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Nuclear DNA 2C-values for 16 species from Timor-Leste increases taxonomical representation in tropical ferns and lycophytes

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Abstract. Knowledge regarding genome size allows us to infer relationships between taxa, address questions related to systematics and contribute to biodiversity studies. However, currently, less than 3% of the described Pteridophyta species have genome size estimates reported in databases, and only around one third of these are tropical species, although the tropics are home of 86% of fern diversity. The region of Timor-Leste, included in one of the 25 hotspots of biodiversity, is considered one of the richest areas of the world in terms of pteridophyte species. Nonetheless, biodiversity-driven research focused on this territory's biodiversity is scarce. This study presents novel 2C-values for 15 species of ferns collected in Timor-Leste, using flow cytometry. Furthermore, one species of the lycophyte *Palhinhaea cernua* (L.) Vasc. & Franco, was also studied and its estimated genome size compared to a previous report. Estimates ranged from 10.45 pg in *Selliguea feei* Bory to 29.7 pg in *Microsorum punctatum* (L.) Copel, and are considered medium-size genomes. The data was compared with previous reports for closely related species. These are the first 2C-values for two families and seven genera of ferns, increasing the number of pteridophytes with reported C-values from 292 to 307.

Keywords: genome size, chromosome, cytogenetics, DNA amount, nuclear DNA content, Malesia, geographical distribution.

INTRODUCTION

Information regarding genome size plays a fundamental role in understanding a species' evolutionary history and is a tool that allows us to infer relationships between taxa, address questions related to cellular and developmental biology and systematics, among others, and contributes to biodiversity studies (Leitch 2005; Kumar et al. 2011). The considerable differences in nuclear DNA content across species can be related to adaptive features, which shows that genome size can be under selective pressure and its variations may be related to the evolutionary history of a given group (Ohri 1998). Currently, flow cytometry is the main technique used to obtain information related to species DNA C-value (Dolezel 2005). However, despite the importance of these studies, and the recent efforts concerning information about genome size in plants, there is still a substantial gap in knowledge, with only a very small portion of species studied, and more research is required.

The majority of values reported in the Plant DNA C-value database (Release 7.1, April 2019:https://cvalues.science.kew.org/) (Leitch et al. 2019) belong to angiosperms. The 2C-value for 10.770 species of angiosperms is known, corresponding to 3.3% of their global diversity (Antonelli et al. 2020). Pteridophytes are even more under-represented, with only 292 species reported in the database. These numbers account for 2.45% of the 11,916 species of pteridophytes described (PPG 2016). In 2001, Bennet & Leitch set the goal of obtaining the C-value for 200 pteridophytes species by 2005, with a special focus on those that maximize systematic and geographic representation (Bennet and Leitch 2001). Although this goal was met, further studies regarding this group are fundamental, since the pteridophytes represent an important evolutionary transition between bryophytes and spermatophytes and, as such, are critical to our understanding of how DNA content has evolved across land plants (Bainard et al. 2011). Furthermore, since the laboratories adequately equipped to make 2C-values estimation are mostly located in temperate climate areas, with more difficult access to tropical fern species, we suspected such species would be underrepresented in the Plant DNA C-value database. Yet, pteridophyte diversity in the tropics is significantly higher than in any other region of the globe. Estimates point to the existence of 4500 species of ferns and lycophytes in Southeast Asia, more than twice the number of species of the entire Holarctic Kingdom (Moran 2008). At the same time, the region of Timor-Leste, located in Southeast Asia, is included in the biogeographic region of Malesia, which is considered one of the richest areas of the world in terms of tropical pteridophyte species diversity (Ebihara and Kuo 2012). Additionally, Timor-Leste is included in Wallacea, an area classified as one of the 25 hotspots of biodiversity identified by Myers et al. (2000) as a priority of conservation at a global scale. Despite the rich biological patrimony of Timor-Leste, research focused on the country's biodiversity and genetic resources is lacking, mainly due to the military occupation of the territory that took place between 1975 and 1999 (Bouma and Kobryn 2004). In this sense, better coverage of pteridophytes nuclear DNA values data in this territory is crucial to understand the mechanisms behind genome size evolution and their relationship with geographic and ecological factors (Dagher-Kharrat et al. 2013).

Therefore, the aims of this paper are: 1. to check what percentage of genome size data from tropical pteridophytes has been estimated, comparing with other biogeographic regions; and 2. to expand knowledge about genome sizes of tropical fern species occurring in Timor-Leste.

MATERIALS AND METHODS

Plant material

Prior to the field work, a search was conducted in the Plant DNA C-value database to establish which pteridophytes species, known to occur in Timor-Leste, had already 2C-values estimations published, and which had not. From the latter list, those species with populations that could more easily be sampled were selected as target species for this study (Table 1). Leaves of 15 ferns and one lycophyte were collected from several field locations in Timor-Leste (Table 1). These samples were kept fresh (at 0-5ºC) for a period no longer than a week and used for flow cytometry analysis. Voucher specimens were prepared and kept in the herbaria of the University of Aveiro (AVE) and Naturalis Biodiversity Center (L). Duplicates were also kept at the National University of East Timor (UNTL, Díli, Timor-Leste).

Nuclear DNA content estimation

The nuclear DNA content of fresh leaf samples was assessed using flow cytometry, currently the most used technique to estimate C/2C-value in plants for its simplicity, accuracy, convenience, and speed (Galbraith et al. 1983, 2009). The methodology used followed Loureiro et al. (2007), which included the preparation of nuclear suspensions by chopping 50 mg of leaf sample tissue and 50 mg of internal standard leaves, *Vicia faba* "Inovec" (2C= 26.90 pg; Dolezel, Sgorbati and Lucretii 1992) or *Pisum sativum* "Ctirad" (2C= 9.09 pg; Dolezel et al. 1992), with a razor blade in a glass Petri dish containing 1 mL of WPB isolation buffer (200 mM Tris.HCl, 4mM $MgCl_2.6H_2O$, 2 mM EDTA $Na_2.2H_2O$, 86 mM NaCl, 10 mM sodium metabisulfite, 1% PVP-10, 1% (v/v) Triton X-100, pH 7.5; Loureiro et al. 2007). The nuclear solution was then filtered through a nylon net of 50 μm, and 50 $\mu g.mL^{-1}$ of propidium iodide (PI, Sigma-Aldrich, St. Lou-

Table 1. Scientific names and localities of samples collected for this study. Voucher specimens are kept in the Herbarium of the University of Aveiro (AVE) and of the Naturalis Biodiversity Center (L). Family circumscription according with PPG (2016).

Taxon	Family	Localities in Timor-Leste
Lycopodiophyta		
Palhinhaea cernua (L.) Vasc. & Franco	Lycopodiaceae	Ainaro, roadside between Maubisse and Turiscai, [8°49'33" S, 125°38'10" E], *Costa et al.* 254 (AVE)
Pteridophyta		
Calochlaena javanica (Blume) M.D.Turner & R.A.White	Dicksoniaceae	Ainaro, roadside from Maubisse to Turiscai, [8°49'22" S, 125°37'01" E], *Costa et al.* 245 (AVE, L.3959675)
Pityrogramma calomelanos (L.) Link	Pteridaceae	Ainaro, roadside between Maubisse and Turiscai, [8°49'33" S, 125°38'10" E], Costa *et al.* 253 (AVE)
Adiantum philippense L.	Pteridaceae	Liquiçá, roadside between Tibar and Faiten, [8°36'59" S, 125°29'09" E], Costa *et al.* 8 (AVE, L.3959700)
Pteris ensiformis Burm.	Pteridaceae	Manufahi, roadside of Laclo, [8°51'28" S, 125°41'36" E], *Costa et al.* 320 (AVE)
Blechnopsis orientalis (L.) C.Presl	Blechnaceae	Ainaro, roadside from Maubisse to Turiscai, [8°49'22" S, 125°37'01" E], Costa *et al.* 244 (AVE)
Diplazium esculentum (Retz.) Sw.	Athyriaceae	Aileu, from Díli to Aileu, after the crossroad to Remexio and Remexio, [8°37'05" S, 125°38'25" E], Costa *et al.* 195 (AVE, L.3959688)
Tectaria melanocaulos (Blume) Copel.	Tectariaceae	Aileu, Asumau, [8°37'19" S, 125°38'37"], Costa *et al.* 200 (AVE, L.3959765)
Oleandra musifolia (Blume) C.Presl	Oleandraceae	Ainaro, roadside between Maubisse and Turiscai, [8°48'57" S, 125°38'39" E], Costa *et al.* 258 (AVE)
Goniophlebium subauriculatum (Blume) C.Presl	Polypodiaceae	Viqueque, on the Waibua forest at foothills of Mundo Perdido mountain, [8°43'59" S, 126°22'10" E], *Costa et al.* 303 (AVE)
Microsorum punctatum (L.) Copel.	Polypodiaceae	Viqueque, on the Waibua forest at the foothills of Mundo Perdido mountain, [8°43'59" S, 126°22'10" E], *Costa et al.* 307 (AVE)
Microsorum scolopendria (Burm.f.) Copel.	Polypodiaceae	Viqueque, on the Waibua forest at foothills of Mundo Perdido mountain, [8°43'59" S, 126°22'10" E], *Costa et al.* 301 (AVE)
Platycerium bifurcatum subsp. *willinckii* (T.Moore) Hennipman & M.C.Roos	Polypodiaceae	Díli, Dare, [8°35'38" S, 125°34'07" E], Costa *et al.* 84 (AVE, L.3959789)
Pyrrosia lanceolata (Wall.) Farw.	Polypodiaceae	Aileu, roadside between Aileu and Maubisse, [8°48'16" S, 125°35'31" E], Costa *et al.* 238 (AVE)
Pyrrosia longifolia (Burm.f.) C.V.Morton	Polypodiaceae	Viqueque, roadside of Urulita, [8°46'21" S, 126°22'11" E], Costa *et al.* 290 (AVE)
Selliguea feei Bory	Polypodiaceae	Ainaro, Maubisse - Turiscai, at Rita-Uruho, [8°49'22" S, 125°37'01" E], Costa *et al.* 243 (AVE)

is, MO, USA) and 50 μg.mL^{-1} of RNAse (Sigma-Aldrich, St. Louis, MO, USA) were added to the sample, to stain nuclear DNA and prevent staining of double stranded RNA, respectively. Samples were analyzed within a 10 min period on an Attune® Acoustic Focusing Cytometer (TermoFisher Scientific) equipped with a 488 nm laser.

For each sample, at least 5,000 nuclei were analyzed. As a quality control, nuclear DNA content estimates were only considered when the coefficient of variation of G_0/G_1 peaks (CV_{peak}) were below 5%. Samples with higher CV_{peak} values were discarded and a new sample was prepared.

For most of the taxa, three to five individuals were analyzed, but for *Selliguea feei* and *Tectaria melanocaulos*, only one individual for each of the species survived the time between sampling in Timor and analysis in Aveiro. The number of individuals measured for each population is provided in Table 1.

Statistical analysis

Descriptive statistics were calculated for each taxa studied namely, mean, standard deviation (SD), coefficient of variation (CV), and minimum and maximum values of the holoploid genome size (2C, pg).

Chromosome number

The median of the chromosome numbers for 14 taxa was obtained from the online Chromosome Counts Database (CCDB) (Rice et al. 2015).

Floristic kingdoms versus *2C values analysis*

The floristic kingdom's classification by Takhtajan (1986) was applied to the Pteridophyta taxa whose DNA C-values are available in the Plant DNA C-value database. For that, Global Biodiversity Information Facility (GBIF, at https://www.gbif.org/, January 2022) was consulted to establish each species' main occurrence. Finally, the distribution of species listed in the Plant DNA C-value database by each floristic kingdom was compared with the equivalent distribution of the total World number of Pteridophyte species given by Moran (2008). For this comparison, the Paleotropical and the Cape floristic kingdoms had to be included in the same group, because Moran (2008) gives a single total number for Africa, without segregating the Cape floristic kingdom. The same was not adopted for the Holantarctic kingdom, because Moran (2008), provides separate figures for New Zealand, which allows some separation from other kingdoms. In South America no separation was possible between the Holantarctic and the Neotropical kingdoms, but since the number of Neotropical species should be much greater than the Holantarctic species present in the region, we assumed that the error would not be critical.

RESULTS

DNA content estimates were obtained for the 16 samples, 15 of them representing taxa with no previous 2C-value reported. These estimates, as well as the chromosome median *2n* value that are described in literature, are presented in Table 2. The 2C DNA content ranged from 10.45 pg in *Selliguea feei* Bory, with the *Vicia faba* standard, to 29.7 pg in *Microsorum punctatum* (L.) Copel. with the *Pisum sativum* standard. The average 2C-value for Polypodiopsida was 20.62 pg, and for Lycopodiophyta, represented only by one taxon, the 2C-value was 25.65 pg.

The coefficients of variation (CVs) for the samples varied between 3.7% and 6.7%.

The list of Pteridophyte taxa for which nuclear DNA 2C-values have been published in the Plant DNA C-value database (Leitch 2019) is presented in the Supplementary Material 1, alongside with the Takhtajan's floristic kingdoms (Takhtajan 1986) embraced by their geographical distributions ranges. This information is summarized in Table 3, alongside with the total world estimated number, and percentage, of Pteridophyte species for each floristic kingdom, according with Moran (2008). We can see in this table, that the Paleotropical+Cape kingdoms, together with the Neotropical floristic kingdoms, with 45% and 42%, respectively, include the vast majority of the world's pteridophyte diversity (87%). Contrariwise, the most diverse group of pteridophytes whose nuclear DNA 2C-values are known is the Holarctic, with 44%, followed by the Paleotropical+Cape, with only 23% and the Neotropical with 18%. With this study, the percentages of Holarctic species is reduced to 42%, and the percentage of species from Paleotropical+Cape area increases to 25%.

DISCUSSION

In spite of the long journey between the field in Timor-Leste and the cytometry laboratory in Aveiro, where the analysis was done, we succeed to analyze, at least, three individuals for 14 of the 16 species, and five/six, for nine of the 16 species.

The higher intraspecific variations detected are, most likely, related to difficulties associated to the flow cytometry technique, since the easiness of obtaining data differs between the taxa, as mentioned by Obermayer, et al. (2002).

Following Leitch, Chase & Bennet (1998) genome size classification, all taxa have "intermediate" genomes ($7<2C\leq28$ pg). The median value established for genome size in ferns is 22.8 pg/2C and it has been related, partially, to variation in post-polyploidization processes- such as additional chromosomes and DNA arising from whole genome duplications-, since diploidization is not linked with genome downsizing in ferns in opposition to angiosperms, a group with smaller genomes (median= 3.4 pg/2C) (Liu et al. 2019). Regarding the lycophytes, the median 2C-value for the group is 0.26 pg (Liu et al. 2019), corresponding to a very small genome (≤2.8 pg) (Leitch et al. 1998). Despite the 2C-value previously reported in the literature of 2.75 pg for *Palhinhaea cernua* (L.) Vasc. & Franco (Kuo et al. 2016), the 2C-value estimated for this species is 25.65 pg, corresponding to the "intermediate" category and to the highest genome size in the Lycopodiaceae family reported until present, more than twice that of *Huperzia lucidula* (Michx.) Trevis., which has 11.28 pg (Bainard et al. 2011) and was the previous highest value reported. Considering that the coefficient of variation for this estimate is 5.5%, it doesn't seem likely that the 2C-value for *P. cernua* was

Table 2. Mean 2C-value estimates (pg) for 15 fern species and 1 lycophyte collected in East-Timor, with standard deviation (SD), minimum and maximum values, average coefficient of variation (CV %) for each taxon. Family circumscription according with PPG (2016). Estimates obtained using the *Vicia faba* standard (2C= 26.90) are identified with "*". The remaining measurements were obtained using the *Pisum sativum* standard (2C= 9.09 pg). Reported chromosome number (median n value) for the taxa available is also presented, according to the CCDB database (release 1.58, http://ccdb.tau.ac.il/).

Taxon	Family	Median 2n value	Genome size (2C, pg)				n. samples
			Mean ± SD	Min.	Max.	Average CV (%)	
Lycopodiophyta							
Palhinhaea cernua (L.) Vasc. & Franco	Lycopodiaceae	208, 220, 272, 312, 330, 340, 416	25.65 ± 0.43	25.32	26.32	5.52	5
Pteridophyta							
Calochlaena javanica (Blume) M.D.Turner & R.A.White	Dicksoniaceae	?	11.41 ± 0.11	11.32	11.43	4.78	5
Pityrogramma calomelanos (L.) Link	Pteridaceae	232, 240	26.41 ± 0.36	26.12	26.85	6.72	4
Adiantum philippense L.		60, 90	21.92* ± 2.3	18.48	23.29	4.03	4
Pteris ensiformis Burm.		58, 87-88, 116, 168, 185	19.15* ± 0.55	18.71	19.81	4.71	5
Blechnopsis orientalis (L.) C.Presl	Blechnaceae	?	13.57* ± 0.11	13.43	13.61	5.91	5
Diplazium esculentum (Retz.) Sw.	Athyriaceae	82	22.68 ± 0.70	22	23.57	5.88	4
Tectaria melanocaulos (Blume) Copel.	Tectariaceae	?	24.68	-	-	3.69	1
Oleandra musifolia (Blume) C.Presl	Oleandraceae	80	13.65* ± 0.08	13.57	13.75	6.11	5
Goniophlebium subauriculatum (Blume) C.Presl	Polypodiaceae	72	21.06* ± 0.38	20.59	21.52	4.98	5
Microsorum punctatum (L.) Copel.		72	29.72 ± 0.44	29.43	30.23	4.56	3
Microsorum scolopendria (Burm.f.) Copel.		36**	24.55 ± 1.92	21.14	25.76	4.53	5
Platycerium bifurcatum subsp. *willinckii* (T.Moore) Hennipman & M.C.Roos		74	28.47 ± 2.88	23.74	30.8	3.93	5
Pyrrosia lanceolata (Wall.) Farw.		74	23.73 ± 0.31	23.47	24.16	5.34	4
Pyrrosia longifolia (Burm.f.) C.V.Morton		74	28.79 ± 3.58	26.06	35.7	4.96	6
Selliguea feei Bory		74	10.45*	-	-	5.44	1

** n value presented, no 2n value reported.

negatively influenced by artefacts such as the presence of interfering secondary metabolites (Hanusová et al. 2014). This novel result shows that genome size within the Lycopodiaceae family may be more variable than what was thought until now In fact, the chromosome numbers reported for this species varies from n=34 to 2n=208-416 (Rice et al. 2015).

Comparing the 2C-value of *Diplazium esculentum* (Retz.) Sw. (22.68 pg) with *Diplazium pycnocarpon* (Sprengel) M. Broun (12.63 pg), the only other species of the same genus that has been screened for its genome size by Bainard et al. (2011), the 2C-value differs by approx. 10 pg. This variation shows that even within the same genus, genome size may vary greatly, regardless of the two species' chromosome number being very similar, with *D. esculentum* (2n=82) and *D. pycnocarpon* (2n=80). The same conclusion can be drawn when comparing our estimate for *Adiantum philippense* L., (2C= 21.9 pg) with previous work on the genus: 2C-value estimates reported for *Adiantum pedantum* L. are 10.16 pg (Bainard et al. 2011) and for *Adiantum aleuticum* (Rupr.) C. A. Paris are 11.42 pg (an approx. difference of 10.5 pg) (Clark et al. 2016). The 2C-value discrepancy between *Adiantum* species may be related, most probably, to differences in chromosome numbers between taxa, since both 2n=60 and 2n=90 have been reported for *A. philippense* in literature. Although 2n=60 is similar to chromosome number for *A. pedantum* and *A. aleuticum* (2n=58), a 2n=90 could be a reason to explain this variation.

The 2C-value discrepancy between *Adiantum* species may also be related, in part, to the different geographical origin of the material. Some evidence points towards the prevalence of smaller genomes in plant species that exist in harsher, drier, environments, with shorter growing seasons (Knight, Molinari and Petrov 2005). But checking this would require investigations out of the scope of this paper.

What we could contribute was towards improving the representation of the most diverse phytogeographical kingdoms for this group (Table 3), following Moran's (2008) suggestion that this group of organisms shows a dominant pattern called "the latitudinal diversity gradient", which means that species diversity in ferns increases from the pole towards the equator (Moran 2008). Despite this pattern, almost half of the studied species found in the Plant DNA C-value database (Leitch et al. 2019) belong to the Holarctic kingdom. Therefore, an already understudied group of plants in terms of genome size lacks, to a great extent, estimates from species of the most representative phytogeographical kingdoms for this group, which we tried to counteract with the new data presented in this study (Table 3).

CONCLUSIONS

The present work includes novel data that contributes to the knowledge regarding genome size of 15 species of ferns and 1 species of lycophytes. Our data increases the taxonomic representation of DNA content in pteridophytes databases by two families- Blechnaceae and Oleandraceae-, as well as seven genera (*Blechnopsis*, *Goniophlebium, Microsorum, Palhinhaea*, *Pityrogramma*, *Pyrrosia* and *Selliguea*). Furthermore, the representation of Paleotropical fern species has increased by 2%. However, with almost 12.000 species of pteridophytes described to date, further work focused on the DNA content of more lycophyte and fern species, especially from tropical regions, is crucial to expand taxonomic representation and fill in the phylogenetic gaps within the group.

Although we could not perform chromosome counts alongside with the 2C value estimations, this should be a future target, allowing to draw more complete conclu-

Table 3. Distribution of the number and percentage of species of Pteridophytes recognized by each of Takhtajan's floristic kingdoms comparing with the same distribution in terms of species with published DNA C-values including the contribution of this study.

Takhtajan's floristic kingdoms	No. (%) of species estimated*	No. (%) of species with known DNA C-values	No. of species added in this study**	Current No. (%) of species with known DNA C-values
Holartic	1470 (9.4)	190 (44)		188 (42)
Neotropical	6500 ((41.7)	76 (18)	4	80 (18)
Paleotropical + Cape	6980 (44.7)	94 (23)	16	110 (25)
Australian	456 (2.9)	37 (8)	5	42 (9)
Holantarctic	193 (1.2)	29 (7)		29 (6)
Total	15599 (100)	429 (100)	25	454 (100)

* Numbers of species estimated to occur taken from Moran (2008: 369); ** the numbers presented exceed the 16 species analyzed, because several of them are distributed among more than one floristic kingdom, as it was also adopted by Moran (2008).

sions about the genome of the studied species, namely, concerning ploidy levels.

Bearing this in mind, in spite of the relatively modest contribution in terms of species numbers (not so modest when we consider the number of new families and genera), this paper increases the representation of tropical Pteridophyte diversity whose nuclear 2C-values are known, and highlights that further studies on genome size in ferns are crucial, especially in species from areas that are considered hotspots of tropical fern biodiversity, such as Timor-Leste. The lack of studies on the country's biodiversity coupled with the human impact in the region, makes the execution of these studies even more important, since genome size data is basic information for an appropriate management and conservation of the plant genetic resources of the area.

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Supplementary Material 1. Distribution of Pteridophyta taxa with studied DNA C-value (from https://cvalues.science.kew.org/) among Takhtajan's Floristic Kingdoms (1986).

Genus	Species	Subspecies/Variety	Phytogeographical region(s)
Acrostichum	*aureum*		Neotropical, Palaeotropical, Australian
Adiantum	*aleuticum*		Holarctic
Adiantum	*capillus-veneris*		Holarctic,Neotropical, Palaeotropical, Australian, Holantarctic
Adiantum	*pedatum*		Holarctic
Adiantum	*venustum*		Holarctic
Alsophila	*spinulosa*		Holarctic, Palaeotropical
Amauropelta	*bergiana*	var. *bergiana*	Palaeotropical
Anemia	*collina*		Neotropical
Anemia	*phyllitidis*		Neotropical
Anemia	*rotundifolia*		Neotropical
Anemia	*tomentosa*		Neotropical
Angiopteris	*latipinna*		Holarctic
Angiopteris	*lygodiifolia*		Holarctic
Angiopteris	*pruinosa*		Palaeotropical
Arthropteris	*orientalis*		Palaeotropical
Asplenium	*achilleifolium*		Neotropical
Asplenium	*adiantum-nigrum*	var. *adiantum-nigrum*	Holarctic, Palaeotropical
Asplenium	*adulterinum*		Holarctic
Asplenium	*aethiopicum*	subsp. *tripinnatum*	Palaeotropical
Asplenium	*Aethiopicum*	subsp. *dodecaploideum*	Palaeotropical
Asplenium	*billotii*		Holarctic
Asplenium	*boreale*		Holarctic
Asplenium	*caucasicum*		Holarctic
Asplenium	*ceterach*		Holarctic
Asplenium	*cristatum*		Neotropical
Asplenium	*cuneifolium*		Holarctic
Asplenium	*dalhousiae*		Holarctic
Asplenium	*daucifolium*		Palaeotropical
Asplenium	*flabellifolium*		Australian, Holantarctic
Asplenium	*griffithianum*		Holarctic, Palaeotropical
Asplenium	*hallbergii*		Neotropical
Asplenium	*hemionitis*		Holarctic
Asplenium	*javorkeanum*		Holarctic
Asplenium	*lividum*		Palaeotropical
Asplenium	*marinum*		Holarctic
Asplenium	*mauritiensis*		Palaeotropical
Asplenium	*myriophyllum*		Neotropical
Asplenium	*neolaserpitifolium*		Palaeotropical
Asplenium	*nidus*		Holarctic,Neotropical, Palaeotropical, Australian
Asplenium	*obtusatum*		Neotropical, Palaeotropical, Australian, Holantarctic
Asplenium	*onopteris*		Holarctic
Asplenium	*quadrivalens*		Holarctic
Asplenium	*rhizophyllum*		Holarctic
Asplenium	*richardii*		Holantarctic
Asplenium	*ruta-muraria*		Holarctic
Asplenium	*scolopendrium*		Holarctic, Holantarctic
Asplenium	*septentrionale*		Holarctic
Asplenium	*subglandulosum*		Australian, Holantarctic
Asplenium	*tenerum compl*		Palaeotropical

Genus	Species	Subspecies/Variety	Phytogeographical region(s)
Asplenium	*trichomanes*		Holarctic, Neotropical, Palaeotropical, Australian, Holantarctic
Asplenium	*trichomanes*	subsp. *quadrivalens*	Holarctic, Palaeotropical
Asplenium	*varians*		Holarctic, Palaeotropical
Asplenium	*Víride*		Holarctic
Asplenium	*viviparum*		Palaeotropical
Asplenium	*x- lolegnamense*		Holarctic
Asplenium	*x-lucrosum*		Holantarctic
Asplenium	*x-poscharskyanum*		Holarctic
Athyrium	*filix-femina*	var. *angustum*	Holarctic
Azolla	*microphylla*		Holarctic, Neotropical
Blechnum	*microphyllum*		Neotropical
Blechnum	*nudum*		Australian, Holantarctic
Blechnum	*spicant*		Holarctic
Bolbitis	*heudelotii*		Palaeotropical
Bolbitis	*singaporensis*		Palaeotropical
Botrychium	*neolunaria*		Holarctic
Botrychium	*alaskense*		Holarctic
Botrychium	*boreale*		Holarctic
Botrychium	*echo*		Holarctic
Botrychium	*hesperium*		Holarctic
Botrychium	*lanceolatum*		Holarctic
Botrychium	*lunaria*		Holarctic, Australian
Botrychium	*matricariifolium*		Holarctic
Botrychium	*michiganense*		Holarctic
Botrychium	*minganense*		Holarctic
Botrychium	*montanum*		Holarctic
Botrychium	*pallidum*		Holarctic
Botrychium	*pinnatum*		Holarctic
Botrychium	*simplex*		Holarctic
Botrychium	*spathulatum*		Holarctic
Botrychium	*virginianum*		Holarctic
Botrypus	*cf. virginianus*		Holarctic, Neotropical
Brainea	*insignis*		Palaeotropical
Calochlaena	*dubia*		Australian
Ceratopteris	*thalictroides*		Holarctic, Neotropical, Palaeotropical, Australian
Ceterach	*officinarum*	subsp. *officinarum*	Holarctic
Cheilanthes	*marantae*		Holarctic
Cibotium	*barometz*		Palaeotropical
Cibotium	*hawaiense*		Palaeotropical
Cryptogramma	*crispa*		Holarctic
Ctenitis	*sinii*		Holarctic
Culcita	*macrocarpa*		Holarctic
Cyathea	*crinita*		Palaeotropical
Cyclosorus	*arbusculus*		Palaeotropical
Cyclosorus	*asperum*		Palaeotropical
Cyclosorus	*dentatus*		Holarctic, Palaeotropical
Cystopteris	*bulbifera*		Holarctic
Cystopteris	*dickieana*		Holarctic
Cystopteris	*fragilis*	agg.	Holarctic, Neotropical, Cape, Holantarctic
Cystopteris	*tenuis*		Holarctic
Danaea	*antillensis*		Neotropical

Genus	Species	Subspecies/Variety	Phytogeographical region(s)
Danaea	*kalevala*		Neotropical
Danaea	*mazeana*		Neotropical
Davallia	*denticulata*	var. *denticulata*	Palaeotropical, Australian
Davallia	*tyermanii*		Holarctic
Dendrolycopodium	*dendroideum*		Holarctic
Dendrolycopodium	*obscurum*		Holarctic
Dennstaedtia	*globulifera*		Neotropical
Dennstaedtia	*wilfordii*		Holarctic
Deparia	*acrostichoides*		Holarctic
Deparia	*boryana*		Holarctic, Palaeotropical
Deparia	*japonica*		Holarctic, Palaeotropical
Dicksonia	*antarctica*		Holarctic, Australian
Dicranopteris	*linearis*		Holarctic, Neotropical, Palaeotropical, Australian, Holantarctic
Diphasiastrum	*alpinum*		Holarctic
Diphasiastrum	*digitatum*		Holarctic
Diphasiastrum	*complanatum*		Holarctic, Neotropical, Palaeotropical
Diphasiastrum	*tristachyum*		Holarctic
Diplazium	*arborescens*		Palaeotropical
Diplazium	*australe*		Palaeotropical, Australian, Holantarctic
Diplazium	*proliferum*		Palaeotropical, Australian
Diplazium	*pycnocarpon*		Holarctic
Diplopterygium	*bancroftii*		Neotropical
Dipteris	*chinensis*		Holarctic
Dracoglossum	*plantagineum*		Neotropical
Drynaria	*heraclea*		Palaeotropical
Dryopteris	*bernieri*		Palaeotropical
Dryopteris	*carthusiana*		Holarctic
Dryopteris	*clintoniana*		Holarctic
Dryopteris	*cristata*		Holarctic
Dryopteris	*cycadina*		Holarctic, Holantarctic
Dryopteris	*dilatata*		Holarctic, Holantarctic
Dryopteris	*filix-mas*		Holarctic, Neotropical, Holantarctic
Dryopteris	*goldiana*		Holarctic
Dryopteris	*intermedia*		Holarctic
Dryopteris	*marginalis*		Holarctic
Elaphoglossum	*aubertii*		Palaeotropical
Elaphoglossum	*crinitum*		Neotropical
Elaphoglossum	*hybridum*		Neotropical, Palaeotropical
Elaphoglossum	*lepervanchii*		Palaeotropical
Equisetum	*arvense*		Holarctic, Holantarctic
Equisetum	*bogotense*		Neotropical
Equisetum	*moorei*		Holarctic
Equisetum	*fluviatile*		Holarctic
Equisetum	*giganteum*		Neotropical
Equisetum	*hyemale*		Holarctic, Neotropical, Australian, Holantarctic
Equisetum	*laevigatum*		Holarctic, Neotropical
Equisetum	*myriochaetum*		Neotropical
Equisetum	*palustre*		Holarctic
Equisetum	*pratense*		Holarctic
Equisetum	*ramosissimum*	subsp. *ramosissimum*	Holarctic, Palaeotropical
Equisetum	*scirpoides*		Holarctic

Genus	Species	Subspecies/Variety	Phytogeographical region(s)
Equisetum	*sylvaticum*		Holarctic
Equisetum	*variegatum*		Holarctic
Gymnocarpium	*dryopteris*		Holarctic
Gymnocarpium	*fedtschenkoanum*		Holarctic
Gymnocarpium	*robertianum*		Holarctic
Gymnosphaera	*podophylla*		Holarctic, Palaeotropical
Huperzia	*lucidula*		holarctic
Hymenophyllum	*badium cf*		Holarctic, Palaeotropical
Hymenophyllum	*sibthorpioides*		Palaeotropical
Isoetes	*engelmannii*		Holarctic
Isoetes	*lacustris*		Holarctic
Lepisorus	*excavatus*		Palaeotropical
Lindsaea	*ensifolia*		Palaeotropical, Australian
Llavea	*cordifolia*		Neotropical
Lonchitis	*occidentalis*		Palaeotropical
Loxsoma	*cunninghami*		Holantarctic
Lycopodium	*annotinum*		Holarctic
Lycopodium	*clavatum*		Holarctic, Neotropical, Palaeotropical
Lycopodium	*dendroideum*		Holarctic
Lycopodium	*obscurum*		Holarctic
Lygodium	*japonicum*		Holarctic, Neotropical, Palaeotropical, Australian
Lygodium	*microphyllum*		Holarctic, Palaeotropical, Australian
Lygodium	*volubile*		Neotropical
Marattia	*purpurascens*		Holarctic
Marsilea	*quadrifolia*		Holarctic, Neotropical, Palaeotropical
Matteuccia	*struthiopteris*	var. *pensylvanica*	Holarctic
Megalastrum	*macrotheca*		Neotropical
Mickelia	*nicotianifolia*		Neotropical, Palaeotropical
Microgramma	*percussa*		Neotropical, Palaeotropical
Microlepia	*speluncae*		Neotropical, Palaeotropical, Australian
Microlepia	*strigosa*		Holarctic, Palaeotropical
Nephrolepis	*biserrata*		Neotropical, Palaeotropical, Australian
Nephrolepis	*cordifolia*	'Duffi'	Holarctic, Neotropical, Palaeotropical, Australian, Holantarctic
Nephrolepis	*exaltata*		Holarctic, Neotropical, Palaeotropical, Australian
Oleandra	*neriiformis*		Palaeotropical, Australian
Onoclea	*orientalis*		Holarctic
Onoclea	*sensibilis*		Holarctic
Onychium	*lucidum*		Holarctic, Palaeotropical
Ophioglossum	*gramineum*		Palaetropical, Australian
Ophioglossum	*pendulum*		Palaeotropical, Australian
Ophioglossum	*petiolatum*		Holarctic, Palaetropical, Holantarctic
Osmunda	*cinnamomea*		Holarctic, Neotropical
Osmunda	*claytoniana*		Holarctic
Osmunda	*regalis*	var. *spectabilis*	Holarctic, Neotropical
Paragymnopteris	*marantae*		Holarctic
Paragymnopteris	*vestita*		Holarctic
Pellaea	*atropurpurea*		Holarctic, Neotropical
Pellaea	*glabella*	subsp. *glabella*	Holarctic
Phegopteris	*connectilis*		Holarctic
Phyllitis	*scolopendrium*	subsp. *scolopendrium*	Holarctic
Plagiogyria	*matsumureana*		Holarctic

Genus	Species	Subspecies/Variety	Phytogeographical region(s)
Platycerium	*coronarium*		Palaeotropical
Pleopeltis	*macrocarpa*		Neotropical, Palaeotropical
Polyphlebium	*capillaceum*		Neotropical
Polypodium	*australe*		Holarctic
Polypodium	*cambricum*		Holarctic
Polypodium	*glycyrrhiza*		Holarctic
Polypodium	*interjectum*		Holarctic
Polypodium	*scouleri*		Holarctic
Polypodium	*virginianum*		Holarctic
Polypodium	*vulgare*		Holarctic, Neotropical, Cape, Holantarctic
Polypodium	*Vulgare x interjectum*		Not defined
Polypodium	*x-font-queri*		Holarctic
Polypodium	*x-mantoniae*		Holarctic
Polypodium	*x-shivasiae*		Holarctic
Polystichum	*acrostichoides*		Holarctic
Psilotum	*nudum*		Holarctic, Palaeotropical, Neotropical, Australian, Holantarctic
Pteridium	*aquilinum*		Holarctic, Neotropical, Palaeotropical, Australian
Pteridium	*revolutum*		Palaeotropical
Pteridium	*subsp. caudatum*	var. *arachnoideum*	Neotropical
Pteridrys	*cnemidaria*		Palaeotropical
Pteris	*croesus*		Palaeotropical
Pteris	*linearis*		Palaeotropical, Neotropical
Pteris	*pseudolonchitis*		Palaeotropical
Pteris	*vittata*		Holarctic, Neotropical, Palaeotropical, Holantarctic, Australian
Ptisana	*salicina*		Holantarctic, Palaeotropical
Pyrrosia	*lingua*		Holarctic, Palaeotropical
Saccoloma	*domingense*		Neotropical
Sadleria	*cyatheoides*		Palaeotropical
Salvinia	*molesta*		Holarctic, Neotropical, Palaeotropical, Holantarctic, Australian
Selaginella	*apoda*		Holarctic, Neotropical
Selaginella	*arenicola*		Holarctic
Selaginella	*arizonica*		Holarctic
Selaginella	*asprella*		Holarctic
Selaginella	*bigelovii*		Holarctic
Selaginella	*braunii*		Holarctic
Selaginella	*cinerascens*		Holarctic
Selaginella	*densa*		Holarctic
Selaginella	*eremophila*		Holarctic
Selaginella	*exaltata*		Neotropical
Selaginella	*extensa*		Neotropical
Selaginella	*flabellata*		Neotropical
Selaginella	*hansenii*		Holarctic
Selaginella	*helvetica*		Holarctic
Selaginella	*involvens*		Holarctic, Palaeotropical
Selaginella	*kraussiana*	var. *poulteri*	Holarctic
Selaginella	*kraussiana*		Holarctic, Neotropical, Holantarctic, Palaeotropical, Australian
Selaginella	*landii*		Neotropical
Selaginella	*lepidophylla*		Holarctic, Neotropical
Selaginella	*leucobryoides*		Holarctic
Selaginella	*martensii*		Neotropical
Selaginella	*moellendorffii*		Holarctic, Holantarctic, Palaeotropical

Genus	Species	Subspecies/Variety	Phytogeographical region(s)
Selaginella	*mutica*		Holarctic
Selaginella	*oregana*		Holarctic
Selaginella	*pallescens*		Holarctic, Neotropical
Selaginella	*peruviana*		Neotropical
Selaginella	*pilifera*		Neotropical
Selaginella	*pulcherrima*		Neotropical
Selaginella	*rupestris*		Holarctic
Selaginella	*rupincola*		Holarctic
Selaginella	*selaginoides*		Holarctic
Selaginella	*sellowii*		Neotropical, Holarctic
Selaginella	*tortipila*		Holarctic
Selaginella	*uncinata*		Holarctic, Palaeotropical
Selaginella	*underwoodii*		Holarctic
Selaginella	*vogelii*		Palaeotropical
Selaginella	*wallacei*		Holarctic
Selaginella	*watsonii*		Holarctic
Selaginella	*weatherbiana*		Holarctic
Selaginella	*willdenowii*		Holarctic, Neotropical, Palaeotropical, Australian
Selaginella	*wrightii*		Holarctic, Neotropical
Serpocaulon	*triseriale*		Neotropical
Sphaeropteris	*lepifera*		Neotropical, Palaeotropical
Spinulum	*annotinum*		Holarctic
Stenochlaena	*tenuifolia*		Palaeotropical
Tectaria	*zeilanica*		Palaeotropical
Thelypteris	*noveboracensis*		Holarctic
Thelypteris	*palustris*	var. *pubescens*	Holarctic
Thyrsopteris	*elegans*		Neotropical
Tmesipteris	*obliqua*		Australian
Tmesipteris	*tannensis*		Holantarctic
Todea	*barbara*		Palaeotropical, Australian, Holantarctic
Trichomanes	*speciosum*		Holarctic
Vandenboschia	*auriculata*		Holarctic, Palaeotropical
Vandenboschia	*davallioides*		Palaeotropical
Vittaria	*lineata*		Neotropical, Palaeotropical
Woodsia	*alpina*		Holartic
Woodsia	*ilvensis*		Holartic
Woodsia	*pulchella*		Holartic
Woodwardia	*fimbriata*		Holartic
Woodwardia	*unigemata*		Holarctic, Palaeotropical