

## CHROMOSOME NUMBERS OF CHILEAN PTERIDOPHYTES: FIRST CONTRIBUTION

### *NUMEROS CROMOSOMICOS DE PTERIDOFITOS CHILENOS: PRIMERA CONTRIBUCION*

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#### ABSTRACT

Mitotic chromosome counts in Chilean pteridophytes confirm the diploidy described for *Equisetum* L. ( $2n = 216$ ) and *Blechnum* L. ( $2n = 66$ ), as well as the tetraploidy of *Asplenium* L. ( $2n = 144$ ). *Megalastrum spectabile* (Kaulf.) A.R.Sm. et R.C.Moran is diploid ( $2n = 82$ ), while in metaphases of *Polystichum subintegerrimum* (Hook. et Arn.) R.A.Rodr. chromosome numbers from ca. 311 to 328 were counted. Tetraploidy was also described in *Adiantum chilense* Kaulf. ( $2n = 116$ ).

The analysis of chromosome numbers represents an important step in studies of genetic variation, phylogeny, taxonomy and evolution, as well as in studies on the structure and diversity of the genomes (e.g., genome sizes, ploidy levels, nuclear architecture). In pteridophytes, chromosome numbers have been documented by Manton (1950), Tryon *et al.* (1975), Löve *et al.* (1977), Smith & Mickel (1977), Walker (1985), Dawson *et al.* (2000), Bennett & Leitch (1997, 2001), Yatabe *et al.* (2001), Widén *et al.* (2001), Hanson & Leitch (2002), Obermayer *et al.* (2002), Tindale & Roy (2002), Buarque *et al.* (2003), Windham & Yatskievych (2003), Perrie *et al.* (2003), Buarque *et al.* (2005), Bennert *et al.* (2005). Others important contributions are available but are not cited here. Based on all these references, we compiled information on chromosome numbers for at least 641 species (7.12% of the species recognized), which inhabit in Europe, Oceania, North America, Asia and Caribbean Island. In South America, chromosome data for 27 species (included in the total above) have been documented for taxa from Amazonia and north-eastern Brazil (Tryon *et al.* 1975, Buarque *et al.* 2003, Buarque *et al.* 2005). However, species from other South American countries have not been analysed.

Among the South American undersampled taxa

are the Chilean pteridophytes. These taxa are all homosporous, and although they are a minor component of the biodiversity of Chilean vascular plants (about 2.3% of vascular plants) (Marticorena 1990), are important due to that historically have been used as medical (Looser & Rodríguez 2004) and ornamental (Macaya 2004) resources. Despite of that, the structure and diversity of their genomes have not been still studied.

In this work chromosome numbers for nine native Chilean species belonging to six genera and five families are shown. Chromosome numbers may be used in first instance to elaborate a chromosome index for Chilean pteridophytes, which may be the basis for future studies on structure, diversity and evolution of the genomes of these vascular plants.

Sporophytes of one accession of each species were obtained from naturally growing populations, and voucher specimens were deposited in the no official herbarium (UCT) of the Escuela de Ciencias Biológicas y Químicas of the Universidad Católica de Temuco (Table I). The taxonomic identification was based on Gunckel (1984) and Rodríguez (1995). In the laboratory, plants were kept with their rhizomes submerged in water subject to constant aeration, in order to favor active growth of adventitious roots. After ten to fifteen

days, 5 mm-long root tips were excised from the rhizomes and treated in a solution of 2 mM 8-hydroxyquinoline for 1 h at room temperature followed by 23 h at 8°C (Buarque *et al.* 2003). They were then fixed in ethanol–acetic acid (3:1 v/v) for at least 24 h, placed in 70% ethanol, and stored at 4°C until required. The mitotic chromosomes were obtained

by squashing of root tips previously stained with the Feulgen reaction, and photographed with a digital camera OLYMPUS C-5050 connected to microscope OLYMPUS CX31. The chromosome counts were made on magnified printed photograph. The chromosome numbers are presented in Table I, and metaphases of four species are shown in Fig. 1.

TABLE I. Somatic chromosome number (2n) of the species studied. x = base number.

TABLA I. Número de cromosomas somáticos (2n) de las especies estudiadas. x = número básico.

Taxon	x	2n
Adiantaceae		
<i>Adiantum chilense</i> var. <i>chilense</i> <sup>1</sup>	29	116
Aspleniaceae		
<i>Asplenium dareoides</i> <sup>3</sup>	36	144
Blechnaceae		
<i>B. chilense</i> <sup>1</sup>	33	66
<i>B. hastatum</i> <sup>1</sup>	33	66
<i>B. mochaenum</i> var. <i>mochaenum</i> <sup>1</sup>	33	66
<i>B. penna-marina</i> <sup>2</sup>	33	66
Equisetaceae		
<i>Equisetum bogotense</i> <sup>1</sup>	108	216
Dryopteridaceae		
<i>Megalastrum spectabile</i> var. <i>spectabile</i> <sup>1</sup>	41	82
<i>Poystichum subintegerrimum</i> <sup>2</sup>	41	ca. 311 - 328

COLLECTION SITES:

<sup>1</sup>CHILE, Región de la Araucanía, Provincia de Malleco, Fundo Niágara, 300 m north of highway between Curacautín and Manzanar, km 15, altitude 380 m, (38°27'S; 71°37'W), 11-03-2005, M. Romero-Mieres.

<sup>2</sup>CHILE, Región de la Araucanía, Provincia de Malleco, Malalcahuello sector road to Lolco, km 3, near to international highway between Malalcahuello and Lonquimay, km 90, altitude 600 m (38°28'S; 71°31'W), 11-03-2005, M. Romero-Mieres.

<sup>3</sup>CHILE, Región de la Araucanía, Provincia de Cautín, Temuco, Monumento Natural Cerro Ñielol, altitude 160 m, (38°75'S; 72°63'W), 06-05-2005, M. Romero-Mieres.

The results of chromosome count for species of each genus are described as follow:

*Adiantum* L. (Adiantaceae): A tetraploid number 2n = 116 was described for *Adiantum chilense* Kaulf. var. *chilense*, and its base number x = 29 is similar to that documented for *A. pedatum* L. (Hafler & Soltis 1986). The chromosome numbers described for these two species are the sole recorded for the genus.

*Asplenium* L. (Aspleniaceae): The tetraploidy documented previously for species of *Asplenium* (Herrero *et al.* 2001, Van den Heede *et al.* 2004) was also found for *A. dareoides* Desv. (2n = 144). The base number for the genus is x = 36. Inter-specific

variations in chromosome numbers in this genus have been compiled by Dawson *et al.* (2000) for New Zealand taxa, whose larger value was recorded for a hexaploid cytotype of *A. trichomanes* L. (2n = 216).

*Blechnum* L. (Blechnaceae): The four species analysed showed a diploid chromosome number 2n = 66, which is similar to the described for fifteen New Zealand species. Besides, a high variation in 2n numbers has been compiled by Dawson *et al.* (2000), with 2n values from 56 chromosomes for three species (x = 28) to 132 for a tetraploid accession of *B. fluviatile* (R.Br.) Salomon (x = 33). However, the most frequent base number for the genus is x = 33.

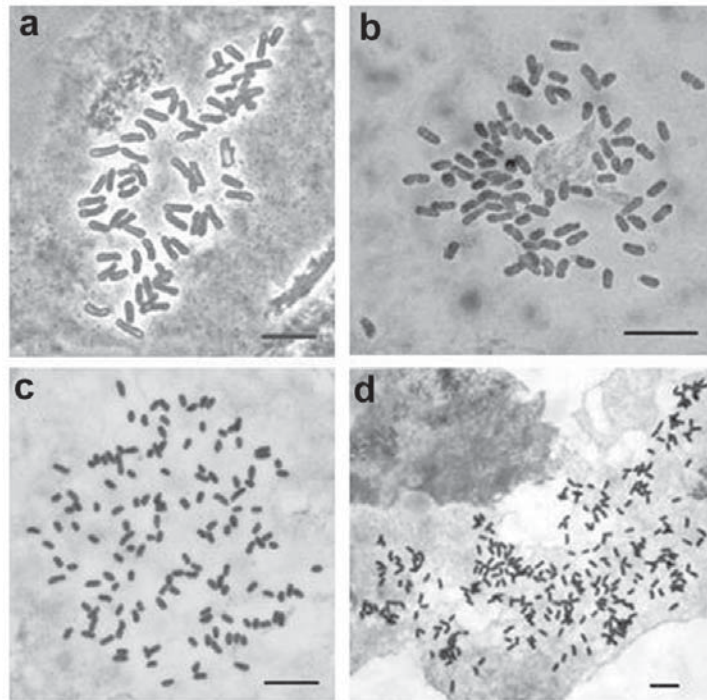


FIGURE 1. Mitotic metaphases of (a) *Blechnum mochaenum* var. *mochaenum*  $2n = 66$ , (b) *Megalastrum spectabile* var. *spectabile*  $2n = 82$ , c) *Asplenium dareoides*  $2n = 144$ , and d) *Polystichum subintegerrimum*  $2n = ca. 311$ . Bar = 10  $\mu\text{m}$ .

FIGURA 1. Metafases mitóticas de (a) *Blechnum mochaenum* var. *mochaenum*  $2n = 66$ , (b) *Megalastrum spectabile* var. *spectabile*  $2n = 82$ , c) *Asplenium dareoides*  $2n = 144$  y d) *Polystichum subintegerrimum*  $2n = ca. 311$ . Barra = 10  $\mu\text{m}$ .

*Equisetum* L. (Equisetaceae): The diploid chromosome number of *Equisetum bogotense* Kunth. ( $2n = 216$ ) was similar to that described for others species of the Subgenus *Equisetum* (Obermayer *et al.* 2002). Additionally, all species of this Subgenus checked to date are uniform with a high base number  $x = 108$ , which is unusual within the pteridophyta. This base number is also presents in hybrid species of the Subgenus *Hyppochaete* (J.Milde) Baker which were found to be triploid with  $2n = 324$  chromosomes (Bennert *et al.* 2005).

*Megalastrum* Holtt. (Dryopteridaceae): The diploid chromosome number  $2n = 82$  described for *Megalastrum spectabile* (Kaulf.) A.R.Sm. et R.C.Moran was similar to that documented previously for its sister genus *Dryopteris* Adans. (Widén *et al.* 2001). The base number for these two genera is  $x = 41$ .

*Polystichum* Roth (Dryopteridaceae): Meta-phases with  $2n$  numbers from *ca.* 311 to 328

chromosomes were observed in *Polystichum subintegerrimum* (Hook. et Arn.) R.A.Rodr. This high  $2n$  number is nearby to that described previously for the octoploid *Polystichum neozelandicum* Fée, with  $n = ca. 164$  bivalents in diakinesis ( $2n = ca. 328$ ) (Perrie *et al.* 2003). Furthermore, Perrie *et al.* (2003) pointed out that others species have been described as tetraploid with  $2n = ca. 164$  chromosomes. The base number for the genus is  $x = 41$ . The remarkable similitude in the base number  $x = 41$  among *Megalastrum*, *Dryopteris* and *Polystichum*, suggest a constancy of base chromosome number in the family Dryopteridaceae.

The high chromosome number described for nine Chilean species of pteridophytes is a typical character for homosporous, whose lowest record is  $x = 27$  (Windham & Yatskievych 2003). On the other hand, the allopolyploidy described in many species (Herrero *et al.* 2001, Van den Heede *et al.* 2004, Perrie *et al.* 2003) together with the different diploid numbers

reported, may be evidence of a high genome diversity in these vascular plants. However, with aprox. 116 species of pteridophytes recognized for continental Chile, much remains to be done. Further studies in others Chilean families will be reported in the future, thus increasing the information on chromosome numbers in homosporous pteridophytes.

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