Chromosome number of two Chilean species of *Nothofagus* (Nothofagaceae)

Número cromosómico de dos especies chilenas de *Nothofagus* (Nothofagaceae)

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RESUMEN

Se documenta un número cromosómico 2n = 26 (x = 13) para *Nothofagus dombeyi* (Mirb.) Oerst. y *N. glauca* (Phil.) Krasser, el cual es similar al descrito previamente para otras especies del género. Se incrementan a 13 las especies de *Nothofagus* con número cromosómico conocido, existiendo dos números básicos (x = 13, 14).

Nothofagus Blume (Nothofagaceae) is a representative genus of the Gondwanean flora that includes 35 species of trans-antarctic and disjunct distribution, which is subdivided into four subgenera (Hill & Read 1991, Hill & Jordan 1993, Manos 1997). In South America the species of *Nothofagus* occur in Southern Chile and Argentina, whereas in Oceania, they occur in New Zealand, Tasmania, East Australia, New Caledonia and New Guinea. In Chile, nine species and one hybrid have been taxonomically recognized inhabiting from 33°S to 56°S, covering the Central Valley, and Andean and Nahuelbutan slopes (Manos 1997, Donoso *et al*. 2004). Systematic studies carried out in *Nothofagus* have analyzed various characters of extinct species and fossils (Jordan & Hill 1999). In this way, the phylogenetic framework of *Nothofagus* is abundant, including morphological and/or molecular analysis (Hill & Jordan 1993, Martin & Dowd 1993, Manos 1997, Setogushi *et al*. 1997, Jordan & Hill 1999, Premoli *et al*. 2012). However, despite these advances, some aspects on the evolution of *Nothofagus* remain obscure, for example, the mechanism on chromosome evolution related with the origin of the genus and species differentiation. On this respect, chromosome numbers and/or qualitative karyotype studies have been previously reported for 11 species of *Nothofagus* (30.5% of the species of the genus), seven of them inhabit various countries from Oceania (Armstrong & Wylie 1965, Wardle 1967, Ono 1977, Carr & McPherson 1986, Oginuma *et al*. 1998, Wiltshire & Jackson 2003), and four occurring in Chile (Ono 1977) (Table I). The most frequent chromosome number described within the genus is 2n = 26 (Ono 1977, Dawson 2000), although one species from Oceania has been reported having a diploid number of 2n = 28 (*N. cunninghamii* (Hook.) Oerst.; Wiltshire & Jackson 2003). Interestingly, all four Chilean species studied so far show a diploid chromosome number 2n = 26. However, it is possible that both 2n numbers (26 or 28) can be present in other species distributed along Chile. To increase the cytogenetic data of *Nothofagus*, in this study the chromosome number of *N. dombeyi* (Mirb.) Oerst. and *N. glauca* (Phil.) Krasser are reported for the first time.

Young plants of *N. dombeyi* and *N. glauca* were obtained from CONAF Nursery, Chile, Región de La Araucanía, Provincia de Cautín, km 28 Temuco-Nueva Imperial road. The voucher specimens were deposited in the Herbarium of the Universidad Católica de Temuco. To obtain mitotic metaphases, the protocol described by Ono (1977) was followed. Root tips were cut in the morning and pretreated with a 2 mM solution of 8-Hydroxiquinoline for 3 h at a temperature of 18°C. After fixation with 45% acetic acid during 0.5-2 h, the material was hydrolyzed with a mixed solution of 1N HCl and 45% acetic acid (1:1 v/v) at 60 °C during 10 min and then stained with acetic orceine 2% by 24 h. The mitotic metaphases were obtained by squashing the root tips and photographed with a digital camera Olympus C-5050 connected to an Olympus microscope CX31. Three plants per each species were examined and the chromosome counts were made in ten metaphases of each of them.

The results of the chromosome count show for *N. dombeyi* and *N. glauca* a number 2n = 26 (Fig. 1a, 1b). The basic number, x = 13, is the most frequent within this genus, being present in 12 species, which is additional to

number x = 14 present in *N. cunninghamii* (Hook.) Oerst. from Tasmania (Armstrong & Wylie 1965, Wardle 1967, Ono 1977, Dawson 2000, Wiltshire & Jackson 2003). These results increase to 13 the species of *Nothofagus* with available chromosome data (36% of the genus), which represent all four subgenera proposed by Hill & Read (1991) (e.g., *Lophozonia*, *Fucospora, Nothofagus,* and *Brassospora*).

In an evolutionary context, Ono (1977) compiled the chromosome data available until then for *Nothofagus*, and resumed some interpretations on possible ancestors. Thus, one hypothesis suggested *Nothofagus* as a specialized offshoot of *Fagus* (Fagaceae) whose basic number is x = 12 chromosomes (Harris 1956, Hair 1966). Other alternative hypothesis suggest *Nothofagus* as a reduced form of an ancestral type with x = 14 chromosomes (Armstrong &

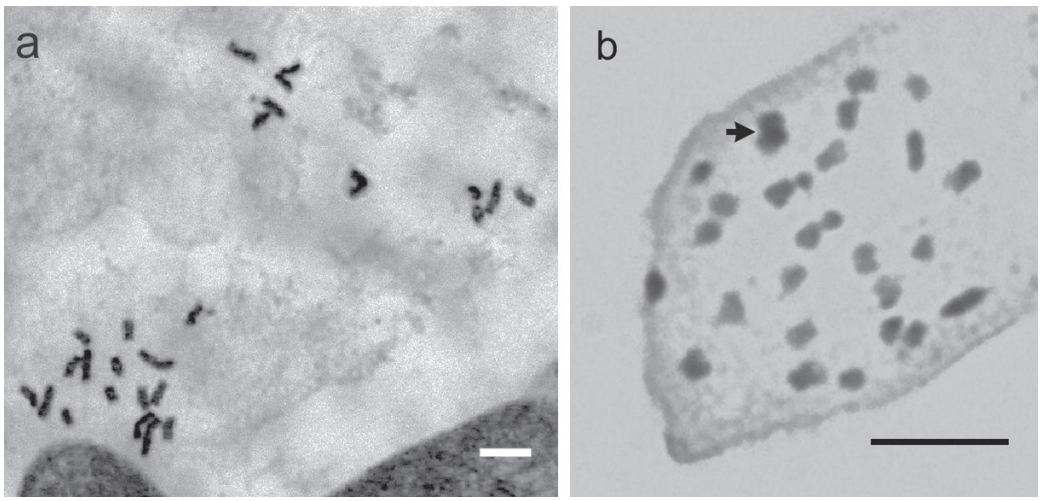

Figure 1. Mitotic metaphases of: a) *N. dombeyi,* and b) *N. glauca*. 2n = 26. Arrow shows two super-imposed chromosomes. Bar = 5 µm.

Figura 1. Metafases mitóticas de: a) *N. dombeyi* y b) *N. glauca*. 2n=26. La flecha muestra dos cromosomas superpuestos. Barra = 5 µm.

Table I. Chromosome number and collection sites of *Nothofagus* species studied so far. The sub-generic classification was based on Hill & Read (1991).

Tabla I. Número cromosómico y sitios de colecta de especies de *Nothofagus* estudiadas a la fecha. La clasificación subgenérica se basa en Hill & Read (1991).

Subgenus / Species	Collection sites	2n	Reference
Lophozonia			
Nothofagus alpina (Poepp. &Endl.) Oerst.	Provincia de Ñuble, Chile	26	Ono (1977)
N. cunninghamii (Hook.) Oerst.	Tasmania	28	Wiltshire & Jackson (2003)
N. glauca (Phil.) Krasser	Temuco, Nursery specimens, Chile	26	Present study
N. menziesii (Hook.) Oerst.	New Zealand	26	Armstrong & Wylie (1965)
N. obliqua (Mirb.) Oerst.	Temuco, Provincia de Cautín, Chile	26	Ono (1977)
Fucospora			
N. fusca (Hook.) Oerst.	New Zealand	26	Armstrong & Wylie (1965)
N. solandri (Hook.) Oerst.	New Zealand	26	Armstrong & Wylie (1965)
N. truncata (Col.) Ckn.	New Zealand	26	NZPCN (2013)
Nothofagus			
N. antarctica (Forst) Oerst.	Antillanca, Provincia de Llanquihue, Chile	26	Ono (1977)
N. dombeyi (Mirb.) Oerst.	Temuco, Nursery specimens, Chile	26	Present study
N. pumilio (Poepp. & Endl.) Krasser.	Antillanca, Provincia de Llanquihue, Chile	26	Ono (1977)
Brassospora			
N. discoidea (Baum.-Bod.) Steenis	New Caledonia	26	Carr & McPherson (1986)
N. grandis Steenis	New Guinea	26	Oginuma *et al*. (1998)

Wylie 1965). On the basis of multiple lines of evidence compiled in later decades the first hypothesis was discard (Hill & Jordan 1993, Manos & Stanford 2001). Regarding the second hypothesis, it may be supported by the current cytogenetic evidence available for *Nothofagus* which shows that one species has 28 chromosomes (*N. cunninghamii* with x = 14), being included within the subgenus *Lophozonia* together with other species having 26 chromosomes (Dawson 2000, Wiltshire & Jackson 2003). Thus, the existence of an ancestor with x = 14 chromosomes is possible, which may be closest to *N. cunninghamii*. Interestingly, this observation is consistent with the topologies described by Setogushi *et al*. (1997) as revealed by molecular phylogenies, hypothesizing that during the evolution of *Nothofagus* the subgenus *Lophozonia* derived first, followed later by *Fucospora*, *Nothofagus* and *Brassospora*, respectively. Then, based on these arguments, it is possible that the subgenus *Lophozonia* retain most ancestral characters, as was described in the male inflorescence (Hill & Jordan 1993), and in the basic number x = 14 present in *N. cunninghami*. Nevertheless, this partial cytogenetic evidence available for *Nothofagus* based upon the chromosome number should be complemented with comparative studies on karyotype morphology including species of the four subgenera. Thus, the hypothesis on reduction of the chromosome number proposed to explain the origin of *Nothofagus* may be robustly tested, and interpreted in a cytogeographic context to shed light on the studies that suggest South America as the most likely ancestral area for the genus, followed by New Zealand as the second most plausible source area (Swenson *et al*. 2000).

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