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Conifer genome sizes of 172 species, covering 64 of 67 genera, range from 8 to 72 picogram

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Nuclear genome size of conifers as measured by flow cytometry with propidium iodide was investigated, striving to collect at least a single species from each genus. 64 out of 67 genera and 172 species were measured. Of the 67 genera, 21 are reported here for the first time and the same is true for 76 species. This nearly doubles the number of measured genera and adds 50% to the number of analyzed species. Conifers have chromosome numbers in the range of n = (7)10–12(19). However, the nuclear DNA content (2C-value) is shown here to range from 8.3 to 71.6 picogram. The largest genome contains roughly 6×10^{10} more base pairs than the smallest genome. Genome sizes are evaluated and compared with available taxonomic treatments. For the mainly (sub)tropical Podocarpaceae small genome sizes were found with a 2C-value of only 8–28 pg, with 13.5 pg on average. For the Taxaceae 2C-values from 23–60 pg were determined. Not surprisingly, the genus *Pinus* with 97 species (39 species measured here) has a broad range with 2C = 38–72 pg. A factor of 2 difference is also found in the Cupressaceae (136 species) with nuclear DNA contents in the range 18–35 pg. Apart from the allohexaploid *Sequoia*, ploidy plays a role only in *Juniperus* and some new polyploids are found. The data on genome size support conclusions on phylogenetic relationships obtained by DNA sequencing. Flow cytometry is applicable even to young plants or seeds for the monitoring of trade in endangered species.

Extant conifers are relict populations of once more widely distributed lineages (Hill 1995). Of the 67 extant genera, 28 have only a single species and a further 11 genera have only two or three species.

Conifers are economically very important. They are not only the sources of timber but also of wood pulp, resins, and edible seeds especially in the genus *Pinus* L. (Zonneveld 2012). Moreover, several deviating forms, especially the dwarf ones, are the mainstay of gardening, providing color and form year-round. Conifers differ from most green plants in that chloroplast are inherited via pollen. Furthermore, in Cupressaceae also mitochondria are inherited via pollen, whereas in Pinaceae they are inherited from the seed parent.

The conifers were divided by Pilger (1926) in seven families, and this treatemt was accepted for a long time. However, in 1976, Eckenwalder argued that the Taxodiaceae should be merged with Cupressaceae and that *Sciadopitys* Siebold and Zucc. should be placed in a separate family. Recently, two valuable books on all conifers appeared: that of Eckenwalder (2009) and that of Farjon (2010). The main differences are at species level: Eckenwalder recognizes 546 species in 67 genera and 6 families, wheras Farjon is more liberal and has 615 species in 70 genera and 8 families.

Farjon recognizes Cephalotaxaceae (Eckenwalder includes them in the Taxaceae) and Phyllocladaceae (Eckenwalder places the only genus *Phyllocladus* Rich. ex Mirb. in the Podocarpaceae). The three 'extra' genera of Farjon are: *Pilgerodendron* Florin (Eckenwalder includes this in *Libocedrus* Endl.); *Xanthocyparis* Farjon and Hiep. (Eckenwalder considers this as *Cupressus* L.) and *Sundacarpus* (Buchholz and Gray) Page that is included by Eckenwalder in *Prumnopitys* Phil. The more conservative approach of Eckenwalder is largely followed here, but the three extra genera of Farjon were also investigated.

The classical taxonomic traits based on morphological characters and geographical distribution and the extensive molecular data (Stefanovic et al. 1998, Cheng et al. 2000, Rai et al. 2008, Xiang et al. 2009) are here supplemented with data on nuclear DNA content. Nuclear DNA content is a specific karyological feature that can be very useful for systematic purposes and evolutionary considerations (Bennett and Leitch 1995). Genome size is essential at the start of whole-genome-sequencing and could be the first step when introducing bar-coding. Nuclear DNA content in e.g. *Capsicum* contributed to their taxonomic grouping and supported previous conclusions on systematic affinities (Moscone et al. 2003).

1 picogram amounts to 10^9 base pairs and, depending on the size of the genome, to several thousand genes. Therefore, the conclusions and suggestions based on genome size and compared with available phylogenetic reconstruction data seem intrinsically more informative than a single morphological character.

Nuclear DNA content can conveniently be measured by flow cytometry using propidium iodide, a stoichiometric DNA stain that intercalates in the double helix. Where many species in a genus have the same chromosome number, differences in DNA 2C-value have proved to be very effective in delimiting infrageneric subdivisions in a number of taxa (Ohri 1998, Zonneveld 2004, 2008, 2009, 2012, Zonneveld and Duncan 2010, Zonneveld and Van lren 2001, Zonneveld et al. 2003). Greilhuber (2005) clearly showed that there is in general much less intraspecific variation of genome size than earlier expected.

The evolution of genome size has received increased attention during recent years. Primitive angiosperms supposedly had small genomes; increases up to a factor 1000 occurred independently in various modern taxa (Leitch et al. 1998). Flow cytometry was successfully used to measure the 2C-value for several genera like *Sesleria* (Lysak and Doležel 1998), *Lactuca* (Koopman 2000), roses (Yokoya et al. 2000), mosses (Voglmayr 2000), *Petunia* (Mishaba et al. 2000), Taxodiaceae (Hizume et al. 2001), Pinaceae (Joyner et al. 2001, Grotkopp et al. 2004), *Capsicum* (Moscone et al. 2003), *Hosta*, *Helleborus*, *Galanthus*, *Narcissus*, *Tulipa* and *Eucomis* (Zonneveld 2004, 2008, 2009, 2012, Zonneveld and Duncan 2010, Zonneveld and Van lren 2001, Zonneveld et al. 2003). Genome sizes of 176 species of conifers have been measured previously (Murray et al. 2010) of which 90 are *Pinus* species. In this paper, genome sizes of 172 different species of which 76 were not measured previously, and 64 out of 67 genera of which 21 were not determined before, were measured to show differences between and within genera of conifers.

Material and methods

Plant material

Plant material was obtained from the collections of Kirstenbosch Botanical Garden, RSA; Anthoine Pinetum, Belgium; Botanical Garden of Bochum, Germany; Arboretum Trompenburg, Pinetum Blijdenstein, Botanical Garden of Leiden, from Pharmacognosy, Leiden and from plant nurseries, all from the Netherlands. Where possible, material of known origin was used, and care was taken to ensure correct identification of all material. Plants are maintained as a living collection in the above arboreta and botanical gardens.

Flow cytometric measurement of DNA 2C-value

Conifer needles are relatively difficult to chop. So, if possible, young leaves or buds were used to isolate nuclei. These were chopped together with a piece of *Agave americana* L. 'Aureomarginata' as an internal standard (below). With the Podocarpaceae *Agave attenuata* Salm (2C = 8.1 pg) was used as the genome sizes of the samples often coincided more or less with *A. americana*. The chopping was done with a new razor blade in a Petri dish in per 20 ml 8 mg RNAse, 0.4 ml Dithiothreitol (1 g/6.5 ml) and 20 ml Polyvinyl pyrolidone (PVP10; 50 g/l) was added (Zonneveld and Van Iren 2001). After adding 1.75 ml propidium iodide solution (50 mg PI l^{-1} in isolation buffer) the suspension with nuclei was filtered through a 30 μm nylon filter. The fluorescence of the nuclei was measured 30 min and 1 h after addition of propidium iodide, using a Partec CA-II flow cytometer. The optical path contained a HBO mercury lamp, filters KG1, BG12, dichroic mirror TK500, filter OG570 and a Leitz 50 × 1 water immersion objective. Data were analyzed by means of DPAC software. The 2C DNA content of the sample was calculated as the sample peak mean, divided by the *Agave* peak mean, and multiplied with the amount of DNA of the *Agave* standard. Usually two different samples, with each at least 5000 nuclei, were measured twice for each accession. Most histograms revealed a coefficient of variation (COV) of less than 5%. For better comparison with published values for *Pinus* in Table 3, these values were recalculated. Grotkopp et al. (2004) used 11.12 instead of 10.0 for their standard *Hordeum vulgare* L. and their results are multiplied by 0.93. Joyner et al. (2001) used 8.22 instead of 8.6 pg for *Pisum sativum* L. and their results are multiplied by 1.1.

It was impossible to compare our data with the other 86 species of the Kew list (Murray et al. 2010) as these only partly overlapped with the present results, came from a large number of different authors, were measured by different methods and with (or without) different internal standards. The present data will be sent to the Kew data base.

Internal standard and absolute DNA content values

When measuring nuclear DNA content by means of flow cytometry, it is necessary to chop tissue from the plant of interest together with an internal standard. This standard must be as close as possible to the plants of interest. In this way, variation in signal intensities due to staining kinetics, to light absorption and quenching by sample components, as well as to instrument and other variables, is reduced to a minimum. *Agave americana* and *A. attenuata* were chosen as internal standard for conifers. They are available year-round, keep fresh several weeks without water and, being large plants, a single specimen can serve a lifetime, thereby further reducing variation in readings. They also have a low background in propidium iodide measurements, and show a single G_0 peak, almost lacking G_2 arrest. Fresh male human leucocytes (2C = 7.0 pg; (1 picogram = 10^{-12} gram = 0.978×10^9 base pairs (Doležel et al. 2003)) were chosen as primary standard (Tiersch et al. 1989). This yields 2C = 15.9 pg for nuclei of *Agave americana* L. Based on a published male human genome size of 6.294×10^9 base pairs, the nucleus was calculated as containing 6.436 pg (Doležel et al. 2003). However this is based on a human sequence where the size of the very large repeat sequences could not accurately be determined. So the true genome size may be closer to 7 pg than now envisioned.

Results and discussion

General

Nuclear DNA content is a specific karyological feature that is very useful for systematic purposes and evolutionary considerations (Bennett and Leitch 1995). Still, genome size does not give direct clues about evolution or relationships.

Similar genome sizes might be just coincidence, but significantly different genome sizes are often indicative of differentiation into separate species and/or different ploidy levels. Moreover, its correlation here with the phylogenies proposed by others based on molecular data is compelling, suggesting that related species often have similar DNA contents.

A C-value is considered deviant if it falls outside the range of the values for the other species. Nuclear DNA content (2C-value) or genome size (= 1C-value) was measured in 224 accessions with in total 172 different species of conifers. Genome size of 64 out of the 67 genera were determined and 21 of the genera were measured for the first time. The three monotypic genera *Austrotaxus* R. H. Compton, *Nototsuga* Hu ex Page and *Parasitaxus* de Laub. were not available for this study. Genome size as investigated here (Table 1–3), complements the work based on morphological characters and the extensive molecular data (Stefanovic et al. 1998, Cheng et al. 2000, Rai et al. 2008, Xiang et al. 2009). In several cases genome sizes of conifer genera, related according to their DNA sequences, differ up to a factor 2 or 3. Such variability is often found within genera (Zonneveld 2004) let alone between genera. This level of differentiation may be explained by the old age and relictual nature of extant conifer lineages.

The species are alphabetically arranged in Table 1. Species in bold are measured for the first time in the current study. As explained above, apart from *Pinus* (Table 3), our data could not be compared meaningfully with the genome sizes of the other 86 species collected by Murray et al. (2010). In Table 2 the genera are arranged according to their systematic classification (Eckenwalder 2009) with the average of their nuclear DNA content in whole picograms. This shows the relationship between the genome sizes found for the different families and subfamilies. Being the largest genus here represented by 39 species, a separate summary is given of the genome sizes of *Pinus* L., arranged according to a subdivision in 4 sections and 11 subsections (Table 3). These genome sizes are compared with the values recalculated from Joyner et al. (2001) and Grotkopp et al. (2004).

The families of conifers

Araucariaceae

With three genera and 35 species Araucariaceae are one of the smaller families. The genome size (2C = 28.4 pg) of the recently discovered *Wolllemia nobilis* W. G. Jones et al. is close to *Agathis dammara* (Lamb.) Rich. & A. Rich. with 27.2 pg and less so to *Araucaria araucana* (Molina) K. Koch with 45.4 pg. This seems to corroborate the molecular phylogeny (Gilmore and Hill 1997, Quinn et al. 2002, Rai et al. 2008) that suggests *Wollemia* to be the sistergroup of *Agathis.*

Cephalotaxaceae, now Taxaceae

The only genus and species in this family is firmly placed in the Taxaceae (Cheng et al. 2000, Quinn et al. 2002, Rai et al. 2008). *Cephalotaxus* is only loosely related to the other genera in the Taxaceae, but the genome size of *Cephalotaxus harringtonii* (Knight ex J. Forbes) K. Koch is with 51.6 pg comparable to some of the other species of Taxaceae.

Cupressaceae

Cupressaceae are the only cosmopolitan family of conifers with 28 genera and 136 species. From all genera at least one species was measured here. If the 28 genera are split between plants from the Northern and Southern Hemisphere, there is hardly any difference in the mean value of their nuclear DNA content: 24.1 vs 23.8 pg.

This second largest family includes the former Taxodiaceae of which Eckenwalder (1976) did show that they, apart from *Sciadopitys*, were not separable from the Cupressaceae.

Cupressus L. is with 17 species the second largest genus within the Cupressacae. The genome sizes for the 3 measured species vary between 21.8 and 22.8 pg. The similar value 22.9 pg was found for *Cupressus* (*Xanthocyparis*) *nootkatensis* D. Don in A. Lambert. So its inclusion by Gadek et al. (2000) in *Cupressus* is endorsed by similar genome size.

Despite the identical chromosome number n = 11 (Khoshoo 1961) (excluding the polyploids), the amount of DNA in the Cupressaceae varies between 18 and 34 pg (average 22.6 pg) with 35.0 pg for the tetraploid *Fitzroya cupressoides* (G. Moline) I. Jonhston (Hair 1968). DNA studies indicate that the Patagonian *Fitzroya* is closest to the Tasmanian *Diselma archeri* J. Hooker with 18.1 pg and the African *Widdringtonia* Endl. with on average 20.3 pg (Eckenwalder 2009).

Calocedrus Kurz contains three species of which two were measured: *C. formosana* (Florin) Florin with 34.0 pg and *C. decurrens* (Torrey) Florin with 30.5 pg. However DNA evidence (Gadek et al. 2000) indicates that *Calocedrus* is related to the Northern Hemisphere *Platycladus orientalis* (L.) Franco (20.3 pg) and *Microbiota decussata* V. Komarov (18.3 pg). *Calocedrus* was originally placed with the Southern Hemisphere *Libocedrus* Endl. with a more similar genome size of 28.1 pg.

Chamaecyparis Spach with 5 species, all measured, varies in genome size between 18 and 21 pg. According to DNA research of Gadek et al. (2000) they are not very closely related to *Cupressus* (23 pg). Its closest relative is the monotypic *Fokienia hodginsii* (S. Dunn) A. Henri & A. Thomas with 22.2 pg. *Actinostrobus pyramidalis* Miquel (21.3 pg) is sister to *Neocallitropsis pancheri* (Carriere) de Laub. (25.5 pg) which is closely related to *Callitris rhomboidea* R. Brown ex Richard (18.0 pg), the latter two occurring in Australia and New Caledonia (Gadek et al. 2000).

The monotypic *Tetraclinis articulata* (Vahl) Mast. (25.7 pg) was originally thought to be related to *Widdringtonia* (21 pg). DNA evidence (Gadek et al. 2000) points to a closer relationship to *Microbiota decussata* V. Komarov (18.3 pg) and *Platycladus orientalis* (L.) Franco (20.3 pg).

All five species of *Thuja* L. were measured, showing a genome size close to 23 pg on average. Most closely related (Gadek et al. 2000) to *Thuja* is *Thujopsis dolabrata* (Thunb. Ex L. f.) Siebold and Zucc. with 23.8 pg.

Table 1. Conifer taxa with their nuclear DNA content (2C) standard deviation and origin of samples analysed. In bold species that have not been measured before (others in Murray et al. 2010).

Species of conifers	pg DNA per nucleus	SD	Origin
Abies balsamea (L.) Mill. ‘Nana’	32.8	0.5	ex commerce
Abies cephalonica **Loudon**	36.2	0.2	Greece
Abies homolepis **Siebold & Zucc.**	39.7	0.3	BG Leiden
Abies koreana **E. H. Wilson**	36.5	0.4	BG Leiden
Abies nordmanniana **(Steven) Spach**	35.2	1.1	BG Leiden
Abies pinsappo **Boiss.**	38.1	0.8	S. Spain
Abies pinsappo **‘Glauca’**	37.8	0.4	ex commerce
Abies procera **Rehd. ‘Glauca’**	34.4	0.6	ex commerce
Acmopyle pancheri **(Brogniart & Gris) Pilger**	17.5	0.1	Pinetum Blijdenstein
Acmopyle sahniana **J. Buchholz**	13.8	0.3	Pinetum Blijdenstein
Actinostrobus pyramidalis Miquel	21.3	0.3	J. de Koning, Zeist
Afrocarpus falcatus **(Thunb.) C. N. Page**	11.6	0.7	BG Barcelona
Afrocarpus mannii **(J. Hooker) C. Page**	10.2	0.2	BG Bochum
Agathis dammara **(Lamb.) Rich. & A. Rich.**	27.2	0.1	BG Leiden
Amentotaxus yunnanensis **var.** ***formosana*** **(H. L. Li) Silba**	60.4	0.0	Pinetum Blijdenstein
Araucaria araucana **(Molina) K. Koch**	45.5	0.1	ex commerce
Athrotaxus cupressoides D. Don	20.2	0.3	Pinetum Blijdenstein
Athrotaxus selaginoides D. Don	20.1	1.2	Pinetum Blijdenstein
Athrotaxus × laxifolius **W. J. Hooker**	20.2	0.2	Pinetum Blijdenstein
Austrocedrus chilensis **(D. Son) Pichi Sermolli**	21.8	0.4	Pinetum Blijdenstein
Callitris rhomboidea R. Brown ex Richard	18.0	0.4	Pinetum Blijdenstein
Calocedrus decurrens (Torrey) Florin	30.5	0.4	Arb. Trompenburg
Calocedrus formosana **(Florin) Florin**	34.0	0.8	Pinetum Blijdenstein
Cathaya argyrophylla **Chun & Kuang**	49.5	0.6	Arb. Trompenburg
Cedrus deodara (Lamb.) G. Don	33.2	0.1	ex commerce
Cedrus libani ssp. *brevifolia* (J. Hooker) Meikle	31.7	2.0	ex commerce
Cedrus libani A. Richard ssp. *libani*	33.0	0.9	BG Leiden
Cedrus libani ssp. *atlantica* (Endl.) Bat. t & Trab. ‘Glauca’	31.1	1.1	ex commerce
Cedrus libani ssp. *atlantica* (Endl.) Bat. t & Trab. ‘Glauca’	32.4	1.2	ex commerce
Cephalotaxus harringtonii **(Knight) Koch ‘Drupacea’**	52.5	0.2	ex commerce
Cephalotaxus harringtonii **‘Fastigiata’**	50.7	1.7	ex commerce
Chamaecyparis obtusa (Siebold & Zucc.) Endl. ‘Nana gracilis’	18.6	0.3	ex commerce
Chamaecyparis formosensis **J. Matsumara**	17.1	0.3	Arb. Trompenburg
Chamaecyparis lawsoniana (A. Murray bis) Parlatore	21.6	0.7	BG Leiden
Chamaecyparis pisifera (Siebold & Zucc.) Endl.	18.5	0.8	BG Leiden
Chamaecyparis pisifera ‘Boulevard’	18.5	0.1	ex commerce
Chamaecyparis pisifera ‘Sacha’	18.4	0.0	ex commerce
Chamaecyparis thyoides **(L.) Britton, Sterns & Poggenb.**	20.8	0.6	ex commerce
Cryptomeria japonica (Thunb. ex L. f.) D. Don	20.6	0.4	BG Leiden
Cunninghamia konishii **Hayata**	25.5	0.4	J. de Koning, Zeist
Cunninghamia lanceolata (Lamb.) Hook	39.6	0.6	W. Snoeijer
Cunninghamia lanceolata (Lamb.) Hook	25.5	0.4	ex commerce
Cupressus lusitanica **P. Miller**	21.5	0.3	Costa Rica
Cupressus arizonica Greene	22.6	0.1	BG Jochumhof
Cupressus sempervirens L.	22.8	0.6	Spain
Cupressus × leylandii	23.7	1.1	ex commerce
Dacrycarpus imbricatus **(Blume) De Laubenfels**	10.7	0.5	Pinetum Blijdenstein
Dacrydium balansae **Brogniart & Gris**	14.1	0.2	BG Bochum
Dacrydium elatum **(Roxburgh) Wallich ex J. W. Hooker**	13.1	0.4	BG Bochum
Dacrydium gracile **de Laubenfels**	13.3	0.2	BG Bochum
Dacrydium naussoriense **de Laubenfels**	14.0	0.3	BG Bochum
Dacrydium nidulum **de Laubenfels**	13.9	0.4	BG Bochum
Diselma archeri **J. Hooker**	18.1	0.3	Pinetum Blijdenstein
Falcatifolium taxoides **(Brogniart & Gris) de Laub.**	22.4	0.4	Pinetum Blijdenstein
Fitzroya cupressoides **(G. Moline) I. Jonhston**	35.0	1.3	Trompernburg
Fokienia hodginsii (S. Dunn) A. Henri & A. Thomas	22.2	0.1	Pinetum Blijdenstein
Glyptostrobus pensilis (Staunton) K. Koch	18.1	0.6	Pinetum Blijdenstein

(Continued)

Table 1. (Continued).

Species of conifers	pg DNA per nucleus	SD	Origin
Halocarpus bidwillii (J. Hooker ex Kirk) Quinn	16.8	0.1	Pinetum Blijdenstein
Juniperus cedrus **Webb & Berthel.**	22.4	0.1	Spain, Tenerife
Juniperus chinensis **L.**	48.8	1.3	Japan, Hiroshima
Juniperus chinensis **‘Stricta Blue’**	50.2	0.9	ex commerce
J. chinensis **var.** ***procumbens*** **(Sieb.) Miq. ‘Nana’**	48.2	1.4	ex commerce
Juniperus communis ‘Compressa’	22.3	1.1	ex commerce
Juniperus communis **L.**	22.9	0.6	China, Bei ma Shan
Juniperus communis **var.** ***saxatilis*** **Pall.**	22.3	0	Suisse, Juf
Juniperus communis **‘Yellow’**	21.2	0.5	ex commerce
Juniperus foetidissima**? Willd.**	26.4	0.5	Greece
Juniperus horizontalis **Moench ‘Glauca’**	24.4	0.2	ex commerce
Juniperus horizontalis **‘Gold Carpet’**	24.0	0.9	ex commerce
Juniperus horizontalis **‘Wiltonii’**	23.5	0.8	ex commerce
Juniperus oxycedrus **L.**	23.4	0.2	Spain
Juniperus pingii **Cheng (as** ***squamata*****) ‘Loderi’**	44.5	0.8	ex commerce
Juniperus phoenicea **L.**	25.8	0.3	Portugal
Juniperus phoenicea **L.**	73.6	2.1	Greece, M. Olympus
Juniperus phoenicea **var.** ***canariense***	19.1	0.1	Sapin, Tenerife
Juniperus sabina **L. ‘Tamariscifolia’**	44.8	1.2	ex commerce
Juniperus scopulorum **C. Sargent**	22.6	0.3	ex commerce
Juniperus scopulorum **‘Blue Arrow’**	24.4	0.1	ex commerce
Juniperus squamata **Buch.-Ham ex D. Don**	24.1	0.4	China, Bai Ma Shan
Juniperus squamata **‘Meyeri’**	24.5	0.6	ex commerce
Juniperus virginiana L. ‘Sky Rocket’	23.9	0.3	ex commerce
Juniperus xpfitzeriana **(Spath) Schmidt ‘Gold Coast’**	45.5	0.9	ex commerce
Juniperus xpfitzeriana **(Spath) Schmidt ‘Mint Julep’**	35.4	0.7	ex commerce
Keteleeria evelyniana **Mast.**	48.4	2.1	BG Leiden
Lagarostrobos franklinii (J. Hooker) Quinn	10.1	0.1	Pinetum Blijdenstein
Larix gmelinii ssp. *olgensis* (A. Henry) Ostenf. & Syrach	25.7	0.4	Pinetum Blijdenstein
Larix decidua Mill. ‘Puli’	26.6	0.6	ex commerce
Larix decidua ‘Corley’	25.4	0.8	ex commerce
Larix griffithii **J. Hooker**	26.8	1.6	Pinetum Blijdenstein
Larix kaempferi **(Lamb.) Carriere**	26.4	1.3	ex commerce
Larix kaempferi **‘Blue Pearl’**	26.4	0.7	ex commerce
Larix kaempferi **‘Blue Rabbit’**	26.4	0.2	ex commerce
Larix kaempferi **‘Diana’**	27.2	0.4	ex commerce
Libocedrus uvifera **(D. Don) Pilger**	28.1	0.1	Pinetum Blijdenstein
Lepidothamnus fonkii **R. Philippi**	9.5	0.1	BG Bochum
Manoao colensoi (W. J. Hooker) Molloy	27.7	0.6	Pinetum Blijdenstein
Metasequoia glyptostroboides Hu & W. C. Cheng	19.3	0.4	Arb. Trompenburg
Microbiota decussata V. Komarov	18.3	0.0	ex commerce
Microcachrys tetragona **J. Hooker**	8.3	0.5	Pinetum Blijdenstein
Microstrobus fitzgeraldii **(Muller) Garden & Johnson**	8.6	0.1	Pinetum Blijdenstein
Microstrobus niphopheles **J. Garden & L. A. Johnson**	8.4	0.2	BG Bochum
Nageia nagi (Thunb.) Kuntze	11.2	0.4	Pinetum Blijdenstein
Neocallitropsis pancheri **(Carriere) de Laubenfels**	25.5	0.9	BG Bochum
Papuacedrus **var.** ***papuana*** **(F. J. Muller) H. L. Li ‘Arfak’**	23.7	0.4	BG Bochum
Papuacedrus **var.** ***papuana*** **(F. J. Muller) H. L. Li**	24.0	0.3	BG Bochum
Phyllocladus asplenifolius (Labillardiere) J. Hooker	15.9	0.3	Pinetum Blijdenstein
Phyllocladus trichomanoides D. Don	14.7	0.1	Pinetum Blijdenstein
Picea abies (L.) H. Karst.	40.7	1.2	ex commerce
Picea (abies?) ‘Glauca Conica’	40.7	0.8	ex commerce
Picea omorika (Pancic) Purk.	37.9	0.7	ex commerce
Picea pungens Engelm. ‘Glauca’	42.1	1.3	ex commerce
Pinus albicaulis Engelm.	64.6	1.3	Arb. Trompenburg
Pinus aristata Engelm.	56.9	1.4	Arb. Trompenburg
Pinus armandii Franchet	65.2	1.2	Arb. Trompenburg
Pinus balfouriana Balf.	48.2	2.3	Arb. Trompenburg

(Continued)

Table 1. (Continued).

Species of conifers	pg DNA per nucleus	SD	Origin
Pinus banksiana A. Lambert	45.5	0.6	Arb. Trompenburg
Pinus bungeana Zuccarini ex Endlicher	65.8	0.5	Pinetum Blijdenstein
Pinus canariensis R. Sweet ex K. Sprengel	64.3	0.8	Arb. Trompenburg
Pinus caribaea P. Morelet	45.3	100%	Manuel Antonio, C. Rica
Pinus cembra L.	59.9	0.3	Arb. Trompenburg
Pinus cembra L. 'Aurea'	63.2	0.1	Arb. Trompenburg
Pinus contorta D. Douglas ex J. C. Loudon 'Latifolia'	38.9	0.6	Pinetum Blijdenstein
Pinus culminicola var. *remota* (E. Little) Eckenwalder	58.4	1.6	Arb. Trompenburg
Pinus densiflora P. Siebold & Zuccarini	50.1	0.6	BG Bochum
Pinus edulis Engelmann	58.8	1.0	BG Bochum
Pinus flexilis E. James 'Pendula'	62.4	1.8	ex commerce
Pinus gerardiana N. Wallich ex D. Don	71.7	0.4	Arb. Trompenburg
Pinus halepensis Mill.	52.8	1.5	S. Spain
Pinus heldreichii H. Christ	60.5	2.4	Greece
Pinus koraiensis Siebold & Zuccarini	62.0	1.4	Arb. Trompenburg
***Pinus longaeva* D. K. Bailey**	52.5	0.7	Athoine
***Pinus massoniana* A. Lambert**	51.4	1.1	Arb. Anthoine
Pinus merkusii Jungh. & de Vriese	61.8	0.3	BG Leiden
Pinus monophylla J. Torrey & Fremont	63.3	0.9	Arb. Trompenburg
Pinus monticola Douglas ex D. Don	61.3	1.2	Arb. Trompenburg
Pinus mugo Turra	45.4	0.6	ex commerce
Pinus mugo 'Mops'	45.9	0.2	ex commerce
Pinus mugo 'Benjamin'	44.4	0.6	ex commerce
Pinus nigra J. F. Arnold (average of 20 accessions)	50.7	0.7	Arb. Trompenburg
Pinus nigra J. F. Arnold 'Frank'	51.4	0.6	Arb. Trompenburg
Pinus nigra J. F. Arnold 'Strypemonde'	50.2	0.8	Arb. Trompenburg
Pinus nigra J. F. Arnold 'Molette'	50.9	1.2	Arb. Trompenburg
Pinus nigra J. F. Arnold 'Black Prince'	50.0	1.6	Arb. Trompenburg
Pinus nigra J. F. Arnold 'Geant de Suisse'	49.7	1.3	Arb. Trompenburg
Pinus nigra J. F. Arnold 'Goldfingers'	51.4	1.1	Arb. Trompenburg
Pinus nigra J. F. Arnold 'Hornibrookiana'	51.3	0.5	Arb. Trompenburg
Pinus nigra ssp. *laricio* (Poir.) Maire 'Maritima'	51.6	1.5	Arb. Trompenburg
Pinus nigra ssp. *nigra*	51.3	1.3	Arb. Trompenburg
Pinus nigra ssp. *pallassiana* (Lamb.) Holboe var. *fastigiata*	50.2	0.6	Arb. Trompenburg
Pinus nigra ssp. *pallasiana* f. *seneceriana*	50.8	1.0	Arb. Trompenburg
Pinus nigra var. *cebennensis* 'Nana'	49.9	1.0	Arb. Trompenburg
Pinus parviflora Siebold & Zucc.	60.8	1.2	ex commerce
Pinus pinaster Aiton	57.8	1.6	Spain, Grazalema
Pinus pinea L.	57.2	1.5	Portugal
Pinus ponderosa Douglas ex C. Lawson var. *ponderosa*	44.6	0.8	Arb. Gimborn
Pinus pumila (P. Pallas) E. Regel	59.4	1.8	coll Japan
Pinus radiata D. Don	48.5	0.5	AGS seed
Pinus roxburghii C. Sargent	61.7	1.5	Pinetum Blijdenstein
***Pinus sibirica* Du Tour**	59.1	2.1	Arb. Anthoine
Pinus strobus L.	58.8	1.2	Arb. Trompenburg
Pinus strobus 'Radiata'	58.0	0.6	ex commerce
Pinus sylvestris L.	48.4	1.1	Arb. Trompenburg
Pinus taeda L.	44.3	0.5	Pinetum Blijdenstein
Pinus thunberghii Parlatore 'Maijita'	50.8	0.1	ex commerce
Pinus virginiana P. Miller	40.7	0.4	Pinetum Blijdenstein
Pinus wallichiana A. B. Jacks.	59.3	1.3	Pinetum Blijdenstein
Platycladus orientalis (L.) Franco	20.3	0.2	China, Peking
Platycladus orientalis 'Aurea Nana'	20.4	0.4	ex commerce
***Podocarpus cunninghamii* Colenso**	16.9	1.4	ex commerce
***Podocarpus gnidioides*? Carriere 'Red Tip'**	17.3	0.8	ex commerce
***Podocarpus lawrencei* J. Hooker**	15.9	0.1	Pinetum Blijdenstein
***Podocarpus macrophyllus* (Thunb.) Sweet**	19.4	0.4	BG Leiden
Podocarpus nivalis Hook.	16.8	0.2	Arb. Trompenburg
***Prumnopitys amara* (Blume) de Laubenfels**	13.6	0.3	BG Bochum
***Prumnopitys ladei* (F. M. Bailey) de Laubenfels**	11.2	0.2	Pinetum Blijdenstein
***Pseudolarix amabilis* (J. Nelson) Rehd.**	52.2	1.7	ex commerce
***Pseudotaxus chienii* (W. C. Cheng) W. C. Cheng**	34.6	0.2	Pinetum Blijdenstein
Pseudotsuga menziesii (Mirb.) Franco	35.2	0.7	ex commerce
***Retrophyllum rospigliosii* (Pilger) C. Page**	11.8	0.0	Pinetum Blijdenstein

(Continued)

Table 1. (Continued).

Species of conifers	pg DNA per nucleus	SD	Origin
Saxegothaea conspicua **Lindley**	10.2	0.4	Pinetum Blijdenstein
Sciadopitys verticillata Siebold & Zuccarini	40.0	0.1	Arb. Trompenburg
Sciadopitys verticillata Siebold & Zucc. 'Yellow'	37.5	0.4	Arb. Trompenburg
Sequoia sempervirens (D. Don) Endl.	57.7	1.1	Arb. Trompenburg
Sequoiadendron giganteum (Lindl.) J. T. Buchholz	20.8	0.4	Arb. Trompenburg
Taiwania cryptomeroides Hayata	23.0	0.1	Pinetum Blijdenstein
Taiwania cryptomeroides Hayata 'Flousiana'	23.7	0.1	Pinetum Blijdenstein
Taxodium distichum (L.) L. Richard var. *distichum*	18.4	0.8	Arb. Trompenburg
Taxodium dist. var. *imbricarium* (Nuttal) H. Croom	18.3	0.9	Arb. Trompenburg
Taxodium mucronatum Tenore	18.1	0.1	Pinetum Blijdenstein
Taxus baccata L.	23.3	0.3	BG Leiden
Taxus baccata 'Amersfoort'?	22.3	0.1	ex commerce
Taxus baccata 'Fastigiata'	22.9	0.2	W. Snoeijer
Taxus baccata 'Standishii'	22.6	0.6	ex commerce
Taxus brevifolia Nuttal	22.9	0.5	W. Snoeijer
Taxus canadensis Marshall	23.2	0.6	W. Snoeijer
Taxus celebica (Wall.) Li	24.3	0.8	W. Snoeijer
Taxus cuspidata Siebold & Zucc. 'Nana'	22.9	0.7	W. Snoeijer
Taxus sumatrana (Miquel) de Laubenfels	22.3	0.8	BG Bochum
Taxus wallichiana Zucc.	23.7	0.3	W. Snoeijer
Tetraclinis articulata (Vahl) Mast.	25.7	0.3	Pinetum Blijdenstein
Thuja koraiensis **T. Nakai**	23.9	0.2	Pinetum Blijdenstein
Thuja occidentalis L. 'Smaragd'	23.2	0.2	ex commerce
Thuja plicata Donn ex D. Don	22.6	0.6	ex commerce
Thuja standishii (G. Gordon) Carriere	24.8	0.4	Pinetum Blijdenstein
Thuja sutchuensis **Franchet**	24.2	0.3	J.de Koning, Zeist
Thujopsis dolabrata (Thunb. ex L. f.) Siebold & Zucc.	23.8	0.7	ex commerce
Torreya californica **Torr.**	44.1	0.8	W. Snoeijer
Torreya nucifera (L.) Siebold & Zucc.	44.6	1.1	W. Snoeijer
Torreya taxifolia G. Arnott	43.2	2.1	Pinetum Blijdenstein
Tsuga × jeffreyi? (A. Henry) A. Henry 'Iron Spring'	34.1	0.8	Arb. Trompenburg
Tsuga canadensis (L.) Carriere	37.2	0.0	Arb. Trompenburg
Tsuga canadensis (L.) Carriere 'Jeddeloh'	38.6	1.3	ex commerce
Tsuga caroliniana Engelmann	41.9	0.3	Arb. Trompenburg
Tsuga chinensis (Franchet) E. Pritzel	41.7	0.6	Arb. Trompenburg
Tsuga heterophylla (Rafinesque) C. Sargent	34.4	1.4	Arb. Trompenburg
Tsuga mertensiana (Bongiard) Carriere	36.3	0.4	Arb. Trompenburg
Tsuga sieboldii Carriere	41.4	0.3	Arb. Trompenburg
Tsuga sieboldii 'Nana'	33.8	0.5	Arb. Trompenburg
Widdringtonia cedarbergensis **J. A. Marsh**	21.5	0.2	BG Kirstenbosch
Widdringtonia nodiflora **(L.) Powrie**	20.8	0.3	BG Kirstenbosch
Widdringtonia schwarzii (Marloth) Mast.	19.6	0.3	Pinetum Blijdenstein
Wollemia nobilis W. G. Jones et al.	28.4	0.1	BG Leiden
Xanthocyparis nootkatensis **(D. Don) Farjon & Harder**	22.8	0.1	Pinetum Blijdenstein
Xanthocyparis nootkatensis **'Pendula'**	23.0	0.4	ex commerce
Welwitschia mirabilis	13.1	0.3	BG Kirstenbosch
Ginkgo biloba	23.5	0.8	BG Leiden

With *Chamaecyperis*, *Thuja* is one of the genera having produced a large number of cultivars. As their juvenile leaves are very similar, non-fruiting cultivars are often difficult to assign even to genus. The difference in genome size, 18–21 pg for *Chamaecyparis* vs 22.6–24.8 pg for *Thuja* makes it possible to discriminate between them.

Juniperus L. is with 54 species by far the largest genus in the Cupressaceae. Nuclear DNA content (2C) varies between 21.2 and 25.8 pg for the diploid species measured. However, a *Juniperus* from Sapin, Tenerife had only 19.2 pg. It is supposed to be a variety of *J. phoenicea* L. that has 25.8 pg. This asks for further investigation of the juniperi of the Canarian Islands. The polyploid junipers are discussed below. *Sequoia sempervirens* (D. Don) Endl. with 57.7 pg is hexaploid (Khoshoo 1961) and has been shown to be an allohexaploid. Two values for nuclear DNA content for *Cunninghamia lanceolata* (Lamb.) Hook were found: 25.5 and 39.6 pg. The nuclear DNA content suggests that the latter is a triploid form.

Phyllocladaceae (also included in Podocarpaceae)

The genus *Phyllocladus* Rich. Ex Mirb. is placed within the Podocarpaceae by Conran et al. (2000) based on the

Table 2. Nuclear DNA content of 64 genera of the Coniferales placed on a schematic tree (Eckenwalder 2009). First time measured genera are printed in bold. n.d. = not determined.

		No. of spec. Farjon 2011	No. of spec. Eckenw. 2009	No. measured Zonneveld 2012	No. of chromosomes (n) Eck. 2009 Khoshoo 1961	Average DNA (2C) per genus
CONIFERALES						
Araucariaceae	3 genera; 35 species					
	Agathis	15	15	1	13	27
	Araucaria	19	19	1	13	45
	Wollemia	1	1	1	13	28
Cupressaceae	28 genera, 135 species					
Athrotaxoideae	*Athrotaxus*	3	2	3	11	20
Cunninghamioideae	*Cunninghamia*	2	2	2	11	25
Cupressoideae	***Actinostrobus***	3	3	1	11	21
	Austrocedrus	1	1	1	11	22
	Callitris	15	17	1	11	18
	Calocedrus	4	3	2	11	33
	Chamaecyparis	5	5	5	11	18–21
	Cupressus	15	17	3	11	23
	Diselma	1	1	1	11	18
	Fitzroya	1	1	1	22	35
	Fokienia	1	1	1	11	22
	Juniperus	53	54	14	11, 22, 33	19–76
	Libocedrus	5	6	1	11	28
	Microbiota	1	1	1	11	18
	Neocallitropsis	1	1	1	11	26
	Papuacedrus	3	1	1	11	24
	Platycladus	1	1	2	11	20
	Tetraclinis	1	1	1	11	27
	Thuja	5	5	5	11	23
	Thujopsis	1	1	1	11	24
	Widdringtonia	4	4	3	11	21
	Xanthocyparis (Cupr.)	2	2	1	11	23
Sequioideae	*Metasequoia*	1	1	1	11	19
	Sequoia	1	1	1	33	58
	Sequoiadendron	1	1	1	11	21
Taiwanioideae	*Taiwania*	1	1	2	11	23
Taxodioideae	*Cryptomeria*	1	1	1	11	21
	Glyptostrobus	1	1	1	11	18
	Taxodium	2	2	2	11	18
Pinaceae	11 genera, 195 species					
Pinoideae	***Cathaya***	1	1	1	12	50
	Picea	38	29	4	12	38–42
	Pinus	113	97	39	12	38–72
Laricoideae	*Larix*	11	10	5	12	27
	Pseudotsuga	4	4	1	13	35
Abietoideae	*Abies*	47	40	7	12	32–40
	Cedrus	3	2	3	12	34
	Keteleeria	3	2	1	12	48
	Nothotsuga	1	1	0	12	n.d.
	Pseudolarix	1	1	1	22	52
	Tsuga	9	8	6	12	33–41
Podocarpaceae	18 genera, 156 species					
	Acmopyle	2	2	2	10	14, 18
	Afrocarpus	5	2	2	12	12
	Dacrycarpus	9	9	2	10	11
	Dacrydium	22	21	5	10	13
	Falcatifolium	6	5	1	10	22
	Halocarpus	3	3	1	9, 11, 12	17
	Lagarostrobos	1	1	1	15	10
	Lepidothamnus	3	3	1	14, 15	10
	Manoao	1	1	1	10	28
	Microcachrys	1	1	1	15	8
	Nageia	5	5	1	10, 13	11
	Parasitaxus	1	1	0	18	n.d.
	Microstrobus	2	2	2	13	8–9
	Phyllocladus	4	5	2	---	15

(Continued)

Table 2. (Continued).

		No. of spec. Farjon 2011	No. of spec. Eckenw. 2009	No. measured Zonneveld 2012	No. of chromosomes (n) Eck. 2009 Khoshoo 1961	Average DNA (2C) per genus
	Podocarpus	97	82	4	10, 11, 17–19	14–17
	Prumnopitys	9	8	1	18,19	11
	Retrophyllum	5	4	1	10	12
	Saxegothaea	1	1	1	12	10
	Sundacarpus (Prumn.)	1	1	1	---	13
Sciadopityaceae	1 genus, 1 species					
	Sciadopitys	1	1	1	10	40
Taxaceae	6 genera, 23 species					
	Cephalotaxus	8	5	1	12	52
	Amentotaxus	6	2	1	7	60
	Torreya	6	6	2	12	45
	Austrotaxus	1	1	0	---	n.d.
	Pseudotaxus	1	1	1	12	35
	Taxus	10	8	5	12	23
GINKGOALES	1 genus, 1 species					
Ginkgoaceae	*Ginkgo*	1	1	1		24

chloroplast gene *rbc*L. However results of Sinclair et al. (2002), based on combined *trn*L-F and ITS2 sequences and of Quinn et al. (2002) based on *rbc*L and *mat*K data, place *Phyllocladus* basal to the Podocarpaceae. Farjon (2010) gives arguments to place/keep it in a separate family. When the genome sizes are compared, the values of 15.9 pg for *Phyllocladus asplenifolius* (Labillardiere) J. Hooker and 14.7 for *Phyllocladus trichomanoides* D. Son are similar to the average of the other Podocarpaceae (13 pg). Thus, the genome size alone does not support a separation from Podocarpaceae.

Podocarpaceae

Podocarpaceae are a large Southern Hemisphere family with 18 genera and 156 species of which *Podocarpus* has the highest number of species (82). According to DNA evidence (Conran et al. 2000, Quinn et al. 2002, Rai et al. 2008) Podocarpaceae are close to Araucariaceae. However the fossil record (Farjon 2010) points to a basal position of Podocarpaceae + Araucariaceae. A small genome size is usually considered as plesiomorphic (Moscone et al. 2003), although there are several exceptions (Doležel et al. 2007). The small genome sizes for the Podocarpaceae (average 13 pg) (but not for the Araucariaceae) might point to an ancient position for the Podocarpaceae. Alternatively, the strong competition in angiosperm-dominated forests might have led to a decrease in genome size for the Podocarpaceae. The nuclear DNA content for the Podocarpaceae ranges from 8 to 18 pg (n = 10–17) with two outliers: *Manoao colensoi* with 27.7 pg (n = 10) (closest relative *Lagarostrobus franklinii* with 10.1 pg, n = 15 and *Parasitaxus usta* n.d.) and *Falcatifolium taxoides* (n = 10) with 22.4 pg (closest relative *Dacrydium* with 13.1–14.1 pg, n = 10). These nuclear DNA contents show that there is no relation between the genome size and chromosome number and indicates a massive increase in the amount of DNA in *Manoao colensoi* and *Falcatifolium taxoides*. The size reported here for *Lagarostrobus franklinii* of 10.1 pg differs considerably from those of Davies (1996) and Davies et al. (1997) of 2C = 30.4 pg. This discrepancy could be due to wrong attribution, a wrong measurement/calculation or to polyploidisation.

Dacrycarpus with 9 species has for *Dacrycarpus imbricatus* (Blume) de Laub. 10.7 pg and is closely related to *Dacrydium* (27 species, average 13.7 pg) and *Falcatifolium taxoides* (Brogniart & Gris) de Laub. (5 species, average 22.4 pg). This means that *Falcatifolium* has nearly double the genome size of the species that are related based on DNA evidence.

Halocarpus bidwillii (J. Hooker ex Kirk) Quinn has a 2C = 16.8 pg. The three species of *Halocarpus*, all from New Zealand, are morphologically close but have each a different chromosome number n = 11, 12 and 13 (Eckenwalder 2009). They are related to *Lagarostrobos franklinii* (J. Hooker) Quinn (10.1 pg), *Manoao colensoi* (W. Hooker) Molloy 27.7 pg) and *Parasitaxus usta* (Vieillard) de Laub. (Sinclair et al. 2002).

Prumnopitys ladei (F. Bailey) de Laub. has a nuclear DNA content of 11.2 pg. DNA studies of Conran et al. (2000) and Sinclair et al. (2002) show that *Sundacarpus* (13.6 pg) is firmly embedded within *Prumnopitys.* The species of *Prumnopitys* are morphologically difficult to distinguish, but their genomic sizes could be used as a supplementary distinguishing character. Measuring their genome size might help. *Parasitaxus usta* (Vieill.) de Laub. is the only conifer that is supposed to be parasitic, but no material was available for this study.

DNA evidence (Conran et al. 2000, Sinclair et al. 2002) indicates that the only species of *Manoao*, *M. colensoi* (W. Hooker) Molloy with 27.7 pg is closely related to *Lagarostrobus franklinii* with 10.1 pg. Of the three species of *Lepidothamnus*, *L. fonkii* R. Philippi has only 9.5 pg. DNA studies (Quinn et al. 2002) shows it is loosely related to the other Podocarpaceae.

Microcachrys tetragona J. Hooker (8.3 pg) is closely related to *Microstrobus fitzgeraldii* (F. Muller) J. Garden and L. A. Johnson (8.6 pg) and *Microstrobus niphopheles* J. Garden and L. A. Johnson (8.4 pg) (Sinclair et al. 2002).

Table 3. Summary of nuclear DNA content (2C) of *Pinus* species, aligned with a classification of Eckenwalder (2009) and compared with data from Grotkopp et al. (2004) and Joyner et al. (2001).

		No. of sp.			Grotkopp et al. 2004	Joyner et al. 2001	Origin
SUBGENUS *Pinus*					×0.93	×1.1	
section *Pinus*	subsp. *Pinaster*	7	*P. halepensis*	52.8	57.8		south Spain
			P. pinea	57.2	54.7		Portugal
			P. pinaster	57.8	55.7		the Netherlands
			P. heldreichii	60.5	60.3		Greece
			P. canariensis	64.3	60.1		BG Barcelona
			P. roxburghii	61.7	63.5		Pin. Blijdenstein
			P. merkusii	61.8			BG Leiden
	subsp. *Pinus*	15	*P. sylvestris*	48.4		51.0	Arb. Trompenburg
			P. mugo	45.2		40.3	ex commerce
			P. nigra	50.7	48.5		Arb. Trompenburg
			P. thunbergii	50.8		50.4	ex commerce
			P. massoniana	51.4			Pin. Anthoine
			P. densiflora	50.1		48.0	Pin. Anthoine
section *Trifoliae*	subsp. *Contortae*	3	*P. banksiana*	45.5	39.6		Pin. Blijdenstein
			P. contorta latif.	38.9	39.8		Pin. Blijdenstein
			P. virginiana	40.7	40.3		Pin. Blijdenstein
	subsp. *Australes*	25	*P. radiata*	48.5	48.6		AGS seed
			P. taeda	44.3	44.2		Pin. Blijdenstein
			P. caribaea	45.3	44.6		Costa Rica
	subsp. *Ponderosae*	14	*P. ponderosa*	44.6	47.2		Gimborn Arb.
SUBGENUS *Strobus*							
section *Parraya*	subsp. *Balfourianae*	3	*P. balfouriana*	48.1	53.9		Arb. Trompenburg
			P. longaeva	52.5			Pin. Anthoine
			P. aristata	56.9	52.4		Arb. Trompenburg
	subsp. *Nelsoniae*	1	*P. nelsonii*	n.d.			
	subsp. *Cembroides*	9	*P. remota*	58.4		58.1	Arb. Trompenburg
			P. edulis	58.8	59.3		Arb. Trompenburg
			P. monophylla	63.3	60.8		Tiogz pas
section *Quinquefoliae*	subsp. *Gerardianae*	3	*P. bungeana*	65.8		65.1	Arb. Trompenburg
			P. gerardiana	71.7	67.8		Arb. Trompenburg
	subsp. *Krempfianae*	1	*P. krempfii*	n.d.			
	subsp. *Strobus*	17	*P. sibirica*	59.1			Pin. Anthoine
			P. cembra	59.9	57.5		Arb. Trompenburg
			P. pumila	59.4		60.3	coll Japan
			P. wallichiana	59.3	52.6		Arb. Trompenburg
			P. parviflora	60.8		61.8	ex commerce
			P. koraiensis	62.0	62.0		Arb. Trompenburg
			P. strobus	58.8	57.2		Arb. Trompenburg
			P. flexilis	62.4	58.4		ex commerce
			P. monticola	61.3		54.4	ex commerce
			P. albicaulis	64.6		59.8	Arb. Trompenburg
			P. armandii	65.2		64.6	Arb. Trompenburg
			P. ×*schwerinii*	59.1			Arb. Trompenburg

These creeping small-sized plants have similar small genome sizes, the smallest genome sizes measured so far for conifers.

The Southern Hemisphere *Podocarpus* L'Her. ex Pers. has with 82 species the largest number of species after the Northern Hemisphere *Pinus* with 97 species and both are widespread. However, whereas species of the genus *Pinus* forms large stands, species of the genus *Podocarpus* mainly grow scattered within forests dominated by broad leaved trees. *Podocarpus* has been divided in two sections by de Laubenfels (1985): section *Podocarpus* and section *Foliolatus*. The four species of section *Podocarpus* here measured have 15.9 to 17.3 pg whereas the only species here measured of section *Foliolatus* has 19.4 pg. *Saxagothaea conspicua* Lindley has a nuclear DNA content of 10.2 pg. DNA studies (Conran et al. 2000, Sinclair et al. 2002), are inconclusive with respect to its taxonomic position in the Podocarpaceae.

Sciadopityaceae

Sciadopitys verticillata Siebold & Zucc. with 40.0 pg is basal to Cupressaceae and Taxacae. Its 10 cm long 'needles' have been explained as short branches, the true leaves being reduced to small scales.

Taxaceae

The Taxaceae have 23 species and 6 genera if *Taxus* is included. *Austrotaxus* was not available for study. The inclusion of *Cephalotaxus* in Taxaceae as sister group to *Taxus* is only weakly supported (Rai et al. 2008). A distant relationship is likewise suggested by a genome size of 2C = 51.6 pg that is more than twice larger than those of *Taxus* (average 23 pg). The Taxaceae can be divided in three subfamilies with 1) *Cephalotaxus*, 2) *Amentotaxus*/*Torreya* and 3) *Austrotaxus*/*Pseudotaxus*/*Taxus* (Cheng et al. 2000). The five genera of the Taxaceae measured in this study vary from 23 pg in *Taxus* L. to 60 pg in *Amentotaxus*. For conifers, this is a rather wide range for such a small number of species, larger than for the 60 species in Cupressaceae measured so far. The number of accepted species of *Amentotaxus* varies from 2 (Eckenwalder 2009) to 6 species (Farjon 2010). The large genome size (60.4 pg) for *Amentotaxus formosana* (H. L. Li) Silba is remarkable as it is the only genus in this family with x = 7 instead of x = 12, suggesting a massive chromosome fusion and a strong increase in the amount of DNA. DNA studies of Cheng et al. (2000) show that *Amentotaxus* is most closely related to *Torrya* with 45 pg on average and *Cephalotaxus harringtonii* (Knight ex J. Forbes) K. Koch with 51.6 pg. The other genera within Taxaceae have lower amounts of DNA with *Taxus* with 23 pg on average, and *Pseudotaxus chienii* (W. C. Cheng) W. C. Cheng with 34.6 pg. Genome sizes within *Taxus* are very similar with, for the 6 'species' measured, between 22.3 and 24.2 pg. The different opinions on the number of species vary from 1 to 10 species.

Pinaceae

Pinaceae is a Northern Hemisphere family and is also the largest family with 11 genera and 195 species. In Table 3, they are divided in three subfamilies and 11 subsections (Eckenwalder 2009) of which 9 subsections were measured. In the subfamily Pinoideae three genera are included: *Picea* (29 species), *Pinus* (97 species) and the recently discovered *Cathaya* with 1 species. Some can become very old like *Pinus longaeva* DK Bailey living more than 4000 years. Its nuclear DNA content (52.3 pg) is not that different from the two other members of the subsection *Balfourianae*. From most of the species here reported for *Pinus*, the genome sizes have been measured earlier (Joyner et al. 2001, Grotkopp et al. 2004, Murray et al. 2010). For comparison with the data for *Pinus* (Table 3) their results have been recalculated (see Material and methods). It shows that these are mostly in line with the data presented here, in strong contrast to the values for all *Pinus* species as recorded in Murray et al. (2010) where *Pinus* varies from 0.2 (!) to 87 pg, both unlikely values. A similarly unrealistic range of values within species is presented also for *P. lambertiana* (21.2–87.7 pg).

Therefore, I have refrained from including in Table 3 other data than those of Joyner et al. (2001) and Grotkopp et al. (2004). The genus *Pinus* is traditionally divided in the subgenera *Pinus* and *Strobus* with in total 11 subsections (Table 3). If we look at the genome sizes (Table 3), the subgenus *Pinus* vary between 38 and 51 pg and the subgenus *Strobus* between 58 and 72 pg. However this is only true in both subgenera if one subsection is left out: subsection *Pinaster* (all 6 species) of subgenus *Pinus* vary between 53 and 64 pg and subsection *Balfouriana* (all three species) of subgenus *Strobus* vary between 48 and 56 pg. So looking at the DNA only, both seem better to fit in the other subgenus, but that is not proposed here as there seem to be no other arguments to do so. So it is better to say that subgenus *Pinus* vary between 39 and 51(64) pg and subgenus *Strobus* between (48)58 and 72 pg. However, further research may be warranted. *Pinus* vary between 38 and 72 pg and this seems to point to polyploidy. However, the high values are backed by published diploid chromosome counts (Grotkopp et al. 2004), there are many intermediate *Pinus* values and a range of a factor 2 for such a large genus has been found in several other diploid genera like *Helleborus* (Zonneveld 2001).

The monotypic *Cathya argyrophylla* Chun and Kuang (49.5 pg) seems morphologically most related to *Larix* Mill. (27 pg) and *Pseudotsuga* Carriere (35 pg). However, DNA evidence of Wang et al. (2000) points to a closer relationship of *Cathya* with *Picea* A. Dietr. (38–42 pg) and *Pinus* (38–76 pg). Genome size seems to support the latter placement.

Larix species have similar genome sizes between 25.7 and 26.4 pg for four of the ten species.

Pseudotsuga with four species is closely related to *Larix*, not to *Tsuga* (Endl.) Carriere according to Wang et al. (2000); *Tsuga* belongs to a different subfamily. *Pseudotsuga menziesii* (Mirb.) Franco (35.2 pg), the Douglas fir, is the economically most important tree in plantation forestry and can be found now throughout temperate climates. It is unusual among all other Pinaceae as it is the only species with x = 13 instead of x = 12 and it cannot be crossed with any of the other 3 pseudotsugas with x = 12. Moreover, it was the first conifer (later confirmed in other conifers) whereby it was shown that an inverted repeat was missing from the chloroplast that is present in all other green land plants except some members of the pea family (Eckenwalder 2009).

The subfamily Abetoideae has 6 genera and 54 species. The amount of DNA varies from 32 to 52 pg. *Abies* Mill. is the second largest family in the Pinaceae with 40 species. The high chromosome number for *Pseudolarix amabilis* (J. Nelson) Rehd. (n = 22) coupled with a high nuclear DNA content of 52.2 pg suggest an ancient doubling of the chromosomes. However Khoshoo (1961) considered that it was not a polyploid because its karyotype structure with 20 pairs of terminal or subterminal chromosomes and 2 pairs of median chromosomes was more indicative of Robertsonian fission when compared with the largely metacentric karyotypes of the remaining Pinaceae. Moreover, in the related *Keteleeria evelyniana* Mast. with n = 12 also a high amount of nuclear DNA (48.4 pg) is found. Despite its name, *Pseudolarix*, DNA evidence shows that it is closer to the genera of the Abietoideae and especially to *Tsuga* (33–41 pg) and *Nothotsuga* (not available) than to *Larix* (27 pg). This is also suggested by its genome size.

Keteleeria evelyniana Mast. with its 48.4 pg is in this respect more similar to *Pinus* (38–72 pg) than to *Abies*

(38–42 pg). Yet, DNA evidence (Wang et al. 1999) and morphological characters place it closer to *Abies*.

Although the monotypic genera *Welwitschia mirabilis* (2C = 13.1 pg) and *Ginkgo biloba* (2C = 23.5 pg) are gymnosperms but not conifers, their 2C-values are added for comparison as they are often mentioned together with conifers.

Polyploidy

Ploidy seems to play a more important role than earlier envisioned in the speciation of conifers. Earlier cytological investigation of conifers has shown that *Juniperus chinensis* L., *Juniperus sabina* L. and *Juniperus squamata* 'Meyeri' are tetraploid and *Sequoia sempervirens* is hexaploid (Khoshoo 1961). In addition, the amount of nuclear DNA in *J. pingii* Cheng (received as *J. squamata* 'Loderi') was found here to suggest a tetraploid ploidy level. *Cryptomeria japonica* (Thunb. ex L. f.) D. Don, *Larix decidua*, *Pinus densiflora* Siebold and Zucc. and *P. radiata* are diploids, but can be tetraploids locally or in nurseries (Khoshoo 1959, 1961). The *J. squamata* 'Meyeri' measured here was diploid. However, a plant with the same name mentioned by Khoshoo (1961) was tetraploid, suggesting that both ploidy levels occur or that one of the accessions was incorrectly determined. *Juniperus squamata* and *J. pingii* are very difficult to distinguish morphologically, but the difference in ploidy might be used as an easy distinguishing character. On Mount Olympus (Greece) a specimen of *Juniperus phoenicea* was found with an amount of nuclear DNA that suggests it to be a hexaploid, the second hexaploid conifer ever found, but that conclusion need to be confirmed cytologically. One of the cultivars of *J.* ×*pfitzeriana* (*J. phoenicea* × *sabina*) i.e. 'Goldcoast' was found to have a 2C-value of 45.5 pg suggesting that it is a tetraploid as are the parents. The other cultivar studied here (i.e. *J.* ×*pfitzeriana* 'Mint Julep') had a lower 2C-value of 35.4 pg, which could be indicative of triploidy.

It is peculiar that neither Eckenwalder (2009) nor Farjon (2010) mention the tetraploidy of *Fitzroya cupressoides* (Hair 1968). Its ploidy level is also suggested by the high nuclear DNA content of 35.0 pg, twice that of the related *Diselma*. *Fitzroya* is, after *Pinus longaeva*, the second oldest living tree reaching more than 3600 years of age. Khoshoo (1961) mentions that stray polyploid conifer seedlings among normal diploids rarely will survive in nature because they are extremely slow growing. This slow growth in combination with a possibility for vegetative reproduction might have contributed to the longevity of these two species (Ahuja 2005). Finally, two values for nuclear DNA content for *Cunninghamia lanceolata* (Lamb.) Hook were 25.5 and 39.6 pg. This suggests that the latter is a triploid form. Triploids and even tetraploids of *Cryptomeria japonica* are also recorded (Chiba 1950). Triploidy is also suggested for a form of *Taiwania cryptomerioides* with 38.1 pg instead of 25.8 pg (our value 22.9 pg) found by Hizume et al. (2001).

Pseudolarix amabilis has n = 22 and 52.2 pg. Both suggest polyploidy but cytological examination of Khoshoo (1961) found no evidence for polyploidy. *Manoao*, *Cephalotaxus* and *Amentotaxus* have low chomosome numbers, but high amounts of nuclear DNA, twice the average of Podocarpaceae respectively, suggesting an ancient doubling of the DNA or a fusion of chromosomes. The low incidence of polyploidy might have to do with the fact that conifers in general have already a high amount of nuclear DNA, roughly 40 times more than the average angiosperm tree (Ahuja 2005).

The amount of nuclear DNA (2C-value) of conifers ranges from 8 to 72 pg. This nearly tenfold difference in DNA content without much difference in the number of chromosomes must be the result of a vast number of genomic changes including strong genome size increase by LTR retrotransposons (Vitte and Panaud 2005). Depending on the size of the total genome, 1 pg amounts to several thousand genes. Hence the acquisition or loss of 1 pg DNA far surpasses a few gene insertions or deletions. The data presented here for genome sizes agrees in most respects with recent classification of conifers. Flow cytometry as a taxonomic and diagnostic tool is applicable even in the case of seeds (Sliwinska et al. 2005, 2009, Zonneveld 2012) or juvenile plants, and therefore has applications for conservation monitoring and health.

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