ABSTRACT

An analysis was made of the correspondence between species diversity and chromosome number (CN) diversity across 13 Protected Wild Areas (PWA) in the Araucanía Region of southern Chile, encompassing 84 plant species with available cytogenetic data. Our aim was to establish whether higher species diversity within a PWA entails higher CN variation as based on the index of chromosome number heterogeneity (ICNH). The CN data were extracted from databases for Chilean plants, and the ICNH for the flora of each PWA was calculated. Results showed that in nine PWA the species diversity clearly correlates with CN diversity. However, four PWA do not fit this trend. The percentage of species with CN data varied between 9.6% and 24.5% among PWA, with 11 PWA presenting percentages higher than 11%. A 27.3% of the Chilean vascular plant species with available cytogenetic data were studied here for the 13 PWA. The results obtained by studying one part of the flora with available CN data suggest that the PWA could be an important reservoir of genetic diversity at a chromosome level, thus justifying the protective role of the PWA as biodiversity conservation sites.

Key words: Chromosome number heterogeneity; floristic diversity; Chilean flora.

RESUMEN

Se realizó un análisis de la correspondencia entre la diversidad de especies y la diversidad de números cromosómicos (CN) en 13 Áreas Silvestres Protegidas (PWA) en la Región de La Araucanía en el sur de Chile, incluyendo 84 especies de plantas con datos citogenéticos disponibles. El objetivo fue establecer si una mayor diversidad de especies dentro de un PWA implica una mayor diversidad en CN expresado en base al Índice de Heterogeneidad Cromosómica (ICNH). Los CN de cada especie se extrajeron de bases de datos para plantas chilenas y se calculó el ICNH para la flora de cada PWA. Los resultados mostraron que en nueve PWA la diversidad de especies se correlaciona claramente con la diversidad de CN. Sin embargo, cuatro PWA no se ajustan a esta tendencia. El porcentaje de especies con datos de CN varió entre 9.6% y 24.5% entre PWA, con 11 PWA presentando porcentajes superiores al 11%. Un 27.3% de las especies de plantas vasculares chilenas con datos citogenéticos disponibles fueron estudiadas para las 13 PWA. Los resultados obtenidos al estudiar parte de la flora sugieren que las PWA serían un reservorio importante de diversidad genética a nivel cromosómico como se muestra aquí, justificando así el papel protector de las PWA como sitios de conservación de la biodiversidad.

Palabras clave: Heterogeneidad del número cromosómico; diversidad florística; flora chilena.
INTRODUCTION

The Chilean Protected Wild Areas (PWA) system started up in 1984 as a dependent institution of SNASPE (National System of State Protected Wild Areas) (Pauchard and Villarroel, 2002) encompassing 105 terrestrial PWA throughout Chile which are currently managed by the National Forest Corporation (Corporación Nacional Forestal–CONAF). Since their inception the PWA have been understood to be high biodiversity sites along the length of the Chilean territory and many of them are relics of extensive old forests, including taxa from multiple geographical origins (Troncoso et al., 1980; van der Hammen and Cleef, 1983; Villagrán and Hinojosa, 1997; Moreira Muñoz, 2011; Armesto et al., 2010; Scherson et al., 2017). The vascular flora of the PWA is known to be one of the most visible forms of life in the forests that contain them and plant species are vertically organised as herbaceous, shrub and arboreal strata (Smith, 1973; Ramírez et al., 1990), giving rise to environments that harbour an important diversity of organisms belonging to different kingdoms (Smith Ramírez et al., 2007; Marin et al., 2017).

The genetic diversity of the Chilean flora is a heritage that is important to conserve, and for this reason its study requires the use of multiple tools to facilitate its description (Jara Seguel and Urrutia, 2012). The genetic diversity of Chilean plants was initially analysed using isozyme electrophoresis and later on, with the advent of DNA technologies, fingerprint profiling was conducted in populations of single species (Premoli, 1997; Premoli et al., 2000; 2012; Torres Díaz et al., 2007; Premoli and Mathiasen, 2011; García Gonzales et al., 2008; Martin et al., 2014; Bastías et al., 2016). Other studies have used DNA sequences focused on performing phylogenetic reconstructions including species of various families and orders (Aagesen and Sanso, 2003; Davis et al., 2004; Chacon et al., 2012a; 2012b; Delaveau et al., 2013; Jara Arancio et al., 2013; Givnish et al., 2016). In this context, comprehensive work was carried out reconstructing a spatial phylogenetic tree that included 756 native genera of vascular plants (ca. 87% of the total in Chile) thus evaluating both the phylogenetic diversity and endemism of Chilean flora (Scherson et al., 2017). The Scherson study on Chilean flora does not specify whether the sampled specimens were taken within the PWA mentioned as such by us. However, the geographic coordinates of various sites that they describe coincide with the PWA located in the Andean range, Central valley and Nahuelbuta range (see appendix A with supplementary material in Scherson et al., 2017). An additional organisational level to analyse genetic diversity is the chromosomal (Stebbins, 1971; Levin, 2002; Windham and Yatzkievych, 2003; Severns and Liston, 2008; Peruzzi et al., 2012; Morero et al., 2015).

The chromosome set represented by the CN accounts for the complete genome in addition to the chromosome morphology, thus determining a nuclear architecture that is unique to each species. This nuclei ordering is key to understand the organisation and functionality of the plant genomes both in interphase processes and in cell division (Schneider and Grosschädli, 2007; Heslop Harrison and Schwarzacher, 2011). Specifically, gene expression depends on the ordering of multiple chromosome domains within the nucleus (Fernández Donoso and Berrios, 1985; Gregory, 2001). For decades many studies described the CN independently of the chromosome morphology, using it as a basic genetic character to analyse similarity or variation between species (Peruzzi et al., 2012; 2014). Currently, CN data are available for ca. 307 species of Chilean vascular plants, which represent 135 genera and 60 families (ca. 6.6% of the total; Jara Seguel and Urrutia Estrada, 2020) many of them inhabiting in PWA throughout the continental territory. Based on these data, a high CN variation is observed within Chilean vascular plants along the continent and in insular areas.

The CN could be a good marker to evaluate genetic variation in the flora of the PWA as a whole and not just based on single species, making it possible to overlay the species diversity. Thus, two matrices analysing diversity—the floristic and the chromosomal—can be superimposed. Quantitative analyses based on the index of chromosome number heterogeneity (ICNH) have been recently proposed to compare CN variation among different plant or animal taxa (Peruzzi et al., 2014), which could be used to determine quantitative CN diversity in areas harbouring different species of native plants such as occurs in the PWA.

In the Araucanía Region of southern Chile (from 37º to 39º S) there are 13 terrestrial PWA. Ten PWA are located in the Andean range forming the Araucarias Biosphere Reserve, whereas one PWA is located in the Central valley and two PWA are located in the Nahuelbuta range near the Pacific coast (CONAF, 2013). A recent cadastre carried out only for the 10 PWA of the Biosphere Reserve (Natural Reserve (RN) Malleco, RN Las Nalcas, RN Malalcahuello, RN Alto Biobío, RN China Muerta, RN Villarrica, National Park (PN) Tolhuaca, PN Conguillío, PN Villarrica and PN Huerruehue) recognised 829 species present in these areas (Hauenstein and Saavedra, 2019). Nevertheless, the PWA located in the Central valley (Natural Monument (MN) Cerro Nielol) and Nahuelbuta range (PN Nahuelbuta and MN Contulmo) (Baeza et al., 1999; Arriagada, 2002; Saavedra and Morales, 2008), have been described as presenting high floristic diversity but it have not yet been considered a biosphere reserve. In this context, the high species diversity could be correlated with high genetic diversity, such as was discussed theoretically by Vellend (2005), by simulating correlation models.
between both levels, and could also correspond to a CN variation as a genetic character in PWA. At present, no accurate cadastre of number of species with CN data has been published for the 13 PWA from the Araucanía Region in Chile, although information is available in electronic databases as well as in various printed sources. However, the species diversity for these areas represented by the number of species is well known and has been documented in several cadastres, leading us to query: i) does higher species diversity within a PWA entail higher CN diversity? and ii) are there appreciable differences in mean CN between PWA? In this study, we supply evidence based on the index of chromosome number heterogeneity (ICNH) calculations to provide a partial answer to these questions, using the CN data available for species inhabiting these PWA.

**MATERIALS AND METHODS**

**Study areas**

In this study, we evaluated the 13 PWA present in the Araucanía Region of southern Chile. The Araucanía Region is located between 37° 35´ and 39° 37´ S and from 70° 50´ W to the coast of the Pacific Ocean. Boundaries for each PWA are given here only as a reference since they depend on government definitions rather than geographical vegetation units (Table 1).

**Floristic and cytogenetic data**

Plant species in each PWA were obtained from floristic cadastres (e.g., Baeza et al., 1999; Finckh et al., 1995; Arriagada, 2002; Sepúlveda, 2004; Cortés, 2005; CONAF, 2009; Saavedra and Morales, 2008; Saavedra, 2009a; 2009b; 2009c; Saavedra and Hauenstein, 2010a; 2010b; Hauenstein, 2011a; 2011b). So for each PWA the species were listed and their respective CN were looked up in the CPCD (Chilean Plants Cytogenetic Database; Jara Seguel and Urrutia Estrada, 2020, with cytogenetic data for 402 species). Data on the geographical location (Region and Province) of each species were also obtained from CPCD, as well as the original source where cytogenetic data were published. As a criterion to determine high or low floristic diversity in each PWA we calculated the mean species diversity (+ SD) for all 13 PWA. Thus, values above the mean will have high floristic diversity, and values under the mean will have low floristic diversity.

**Chromosome number variation**

To quantify the CN variation of the species within the PWA we followed all the steps proposed by Peruzzi et al. (2014). We calculated mean CN, standard deviation (SD) and frequency of B and Odd chromosomes. To quantify the variation of CN we calculated the square root of the

| Area Number | PWA       | Surface (Km²) | TFD | NSC | CSS (%) | Mean CN | SD CN | fB+ fOCN | ICNH |
|-------------|-----------|---------------|-----|-----|---------|---------|-------|----------|------|
| 1           | RN Las Nalcas | 175.3        | 137 | 22  | 16.0    | 46.7    | 50.3  | 0        | 30.7 |
| 2           | RN China Muerta | 111.7        | 102 | 25  | 24.5    | 44.6    | 47.4  | 0        | 30.2 |
| 3           | PN Villarrica | 610.0         | 199 | 31  | 15.5    | 45.2    | 43.3  | 0.03     | 29.1 |
| 4           | RN Malalcahuello | 127.9        | 283 | 35  | 12.3    | 56.4    | 69.6  | 0        | 41.2 |
| 5           | PN Tolhuaca   | 633.7         | 324 | 41  | 12.6    | 50.6    | 47.2  | 0.02     | 32.1 |
| 6           | PN Conguillio | 608.0         | 359 | 44  | 12.2    | 53.6    | 62.8  | 0        | 38.1 |
| 7           | PN Huerquehue | 125.0         | 245 | 29  | 11.8    | 55.4    | 54.2  | 0        | 36.0 |
| 8           | RN Malleco    | 166.2         | 289 | 35  | 12.1    | 48.2    | 45.0  | 0.03     | 30.8 |
| 9           | PN Nahuelbuta | 68.3          | 311 | 31  | 9.96    | 41.5    | 40.0  | 0.03     | 26.8 |
| 10          | RN Villarrica | 613.5         | 96  | 16  | 16.6    | 42.8    | 31.0  | 0        | 24.0 |
| 11          | MN Contulmo   | 820.0         | 239 | 33  | 13.8    | 51.7    | 46.9  | 0.03     | 32.4 |
| 12          | MN Cerro Ñielol | 844.0       | 165 | 34  | 20.6    | 55.7    | 61.6  | 0.03     | 38.5 |
| 13          | RN Alto Biobio | 330.0        | 119 | 14  | 11.7    | 33.1    | 38.3  | 0        | 23.4 |
area of the ideal triangle built in a three-variable radar plot, where the vertices of the triangle are defined by mean CN, SD, and % (fB + fOCN). The triangle gives a graphic representation of CN variation in a group (PWA in our study) and its area can be easily seen as the sum of the three areas subtended by the smaller triangles set along the plot axes. We defined this value as the Index of Chromosome Number Heterogeneity (ICNH), which was calculated according to the formula:

$$ICNH = \sqrt{\frac{2\pi}{3} \frac{ab + ac + bc}{2}}$$

where a is the mean chromosome number (CN), b is the standard deviation (SD) of CN, and c is % (fB chromosomes + fOdd CN). The resulting value can vary from 0, if no variation occurs in a group, to +∞, although very high values can only be reached theoretically.

The mean CN±SD calculated for each PWA were then statistically compared between areas. Statistical pre-tests based on Kolmogorov-Smirnov and Shapiro-Wilk (using the same dataset) suggest the use of non-parametric analyses, given that the chromosome numbers showed an abnormal distribution (p>0.05). Thus, mean CN across PWA were compared using the Kruskal–Wallis test.

Correlation coefficients were calculated for mean CN and SD and for fB and fOdd CN, and grouped in three levels; weak (up to 0.3), moderate (0.4–0.7), and strong (>0.7) (Peruzzi et al., 2014).

To determine the floristic similarity among the different PWA, a cluster analysis was carried out using SIMPROF (Similarity Profile) (p<0.05). This analysis is based on the conformation of a matrix consisting of the presence or absence of species, for which the Jaccard similarity index was calculated (Pielou, 1975).

**Spatial analysis**

The identification of geographic locations among the 13 PWA was carried out through spatial analysis while ArcGIS 10.3, Datum WGS 84, and Time Zone 18 S were used for the cartographic analysis. PWA species information was supplied by the Chilean National Forest Corporation (CONAF). The resulting cartography was contrasted with base lines, floristic cadastres, and available literature regarding PWA in order to determine the floristic diversity and relationship with the CN variation of the species obtained from the Chilean Plants Cytogenetic Database and other articles as cited above.

**RESULTS**

The ICNH values and other quantitative parameters for PWA are shown in Table 1. In Figure 1 plots are shown with the respective triangle of three PWA with higher ICNH and three PWA with lower ICNH. In total, we found CN data for 84 plant species present in the 13 PWA, encompassing 57 genera and 36 families belonging to Pteridophytes, Gymnosperms, and Angiosperms (Appendix 1 includes a list of studied species with CN and the PWA where each species occur). The number of species analysed here represent 27.3% of the total Chilean flora with available CN data (ca. 307 species studied to date), covering 12.3% of the total terrestrial PWA established in Chile. In addition, depending on the PWA, different percentages of species have been studied, ranging from 9.9% in PN Nahuelbuta to 24.5% in RN China Muerta.

According to floristic cadastres, PN Conguillío showed the highest floristic diversity among PWA with 359 species, while RN Villarrica was among the lowest with only 96 species. With regard to the number of species with cytogenetic data, the more extreme values were found in PN Conguillio with 44 species and RN Alto Biobío with only 14 species. The percentage of species with CN data (NSC – number of species with CN data) varied between 9.6% (PN Nahuelbuta) and 24.5% (RN China Muerta), although 11 PWA showed percentages higher than 11%. The mean species diversity estimated here for the 13 PWA was 220.6±90.3 species. Thus, seven PWA were higher than this mean value and six PWA were lower.

Only RN Malalcahuello presented very high ICNH (>40) and eight PWA presented high ICNH (>30). In turn, four PWA displayed low ICNH (<30). The coverage of the triangles shows clear differences between the higher and lower ICNH values obtained according to the score. The correlation found between mean CN and SD for the overall dataset was strong and positive (r=0.80), while the correlation between fB and fOCN was not estimated since fOCN was zero in all 13 PWA. Mean CN did not show significant differences between the 13 PWA when compared by means of the Kruskal–Wallis test (with significance at a 0.8011 level).

The floristic similarity cluster presented four significantly different groups with a resemblance higher than 60%: i) RN Alto Biobío, ii) MN Contulmo and MN Cerro Ñielol, iii) RN Villarrica, and iv) the remaining nine PWA (Figure 2A). Thus, the more related PWA groups shared a higher number of species among them. As a reference, only one species (Nothofagus dombeysi) is shared by all 13 PWA, whereas 24 species are shared by seven PWA, i.e. around 50% of the total PWA analysed here.

It is worth noting that, within our dataset, the diploid CN 16, 18, 22, 26, and 28 were observed in all studied PWA, whereas the polyploid CN shared among some PWA were 28, 116, 144, 164, 216, and 328, depending on the species. In total, 28 different CN were found across all 13 PWA, including the 84 species. Additionally, the
modal CN for 12 PWA was 26, with the exception of MN Contulmo, which presented a modal number of 22.

**DISCUSSION**

Correlation analyses between species diversity and genetic diversity as a whole (as a community) have not been previously reported for Chilean plants. We studied the correspondence between both levels - species diversity and genetic diversity - but using the CN diversity as a genetic character with data available up to date in Chilean plants.

Chromosome number diversity

The analyses carried out in this study showed that six PWA with high species diversity (≥220 species) have a clear correlation with very high (>40) and high (>30) ICNH values, according to the scores obtained herein. In turn, three PWA with low species diversity (<220 species) also have a clear correlation with low ICNH values (<30) (See Table 1 and Figure 1 showing PWA with higher and lower ICNH). However, some PWA do not fit into this trend: for example, PN Nahuelbuta has high species diversity (311 species) but low ICNH (value of 26.8), whereas all three, MN Cerro Nielol,
RN Las Nalcas and RN China Muerta, have low species diversity (165 species, 137 species, and 102 species respectively) but high ICNH (value of 38.5, 30.7 and 30.2 respectively). It is important to remark that the flora of MN Cerro Ñielol, a PWA located within the urban radius, is made up mainly of native species remaining from the original forest, but a high number of native species have also been introduced from other nearby areas as a conservation tool (Saavedra and Morales, 2008). Many of these species have CN data available in the databases (Jara Seguel and Urrutia, 2020), thus increasing the CN diversity of the MN Cerro Ñielol flora.

The very high and high ICNH values observed in six PWA is indicative of high CN diversity among them. Nevertheless, all 13 PWA studied here showed higher ICNH than was previously estimated for 243 Chilean species of vascular plants with an ICNH of 22.4 (Peruzzi et al., 2014), but until that date, the databases did not include several polyploid species of pteridophytes which were added in our study. The ICNH for the six PWA mentioned above could be explained by the presence of polyploid species (~25 in total) representing four genera of ferns and 12 genera of angiosperms with variable CN, ranging from 28 to 328, many of them being tetraploid, hexaploid or octoploid (Jara Seguel et al., 2006; Jara Seguel and Urrutia, 2012; Jara Seguel and Urrutia Estrada, 2018; Morero et al., 2015) (Figure 1A, B, and C). It is worth noting that diploid angiosperm species are the predominant plant group within the dataset (49 species in total), although they present a lower CN ranging from 8 to 32 as compared to polyploid taxa. An explanation for all ICNH >30 found in nine PWA (independent of the floristic diversity) may be related to the growth form of the plants. This is so because chromosome evolution proceeds much faster in herbs than in angiosperm shrubs and trees, as well as in conifers, as discussed by Levin and Wilson (1996), who described a net increase in the diversity of chromosome numbers and species numbers over time. A similar relationship may have occurred during the evolution of the flora in Chilean forests. As shown in our results, RN Malalcahuello, MN Cerro Ñielol, and PN Conguillio with higher ICNH (>30) present a high percentage of herbs (between 70% and 85% of herbs including ferns) vis-à-vis shrubs and trees (including conifers) within their flora with available CN data (NSC). In contrast, PN Nahuelbuta, RN Villarrica and RN Alto Biobio with lower ICNH have percentages of herbs lower than 67% (between 43% and 67%). All remaining PWA not mentioned above with ICNH >30 have a percentage of herbs of between 62% and 78%. Thus our findings show a clear relationship between high number of herb species and CN diversity.

All these aspects related to genetic variation are decisive for conservation biology (Severns and Liston, 2008) and represent a primary objective pursued by the PWA system. However, the presence of scant polyploid species with known CN data within the chromosome dataset from PN Nahuelbuta, RN Villarrica, RN Alto Biobio, and PN Villarrica could explain their lower ICNH (Figure 2D, E, and F). In these PWA only a few genera of pteridophytes and angiosperms with polyploidy records are present (Arriagada, 2002; CONAF, 2009) but unfortunately no chromosome counts are available for the species that inhabit these areas.

Other relevant observations are related to the
geographical location of the PWA along three separate longitudinal strips represented by the Andean mountain range, the Central valley, and the Nahuelbuta mountain range. None of these strips show a clear correspondence with high or low ICNH values, despite the differences in their climatic, geographic and geological conditions, which undoubtedly affect the flora (Montgomery et al., 2001; Moreira Muñoz, 2011). As discussed by Levin and Wilson (1996), rates of evolution at both the karyotypic and organisinal levels are related to the breeding structure of species and to environmental predictability. Using this reasoning to understand the cytoevolutionary process in PWA, it is remarkable to observe that different environments exist along their expanse. As such, small populations in variable habitats experiencing fluctuations in habitat hospitality are the most conducive to the fixation of chromosomal novelties (changes in CN). On the contrary, large continuous populations –where climatic and biotic pressures are stable over time– are likely to be more conservative in terms of chromosomal structure (stable CN). These cytogenetic aspects have not been studied for a large part of continental Chilean plants and cytoevolutionary mechanisms have only been described in some detail for some genera of herbaceous species (e.g., Chaetanthera, Alstroemeria; Baexa et al., 2009; 2015; 2018).

With regard to B chromosomes, their occurrence in Chilean plants is very low (Jara Seguel and Urrutia, 2012) and their contribution to the ICNH values is negligible. Only one species within the studied dataset (Lapageria rosea) has been described as having B chromosomes (Jara Seguel and Zúñiga, 2004) in six PWA. Moreover, values of fOCN=0 were obtained in all PWA, since there were no species with an odd number of chromosomes across the dataset.

We also observed that various PWA share several species and therefore their respective CN. This may explain the non–significant differences in mean CN observed among PWA despite the CN heterogeneity described when estimating the correlation between mean CN and standard deviation. For example, seven PWA –ca. 50% of the total areas studied here share the presence of 25 species. The CN shared among PWA were 16, 18, 22, 26, 28, 116, 144, 164, 216 and 328 of a total of 28 different numbers. Similarity analysis showing the relation among PWA support this observation, where areas forming the same group share a higher number of species and therefore their respective CN (Figure 2A). Other PWA share a lower number of species both with other areas and when separated as a single branch in the analysis (RN Alto Biobío and RN Villarrica). In addition, a few species of different genera (taxonomically unrelated) or divisions present in various PWA share the same CN which may additionally support the non–significant differences observed in mean CN. For example, a modal CN of 26 was found in 12 PWA, appearing in three genera of three different divisions within the dataset [e.g., the angiosperm genus Nothofagus (N. alpina, N. antarctica, N. dombeyi, N. obliqua and N. pumilio), the gymnosperm Araucaria araucana, and the pteridophyta Hymenophyllum dentatum and H. tunbrigense (Jara Seguel and Urrutia Estrada, 2020)]. An exception was PN Contulmo (group two in Figure 2A), presenting a high ICNH (>30) with a modal CN of 22, which was predominant over species with 26 chromosomes. The taxonomic composition in PN Contulmo showed a predominance of species with CN=22 (e.g., one species for each of the Ugni, Luma, Galium, Podanthus, and Chaetanthera genera; Jara Seguel and Urrutia Estrada, 2020), unlike other PWA. Biogeographically, it is worth noting that most of the genera shared among the PWA have a Gondwanan distribution, which is present in southern South America and Oceania (Jara Seguel et al., 2006; 2010; 2014; Chacon et al., 2012a; Morero et al., 2015). Some of these genera are part of the paleo–endemic flora, mainly ferns and conifers, displaying a recurrent presence in the region that includes the PWA studied here (Southern region according to Scherson et al., 2017). Thus, we suggest that many of the CN found here could be plesiomorphic features within some families, specifically those containing species representative of paleo–endemic flora. Many of these CN, as well as the genera and family that contain them, are also shared with New Zealand and Australian vascular flora (Jara Seguel et al., 2006; 2010; 2014; Morero et al., 2015).

As mentioned above, CN data are available for ca. 6.6% of Chilean vascular plant species of which only 84 species present in the 13 PWA studied here have available CN data. Based on our results, it is possible that the real CN variation in these PWA might be even greater than the one estimated in this study, because a vast part of native species has not yet been cyogenetically studied, i.e. between 75% and 90%, depending on the PWA (CSS in Table 1). This CN diversity could also be superimposed on gene variation among species, thus adding a new matrix of analyses (molecular) to the two described here. In this way, previous studies of the vascular flora of Chile studied as a community, e.g. Scherson et al. (2017), based on DNA sequences and the present work based on CN diversity, provide a robust framework to continue studying the correlation between floristic diversity and genetic diversity at various levels, thus highlighting the genetic diversity present in the Chilean flora and also justifying its protection where PWA have a crucial role.
### Appendix 1

Total species analysed in 13 PWA from Araucania Region, Southern Chile (84 species). CN, chromosome number (2n). Numbers represent each PWA as described in Table 1.

| Family          | Species                  | CN | PWA where each species occur |
|-----------------|--------------------------|----|-----------------------------|
| Alstroemeriaceae| *Alstroemeria aurea*     | 16 | 1-2-3-4-5-6-7-8-9-10         |
|                 | *Alstroemeria ligu*      | 16 | 6-11                        |
|                 | *Alstroemeria patagonica*| 16 | 4                           |
|                 | *Alstroemeria pulchra*   | 16 | 12                          |
|                 | *Bomarea salsilla*       | 18 | 11-12                       |
|                 | *Luzuriaga radicans*     | 20 | 5-6-7-8-9-11-12             |
| Amaryllidaceae  | *Phycella ignea*         | 16 | 9                           |
|                 | *Rhophiala advena*       | 18 | 4-8                         |
|                 | *Rhophiala andicola*     | 16 | 1-2-3-4-6-13                |
|                 | *Rhophiala montana*      | 18 | 2-13                        |
| Araucariaceae   | *Araucaria araucana*     | 26 | 1-2-3-4-5-6-7-8-9-10-12-13  |
| Aspleniaceae    | *Asplenium dareoides*    | 144| 1-2-3-4-5-6-7-8-10-11       |
| Asteraceae      | *Baccharis patagonica*   | 18 | 4-5-6-7-9                   |
|                 | *Chaetanthera chilensis* | 22 | 1-4-5                       |
|                 | *Chaetanthera elegans*   | 22 | 11                          |
|                 | *Erigeron andicola*      | 36 | 5-13                        |
|                 | *Gnaphalium viravira*    | 28 | 6                           |
|                 | *Haplopappus glutinosus* | 10 | 6-7                         |
|                 | *Haplopappus grindelioides* | 10 | 4                           |
|                 | *Hypocharis acaulis*     | 8  | 1-4-13                      |
|                 | *Hypocharis tenuifolia*  | 8  | 3-4-6                       |
|                 | *Lagenophora hariotii*   | 18 | 2-7                         |
|                 | *Podanthus ovatifolius*  | 22 | 11                          |
|                 | *Senecio subumbellatus*  | 80 | 4                           |
| Berberidaceae   | *Berberis empetrifolia*  | 14 | 1-2-3-4-5-6-10-13           |
|                 | *Berberis ilicifolia*    | 28 | 8                           |
|                 | *Berberis microphylla*   | 28 | 1-2-3-4-5-6-7-8-9-10-12-13  |
| Berberidopsidace| *Berberidopsis corallina*| 42 | 11                          |
| Blechnaceae     | *Blechnum chilense*      | 66 | 4-5-6-7-8-9-10-12           |
|                 | *Blechnum hastatum*      | 66 | 1-2-3-4-5-6-7-8-9-10-11-12  |
|                 | *Blechnum mochaenium*    | 66 | 5-6-11-12                   |
|                 | *Blechnum penna-marina*  | 66 | 1-2-3-4-5-6-7-8-9-12        |
| Celastraceae    | *Azara serrata*          | 18 | 9                           |
| Cupressaceae    | *Austrocedrus chilensis* | 22 | 2-5-6-8-13                  |
| Dryopteridaceae | *Megalastrum spectabile* | 82 | 6-7-9-11-12                 |
|                 | *Polystichum andinum*    | 164| 13                          |
|                 | *Polystichum chilense*   | 164| 3-4-5-6-7-8-11              |
|                 | *Polystichum plicatum*   | 164| 3-4-5-6-7                   |
|                 | *Polystichum subintegrerrimum* | 328| 4-6-12                     |
Appendix 1. Continuation. Total species analysed in 13 PWA from Araucania Region, Southern Chile (84 species). CN, chromosome number (zn). Numbers represent each PWA as described in Table 1.

| Family          | Species                        | CN  | PWA where each species occur   |
|-----------------|--------------------------------|-----|--------------------------------|
| Equisetaceae    | *Equisetum bogotense*          | 216 | 1-2-4-5-6-7-8-9-11-12          |
| Eremolepidaceae | *Lepidoceras chilense*         | 72  | 5-6-7                          |
| Fabaceae        | *Adesmia boronioides*          | 20  | 5                              |
| Grossulariaceae | *Ribes magellanicum*           | 32  | 1-2-3-4-5-6-8-9-13             |
| Hymenophyllaceae| *Hymenoglossum cruentum*       | 72  | 11-12                         |
|                | *Hymenophyllum caudiculatum*   | 72  | 6-7-12                        |
|                | *Hymenophyllum dentatum*       | 26  | 3-6-11-12                     |
|                | *Hymenophyllum ferrugineum*    | 72  | 5                              |
|                | *Hymenophyllum tenuigrissense* | 26  | 11                            |
| Iridaceae       | *Herbertia lahue*              | 42  | 11-12                         |
|                | *Libertia chilensis*           | 72  | 5-6-8-9-11-12                 |
| Lardizabalaceae | *Lardizabala biternata*        | 28  | 3-8-12                        |
| Lauraceae       | *Persea lingue*                | 24  | 3-4-5-7-9-12                  |
| Lentibulariaceae| *Pinguicula antarctica*        | 16  | 9                             |
| Loasaceae       | *Loasa acanthifolia*           | 38  | 2-3-5-6-8-11-12               |
| Loranthaceae    | *Desmaria mutabilis*           | 32  | 5-6-8-9                       |
|                | *Notanthera heterophylla*      | 24  | 12                            |
|                | *Tristerix corymbosus*         | 24  | 2-5-6-8-9-12                  |
| Monimiaceae     | *Peumus boldus*               | 78  | 3-11-12                       |
| Myrtaceae       | *Luma apiculata*               | 22  | 3-4-5-6-7-8-9-11-12           |
|                | *Myrteola nummularia*          | 44  | 5-6                            |
|                | *Ugni molinae*                 | 22  | 3-8-11-12                     |
| Nothofagaceae   | *Nothofagus alpina*            | 26  | 1-2-3-4-5-6-7-8-9-10-11-12    |
|                | *Nothofagus antarctica*        | 26  | 1-2-3-4-5-6-7-8-9-10-11-13    |
|                | *Nothofagus dombeyi*           | 26  | 1-2-3-4-5-6-7-8-9-10-11-12-13 |
|                | *Nothofagus obliqua*           | 26  | 1-2-3-4-5-6-8-9-10-11-12      |
|                | *Nothofagus pumilio*           | 26  | 1-2-3-4-5-6-7-8-9-10-13       |
| Onagraceae      | *Fuchsia magellanica*          | 44  | 1-2-3-4-5-6-8-9-10-12         |
| Philesiaceae    | *Lapageria rosea*              | 30+2B | 3-5-8-9-11-12               |
|                | *Philesia magellanica*         | 30  | 8                             |
| Plantaginaceae  | *Ourisia coccinea*             | 16  | 3-6-7                         |
| Poaceae         | *Danthonia araucana*           | 24  | 9                             |
|                | *Danthonia malacantha*         | 48  | 11                            |
|                | *Festuca gracillima*           | 42  | 10                            |
| Proteaceae      | *Orites myrtoidea*             | 28  | 4-5                           |
| Pteridaceae     | *Adiantum chilense*            | 116 | 1-2-3-4-5-6-7-8-9-11-12      |
| Rubiaceae       | *Galium araucanum*             | 22  | 11                            |
|                | *Galium hypogercium*           | 22  | 1-2-3-5-6-7-8-9-11-12-13     |
|                | *Nertera granadensis*          | 44  | 1-2-3-4-5-6-7-8-9-10-11-12   |
Appendix 1. Continuation. Total species analysed in 13 PWA from Araucania Region, Southern Chile (84 species). CN, chromosome number (2n). Numbers represent each PWA as described in Table 1.

| Family            | Species                        | CN | PWA where each species occur |
|-------------------|--------------------------------|----|-----------------------------|
| Rutaceae          | Pitavia punctata               | 36 | 8                           |
| Solanaceae        | Solanum crispum               | 24 | 8-10-11                     |
|                   | Solanum etuberosum            | 24 | 5                           |
|                   | Solanum tuberosum             | 24 | 4-6                         |
| Tecophilaeaceae   | Conanthera bifolia            | 28 | 11                          |
| Verbenaceae       | Rhaphithammus spinosus         | 18 | 1-3-4-5-6-7-8-9-11-12       |

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