMS and MATTFELD J. (eds.), "Die Natürlichen Pflanzenfamilien", pp. 36-86. Duncker & Humblot, Berlin.

LOIZEAU P.-A., 1994 – *Les Aquifoliaceae péruviennes (Eléments pour une révision des Aquifoliaceae néotropicales)*. Boissiera, 48: 306 pp.

NIKLAS C.O., 1987 – *Estudios embriológicos y citológicos en la yerba mate Ilex paraguariensis (Aquifoliaceae)*. Bonplandia, 6: 45-56.

SAURA F., 1944 – *Los cromosomas de la yerba mate*. Asociación Rural Yerbatera Argentina, Posadas.

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