



## Leaf morphological and genetic divergence in populations of *Drimys* (Winteraceae) in Chile

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**ABSTRACT.** The genus *Drimys* is distributed in Chile from semi-arid zones to sub-Antarctic forests; there are three species of this tree, *D. andina*, *D. confertifolia* and *D. winteri*, the latter with varieties *chilensis* and *winteri*. Northern populations are found in small disjunct natural refuges, specifically mountain cloud forests and the bottom of ravines. The size and continuity of populations are greater in the south, where wetter conditions prevail. Morphological differences between populations have been observed, particularly between the northern populations of Fray Jorge and Talinay. This observation, led to the following questions: a) what is the level of morphological and

genetic divergence among the populations of *Drimys* in Chile? and b) do the populations from Fray Jorge/Talinay, currently classified as *D. winteri* var. *chilensis*, differ genetically from the other populations of this variety? To answer these questions, we collected leaf samples from 37 populations of all Chilean *Drimys*, performed leaf morphology analysis and estimated genetic divergence using RAPD markers. We found a high degree of leaf morphological and genetic divergence between the populations of Fray Jorge/Talinay and the other Chilean species of *Drimys*. The morphological and genetic divergence among varieties of *D. winteri* was greater than that among the species of *Drimys*, which may indicate problems with their taxonomic classification.

**Key words:** *Drimys*; RAPD; Fray Jorge/Talinay; Divergence

## INTRODUCTION

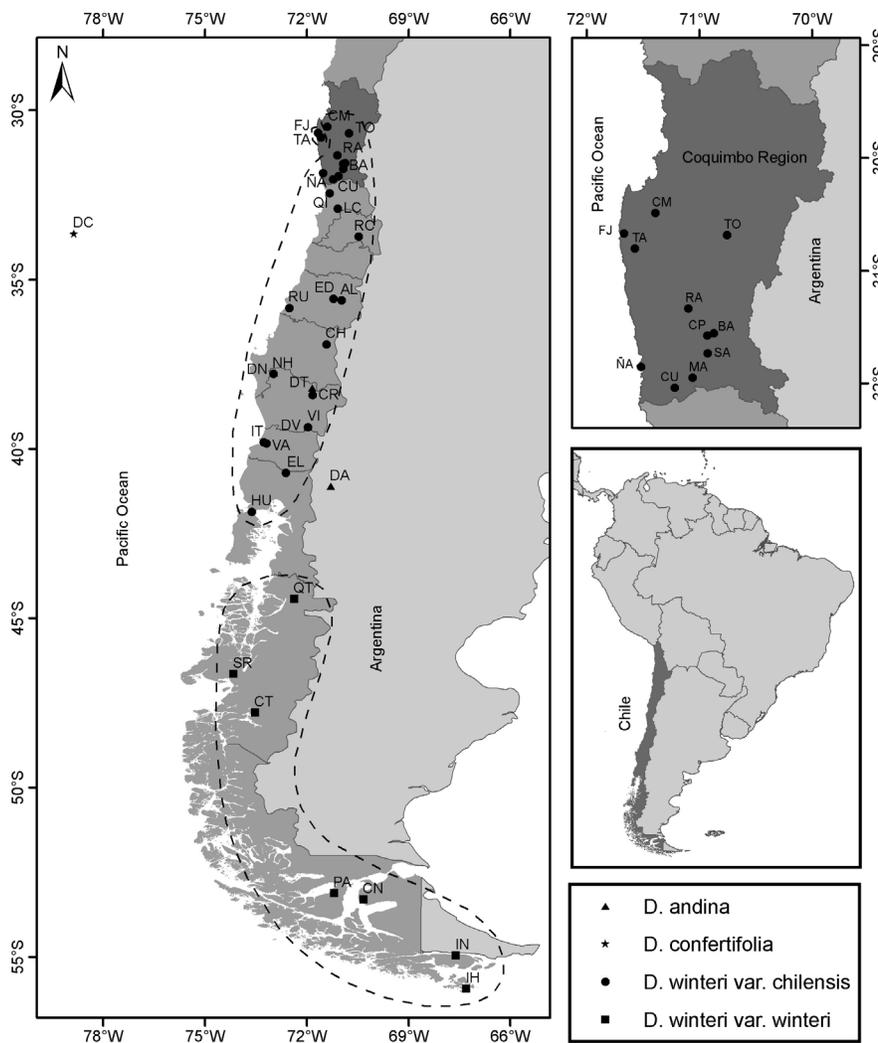
In Chile, the genus *Drimys* (canelo) is distributed discontinuously from the semi-arid zone (30°47'S) to the sub-Antarctic forest (55°57'S). Rodríguez and Quezada (2001) recognized three species in Chile, which are differentiated principally by type of habitat, floral and leaf structures, although there is considerable overlapping of character states. These species are a) *Drimys winteri* J.R. Forst. and G. Forst., with two varieties: *D. winteri* var. *chilensis* (DC) A. Gray, endemic to Chile (30°20' to 46°25'S) and *D. winteri* var. *winteri*, endemic to sub-Antarctic forests (45°44' to 55°58'S); b) *Drimys andina* (Reiche) Rodríguez and Quezada (2001), a shrub endemic to sub-Antarctic forests (37°43' to 41°34'S), and c) *Drimys confertifolia* Phil., endemic to the Juan Fernández Archipelago (33°38'S-78°49'W) (Rodríguez and Quezada, 2001). Among the populations of *D. winteri* var. *chilensis* are two isolated mountaintop populations in Fray Jorge/Talinay whose leaf morphology is highly divergent from the rest of the species of the genus in Chile. These populations date back to the formation, development and paleodistribution of Chilean forests during the Tertiary (Croizat, 1962). Villagrán et al. (2004) suggested that the populations of Fray Jorge/Talinay are the product of a migration before the differentiation of the Chilean-Argentinean species and of the varieties described for *D. winteri*. In a study of Chilean populations of *Drimys* from its entire distribution range and analysis of individuals grown in a common garden, we observed a large divergence in leaf morphology both within species and between populations, particularly between the populations of Fray Jorge/Talinay and other populations of *D. winteri* var. *chilensis*.

Based on these observations and the background information mentioned above we asked two questions: a) what is the level of genetic and leaf morphological divergence among populations of *Drimys* in Chile? b) are the populations of Fray Jorge/Talinay genetically differentiated from other populations of *D. winteri* var. *chilensis*? To answer these questions we collected samples from 37 populations of all Chilean *Drimys*, performed leaf morphology analysis and estimated genetic divergence using RAPD markers both within and between populations. We also examined the congruence between leaf morphological and genetic divergence.

**MATERIAL AND METHODS**

**Study sites**

We collected samples of 32 Chilean populations of *D. winteri* between 30°47' and 55°57'S (25 of *D. winteri* var. *chilensis* and seven of *D. winteri* var. *winteri*). We also collected samples from four populations of *D. andina* (three from Chile and one from Argentina) and one sample of *D. confertifolia* (Robinson Crusoe Island, Archipelago Juan Fernández) (Figure 1 and Table 1).



**Figure 1.** Distribution map of the 37 populations studied of the genus *Drimys*. The Coquimbo Region, with the 11 populations of *D. winteri* var. *chilensis*, is amplified at the upper right. Population abbreviations are in Table 1.

**Table 1.** Geographic location of the 37 populations studied of *Drimys* with their taxonomic classification according to Rodríguez and Quezada (2001).

Populations	S	R	Coordinates		Populations	S	R	Coordinates	
<i>D. winteri</i> var. <i>chilensis</i>					<i>D. winteri</i> var. <i>winteri</i>				
Camaronés	CM	IV	30° 29'	71° 23'	Curacautín	CR	IX	38° 24'	71° 49'
Tomé de Rapel	TO	IV	30° 40'	70° 45'	Villarrica	VI	IX	39° 21'	71° 57'
Fray Jorge	FJ	IV	30° 40'	71° 39'	Isla Teja	IT	X	39° 48'	73° 15'
Talinay	TA	IV	30° 48'	71° 34'	Valdivia	VA	X	39° 49'	73° 11'
Rabanales	RA	IV	31° 20'	71° 05'	Entre Lagos	EL	X	40° 41'	72° 37'
La Capilla	CP	IV	31° 34'	70° 55'	Hueldén	HU	X	41° 51'	73° 36'
El Bato	BA	IV	31° 33'	70° 52'	<i>D. winteri</i> var. <i>winteri</i>				
San Agustín	SA	IV	31° 43'	70° 55'	Queulat	QT	XI	44° 25'	72° 21'
Nague	NA	IV	31° 51'	71° 30'	San Rafael	SR	XI	46° 38'	74° 09'
El Mauro	MA	IV	31° 56'	71° 03'	Caleta Tortel	CT	XI	47° 47'	73° 31'
Culimo	CU	IV	32° 02'	71° 12'	Punta Arenas	PA	XII	53° 06'	71° 11'
Quintero	QI	V	32° 26'	71° 19'	Los Canelos	CN	XII	53° 17'	70° 19'
La Campana	LC	V	32° 53'	71° 05'	Isla Navarino	IN	XII	54° 56'	67° 35'
Río Clarillo	RC	RM	33° 43'	70° 27'	Isla Hornos	IH	XII	55° 55'	67° 17'
Altos de Lircay	AL	VII	35° 36'	70° 58'	<i>D. confertifolia</i> and <i>D. andina</i>				
El Durazno	ED	VII	35° 34'	71° 12'	<i>D. confertifolia</i> (A.J. Fernández)	DC	V	33° 38'	78° 51'
Los Ruiles	RU	VII	35° 50'	72° 30'	<i>D. andina</i> (Argentina)	DA	Ar	41° 06'	71° 17'
Chillán	CH	VIII	36° 54'	71° 24'	<i>D. andina</i> (Villarrica)	DV	VIII	39° 21'	71° 57'
Nahuelbuta	NH	IX	37° 47'	72° 59'	<i>D. andina</i> (Tolhuaca)	DT	IX	38° 12'	71° 50'
					<i>D. andina</i> (Nahuelbuta)	DN	IX	37° 45'	72° 58'

S = acronym; R = Chilean political regions (including one population from Argentina = Ar).

## Leaf morphological analysis

We randomly collected 10 mature (completely expanded) but not senescent leaves reachable by hand from 10 individuals per population, both from the borders and interior of forests, with a minimum distance of 50 m between individuals. We studied seven variables of leaf morphology in *Drimys*: aspect (blade form), roundness (both calibrated by leaf forms established in Hickey and King (1998)), specific weight, thickness, leaf length/width, and length and width of the petiole. For comparisons we used the median value of each variable per population. To determine differences among populations, we performed three analyses. First, we determined the latitudinal tendency of each variable at the population level using linear regression (SigmaStat 2.0). Second, we performed a cluster analysis (MVSP 3.12). We calculated the Euclidean distance between populations and applied the UPGMA algorithm to construct a dendrogram; to have a critical distance to define significant groups we used the minimum Euclidean distance expected by chance, using the 5% percentile of the distribution (PopTools 2.7). Finally, we conducted a principal components analysis (PCA).

## Molecular analysis

To estimate the levels of genetic divergence within and between populations of Chilean *Drimys* we obtained RAPD markers. DNA was extracted from leaf tissue conserved on silica gel, using the protocol of JanBen (1997). To obtain RAPD markers we used the modified protocol of Williams et al. (1990); PCRs used a total volume of 25 µL, which contained 5 µL ADN (~5 ng), 10.8 µL purified sterile water, 3.2 µL 25 mM MgCl<sub>2</sub>, 2 µL 10X buffer, 2 µL 1 mM dNTPs (Invitrogen), 1 µL 10 µM primer (Invitrogen), and 1 µL 1 U/µL Taq polymerase

(Promega). PCR was performed in a Mastercycler gradient (Eppendorf) thermocycler, with an initial temperature of 94°C, followed by 34 cycles of 5 min of denaturing at 94°C, 1 min of alignment at 38°C and 1 min of extension at 72°C, with temperature changes of 1° per second, and a final extension for 10 min at 72°C.

We used 16 primers (see Table 2). PCR products were separated by horizontal electrophoresis at 55 V for 2 h and 30 min on 2% agarose gels with ethidium bromide in 1X TBE buffer, pH 8.0. From the banding patterns we created a binary matrix of the presence/absence of each band for each individual. We restricted the analysis to 142 fragments (113 polymorphic and 29 monomorphic) with sizes from 141 to 1420 bp. With this matrix we performed the following analyses: a) dendrograms (MVSP 3.12): we used the similarity coefficient of Nei and Li (1979) with the UPGMA algorithm, confidence levels were estimated with a bootstrap method with 1000 iterations (FreeTree 0.9.1.5.0); b) principal coordinates analysis (PCO; MVSP 3.12); c) molecular analysis of variance (AMOVA 1.55) at three hierarchical levels: among groups (all species and Fray Jorge/Talinay)  $\Phi_{ct} = S^2_R / (S^2_w + S^2_p + S^2_R)$ , among populations within groups  $\Phi_{sc} = S^2_p / (S^2_w + S^2_p)$  and within populations  $\Phi_{st} = 1 - ((1 - \Phi_{ct}) * (1 - \Phi_{sc}))$ , and d) population structure (POPGENE 1.3.2) among populations of *D. winteri*. We estimated the percentage of polymorphic loci, number of genotypes and the Shannon index (Lewontín, 1972). We also applied the correction of Lynch and Milligan (1994) to the matrix obtained to evaluate the effect of using the loci with little variability.

**Table 2.** Decanucleotide primers (P) used in the RAPD analysis for 37 populations of *Drimys*.

P	Sequence	P	Sequence	P	Sequence	P	Sequence
A01	CAGGCCCTTC	C07	CCTGGATTCC	H15	AATGGCGCAG	O03	CTGTTGCTAC
B14	TCCGCTCTGG	F06	GGGAATTCGG	M07	CCGTGACTCA	O10	TCAGAGCGCC
B17	AGGGAACGAG	F12	ACGGTACCAG	M13	GGTGGTCAAG	O16	TCGGCGGTTC
B20	GGACCCTTAC	H02	TCGGACGTGA	M17	TCAGTCCGGG	X07	GAGCGAGGCT

## Comparative analysis of leaf morphology and RAPD

We performed two complementary analyses to evaluate the association among morphological, molecular and geographic variables: a) the Mantel test (MVSP 3.12), comparing the matrixes of genetic, geographic and Euclidean morphological distances, evaluating the statistical significance of the correlation coefficient between each pair of matrixes by a randomization procedure repeated 2000 times, using both tails ( $P_{2 \text{ tails}}$ ) (PopTools 2.7), and b) discriminant analysis (MVSP 3.12) to determine the relationship between morphological data and the groups obtained in the genetic analysis.

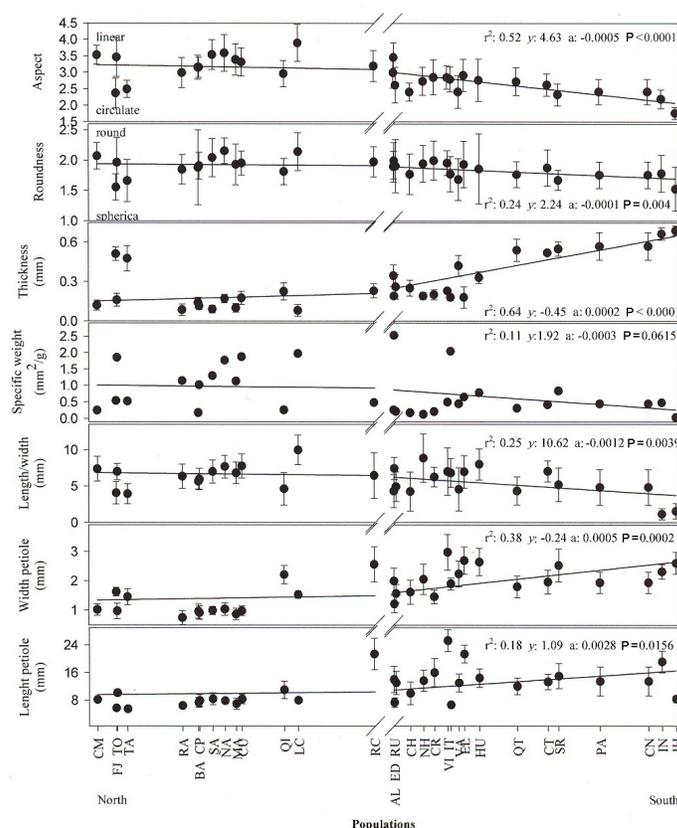
## Reproducibility of RAPD

Since some authors question the reproducibility of RAPD (Xu et al., 1995; Rieseberg, 1996) we evaluated the confidence of our analysis. We randomly selected 48 samples of the total of 363, dividing them randomly into 16 groups (one primer per group) in which we repeated the analyses with one year old and with recently prepared DNA and chemical components.

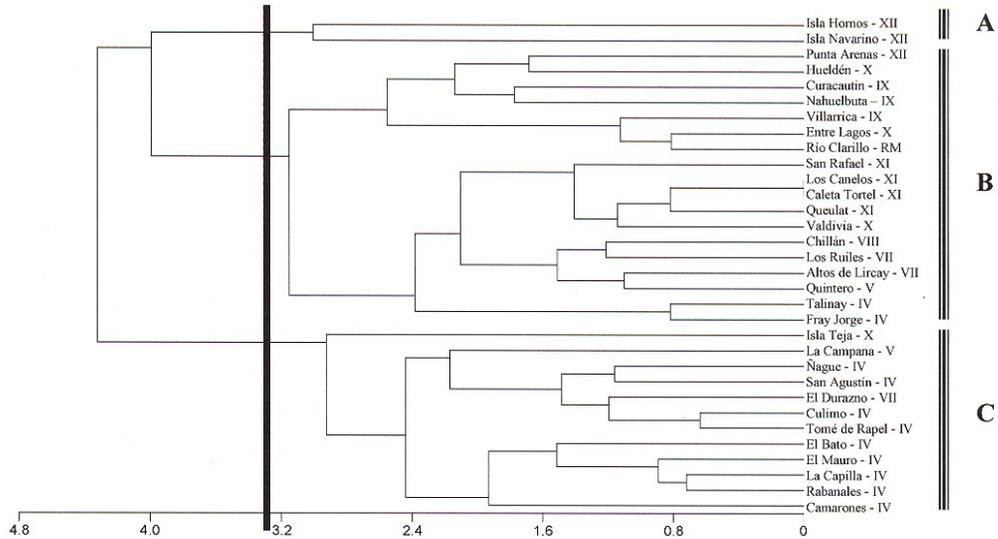
## RESULTS

### Leaf morphological analysis

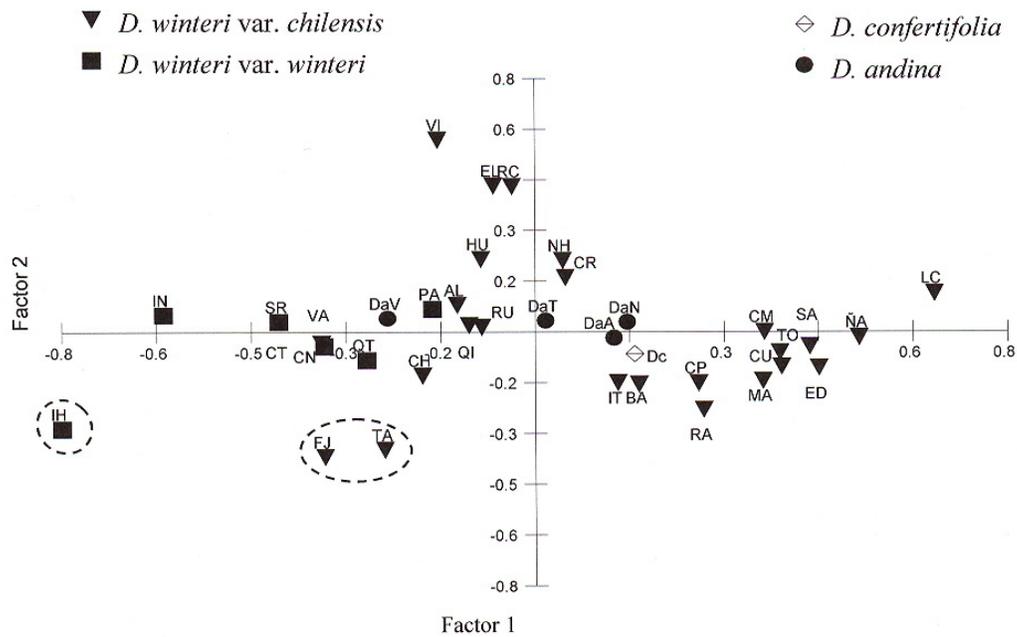
There were clear latitudinal trends in leaf morphology; leaves of *D. winteri* populations were more oblong, spherical, and thicker, and petioles were longer and thicker the farther south the population was located. Differences in specific weight, however, were not quite significant ( $P = 0.062$ ) (Figure 2). Three groups of populations were found by cluster analysis: a) *D. winteri* var. *winteri* with *D. winteri* var. *chilensis* from Fray Jorge/Talinay, b) *D. andina* with *D. confertifolia*, and c) the other *D. winteri* var. *chilensis* populations (Figure 3). The first two axis of the PCA explained most of the total variance (75.8%). The varieties of *D. winteri* were somewhat separated on the first axis, whereas *D. andina* and *D. confertifolia* were not separated from the *D. winteri* (Figure 4). On the second axis, the southern most population of *D. winteri* var. *winteri* (Isla Hornos) and *D. winteri* var. *chilensis* from Fray Jorge/Talinay were separated from the other populations, indicating morphological similarities between populations separated by more than 2800 km (Figure 4).



**Figure 2.** Latitudinal trends leaf morphological of *Drimys winteri*. Lines show model of linear regression. Values of significance are on the right side. For population abbreviations, see Table 1.



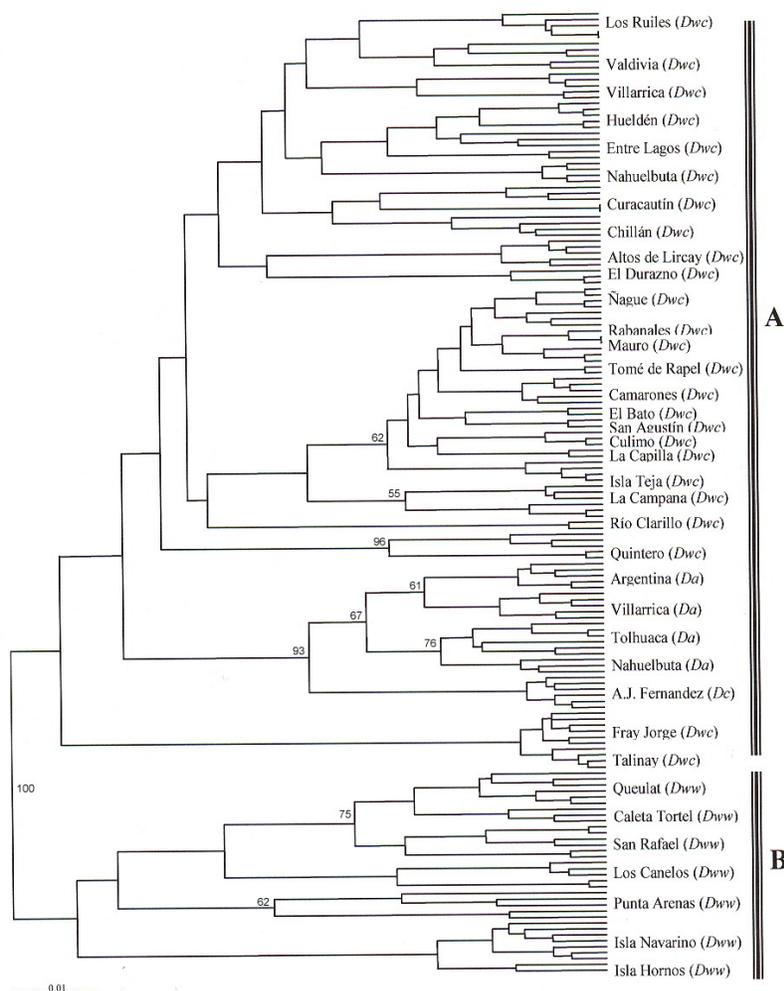
**Figure 3.** Cluster analysis of *Drimys winteri* based on seven leaf morphology characters. Line indicates confidence limit, which determines differences between populations. For population abbreviations, see Table 1. **A.** Isla Hornos and Isla Navarino (XII Region). **B.** Zone center/Fray Jorge/Talinay. **C.** Zone center/north/Isla Teja.



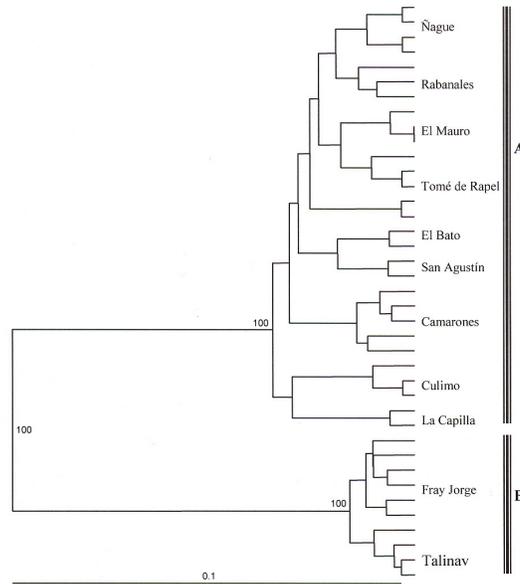
**Figure 4.** PCA of *Drimys* based on seven leaf morphology characters. For population abbreviations, see Table 1.

## Molecular analysis

Based on molecular analysis, the dendrogram for all the 37 populations studied shows a separation between *D. winteri* var. *winteri* and the rest with 100% support (Figure 5). Inside of group A, there are potentially three subgroups without support: a) populations from Fray Jorge/Talinay, b) the rest of population of *D. winteri* var. *chilensis* with respect to *D. andina* and *D. confertifolia*. Finally, *D. andina* is consistently separated from *D. confertifolia*. A detail analysis in *D. winteri* var. *chilensis* from Coquimbo Region shows the clear separation of Fray Jorge/Talinay populations from the rest (Figure 6).

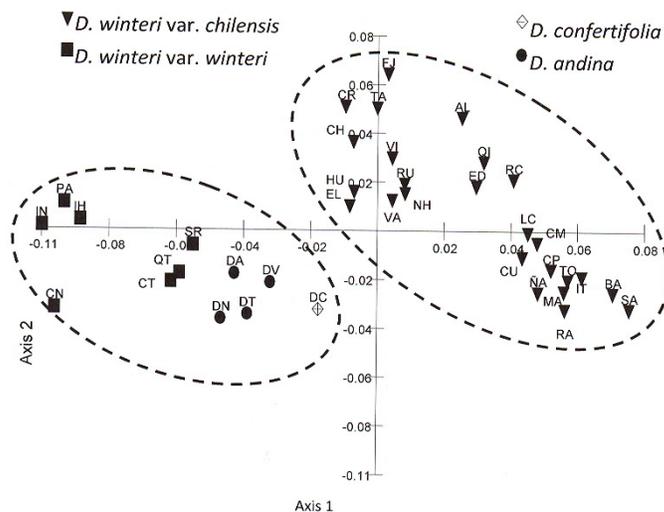


**Figure 5.** Dendrogram for molecular analyses using RAPD markers generated from Nei and Li (1979) distances using the UPGMA algorithm for populations of *Drimys*. Bootstrap values above 50% are shown. **A.** *D. winteri* var. *chilensis*/Fray Jorge-Talinay/*D. andina*/*D. confertifolia*. **B.** *D. winteri* var. *winteri*. Dwc = *D. winteri* var. *chilensis*; Dww = *D. winteri* var. *winteri*; Da = *D. andina*; Dc = *D. confertifolia*.

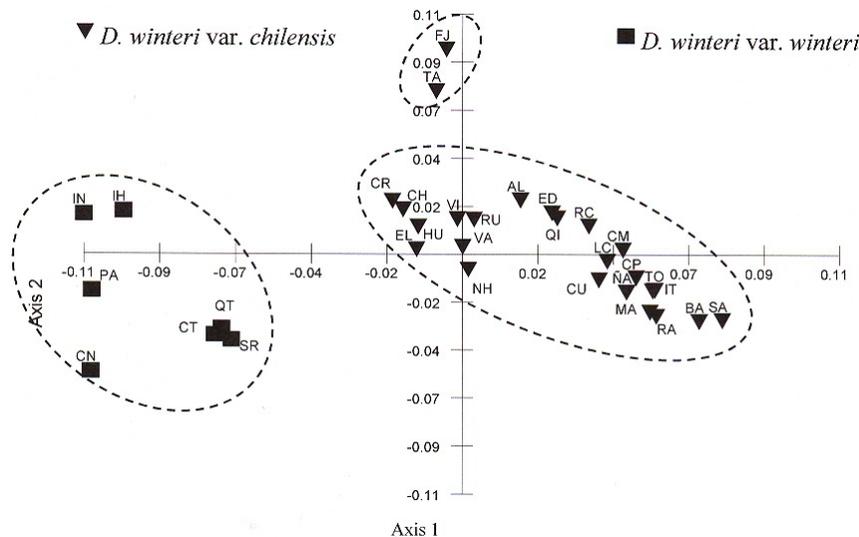


**Figure 6.** Dendrogram for molecular analyses using RAPD markers generated from Nei and Li (1979) distances using the UPGMA algorithm for 11 populations of *Drimys* var. *chilensis* from Coquimbo Region. Bootstrap values above 50% are shown. **A.** Nine populations of *Drimys* var. *chilensis* from Coquimbo Region. **B.** Fray Jorge/Talinav.

On the other hand, the PCO shows two groups of populations: 1) *D. winteri* var. *chilensis* (including Fray Jorge/Talinav populations), and 2) *D. andina*, *D. confertifolia* and *D. winteri* var. *winteri* (explained the variance of 51.3%) (Figure 7). Analyzing only the populations of *D. winteri*, the separation among varieties was more evident, with the populations of Fray Jorge/Talinav separated from the rest (explained the variance of 54.4%) (Figure 8).



**Figure 7.** PCO analysis of the species of *Drimys* based on genetic data. For population abbreviations, see Table 1.



**Figure 8.** Principal coordinates analysis analysis of *Drimys winteri* species based on genetic data. For population abbreviations, see Table 1.

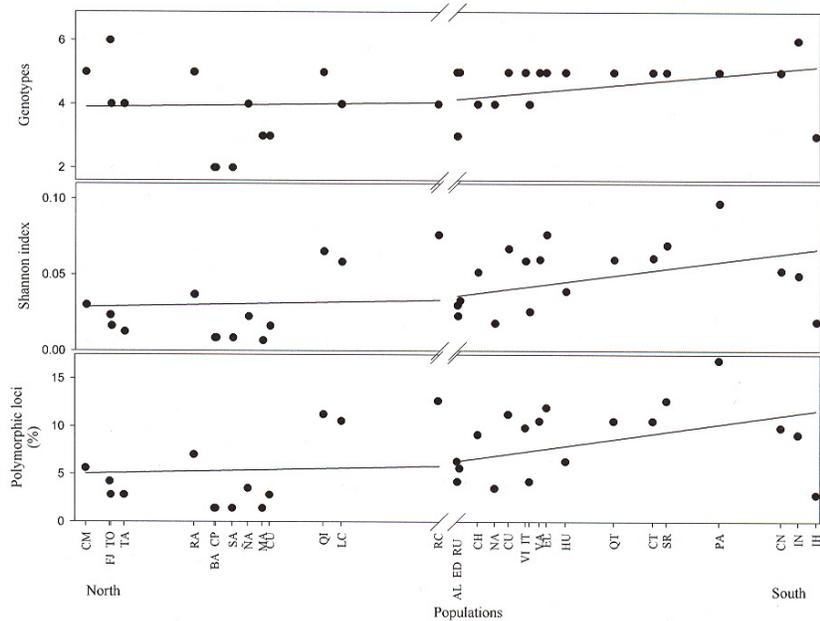
AMOVA found significant differences among all the groups of *D. winteri* and the species of *Drimys* ( $P < 0.001$ ). The  $\Phi$  statistic values among groups and pairs of groups were all above 0.25, which by the criterion of Wright (1951) indicates that there is a differentiation at all hierarchical levels (Table 3). Comparing among groups, there was a large genetic differentiation especially between Fray Jorge/Talinay with the rest of *D. winteri* var. *chilensis* (0.40) and *D. winteri* var. *winteri* (0.48). There was also considerable differentiation among the varieties of *D. winteri* (0.35).

**Table 3.** Values of variance and  $\Phi$  statistics generated by AMOVA for groups composed of populations of *Drimys winteri* and species of the genus *Drimys*.

Variation level	d.f.	Variance	% total variance	$\Phi$ PHI	Statistic
<i>Among varieties of D. winteri</i>					
Among groups ( $S^2_R$ )	1	6.14	35.19	( $\Phi_{ct}$ )	0.35
Among populations within groups ( $S^2_p$ )	29	8.06	46.17	( $\Phi_{sc}$ )	0.71
Within populations ( $S^2_w$ )	125	3.25	18.64	( $\Phi_{st}$ )	0.81
<i>Populations D. winteri from Coquimbo Region</i>					
Among groups ( $S^2_R$ )	1	11.28	70.55	( $\Phi_{ct}$ )	0.71
Among populations within groups ( $S^2_p$ )	10	3.12	19.52	( $\Phi_{sc}$ )	0.66
Within populations ( $S^2_w$ )	38	1.59	9.92	( $\Phi_{st}$ )	0.90
<i>Between FJ/TA and D. winteri var. chilensis</i>					
Among groups ( $S^2_R$ )	1	7.08	40.25	( $\Phi_{ct}$ )	0.40
Among populations within groups ( $S^2_p$ )	24	7.82	44.45	( $\Phi_{sc}$ )	0.74
Within populations ( $S^2_w$ )	101	2.69	15.30	( $\Phi_{st}$ )	0.85
<i>Between FJ/TA and D. winteri var. winteri</i>					
Among groups ( $S^2_R$ )	1	9.62	47.71	( $\Phi_{ct}$ )	0.48
Among populations within groups ( $S^2_p$ )	8	6.91	34.26	( $\Phi_{sc}$ )	0.66
Within populations ( $S^2_w$ )	43	3.63	18.03	( $\Phi_{st}$ )	0.82

d.f. = degrees of freedom; FJ/TA = Fray Jorge/Talinay.  $\Phi_{ct}$  = variance component of groups with respect to the total variance;  $\Phi_{sc}$  = variance component within groups with respect to variance among populations;  $\Phi_{st}$  = variance component among populations with respect to the total variance.

Finally, the analysis of population structure revealed a tendency to increase the percentage of polymorphic loci, number of genotypes and the Shannon index towards the south (Figure 9).



**Figure 9.** Analysis of the species of *Drimys winteri*, based on genetic data, revealed latitudinal trends to increase the percent polymorphic loci, number of genotypes and the Shannon index towards the south. For population abbreviations, see Table 1.

### Comparative analysis of leaf morphology and RAPD

The Mantel test indicated that at the species-level there was a positive and significant association among leaf morphological, genetic and geographic distances. That is, the greater the geographic distance between populations, the greater the morphological divergence ( $r = 0.196$ ;  $P_{2 \text{ tails}} = 0.031$ ) and the genetic divergence ( $r = 0.381$ ;  $P_{2 \text{ tails}} < 0.001$ ), and greater genetic divergence is accompanied by greater morphological divergence ( $r = 0.119$ ;  $P_{2 \text{ tails}} = 0.032$ ). The discriminant analysis classified correctly 86% of the individuals in the populations of *D. winteri* and 81% were correctly classified to species. The percentages of incorrect classification are due to the populations of Fray Jorge/Talinay, which are not in the group of *D. winteri* var. *chilensis*, and to the fact that *D. andina* and *D. confertifolia* did not disassociate.

### Correction of Lynch and Milligan (1994) and analysis of reproducibility

The correction of Lynch and Milligan (1994) reduced the bands from 142 to 92, with which we obtained results equivalent to those described above. The reproducibility was 93.75%.

## DISCUSSION

Currently, the populations of *Drimys* have a disjunct distribution in Chile, especially in the northern part of its distribution. This disjunction has been explained by fragmentation of the west coastal strip along the subtropical South America that occurred during the Miocene (Villagrán et al., 2004), and by the intensification of aridity due to the formation of the Arid Diagonal (Garzzone et al., 2008). Maldonado and Villagrán (2001) documented expansions and contractions of the formation *Luma chequen*, *Escallonia revoluta* and *D. winteri* of the Ñague forest, located in southern Coquimbo Region, over the last 5300 years, associated with changes in the water table produced by regional variation in precipitations. These authors also found that during the last 10,000 years the extension of these forests has been considerably reduced due to human activity.

One of the largest genetic divergences found was among the varieties of *D. winteri* that may be attributed principally to the arid conditions, which fragmented its habitat and eliminated gene flow, forcing the populations to evolve independently. However, at the within population level, Shannon's divergence index in *D. winteri* varied from 0.007 (El Mauro "MA", at 32°S) to 0.097 (Punta Arenas "PA", at 50°S), with a mean of 0.04. This value is much lower than those found for other species, which inhabit the same areas; *Aextoxicon punctatum*, 0.36 (Nuñez-Ávila and Armesto, 2006); *Araucaria araucana*, 0.65 (Bekessy et al., 2002); *Fitzroya cupressoides*, 0.54 (Allnutt et al., 1999); *Podocarpus salignus*, 0.64 (Allnutt et al., 2001); *Pilgerodendron uviferum*, 0.57 (Allnutt et al., 2003), and *Populus tremuloides*, 0.65 (Yeh et al., 1995). This difference may be due to the form of propagation of *Drimys*, which is mainly vegetative due to poor seed germination (Figueroa and Castro, 2002) or to high mortality of young plants within the small forests. This clonal reproduction would impede recombination and the increase in the gene pool.

The greatest genetic and leaf divergence was found between Fray Jorge/Talinay and the rest of the populations studied. A similar result was reported for *A. punctatum* by Nuñez-Ávila and Armesto (2006), who found high genetic divergence between the relict forests of north-central Chile (Fray Jorge/Santa Inés) and the rest of the populations of this Chilean species. Fray Jorge is the northernmost forest in Chile; it is a relict forest of hygrophilic vegetation in an area of arid shrublands, which is maintained by fog condensation (Squeo et al., 2004, 2005). This forest has a floristic similarity with the temperate rainforests of the Valdivian and north-Patagonian type in southern Chile and with the continental and oceanic islands of the Chilean (Pacific) coast (Villagrán et al., 2004); it appears to be a relict of the ancient biota, which populated Chile. Croizat (1962) concludes that the origin of the Fray Jorge flora is linked to the formation, development and paleodistribution of forests in Chile during the Tertiary. The genetic divergences found between the Fray Jorge/Talinay populations compared with other *D. winteri* var. *chilensis* ( $\Phi = 0.40$ ) and *D. winteri* var. *winteri* ( $\Phi = 0.48$ ) are very high compared to the other divergences that we found within and between species of *Drimys*, which were all less than 0.34. The genetic divergences between the populations of Fray Jorge/Talinay and other populations of *D. winteri* var. *chilensis* from north-central and southern Chile suggest two phenomena: first, an early separation of Fray Jorge/Talinay populations associated with the slow tectonic lift of the Alturas de Talinay, which includes the forests of Fray Jorge, Talinay and Santa Inés, during the early to middle Pleistocene. This is consistent with Villagrán et al. (2004) who suggested that Fray Jorge is the product of a migration, which was produced

before the differentiation of the Chilean and Argentinean species and the described varieties of *D. winteri*. Currently, these relict forests are isolated and persist at the tops of coastal mountains due mainly to the fog they intercept, which may provide up to 1000 mm annual precipitation to the forests (Pérez and Villagrán, 1994). Additionally, a secondary colonization of other populations located in the Coquimbo Region from south-central Chile occurred later. No evidence of recent gene flow between these two groups of populations exists. The high genetic divergence may be influenced by isolation, which impedes gene flow and favors independent population evolution (Grant, 1998; Traveset, 2001).

Our results are in discrepancy with the current classification of the genus by Rodríguez and Quezada (2001). The information we present opens a large number of questions, especially concerning the origin of the populations of *Drimys* in Chile and the antiquity of the populations of Fray Jorge/Talinay. Our results may be the base for a new phylogenetic study, which will help answer these questions and re-evaluate the classification of the genus *Drimys* in Chile.

There have been several published phylogenetic studies of *Drimys*; however, these present some limitations, such as incomplete phylogenies where only one or two species were used (Suh et al., 1993; Karol et al., 2000; Doust and Drinnan, 2004), or lack of robustness in the nodes, such as the study of Ruiz et al. (2008). These authors used a nuclear gene and concluded that the genus *Drimys* is monophyletic, but found no support for internal resolution between species. Another study with nuclear and plastid genes at the family level estimated that the divergence between *Drimys* and its sister clade *Pseudowintera* + *Zygogynum s.l.* occurred in the Cretaceous, but the divergence between the two subclades of *Drimys* (*D. roraimensis*, *D. brasiliensis*, *D. angustifolia*, *D. granadensis*, *D. confertifolia*, *D. winteri*, *D. andina*) occurred from 18.5-15.5 Ma (Marquínez et al., 2009). This range of dates coincides with the formation of the South American Arid Diagonal, which increased the aridity in the north-central and north Chile; this event dates back to at least the upper Miocene, when the Andes reached sufficient height to generate an effective block to the eastern winds and increase the vigor of the cold Humboldt current and the South Pacific Subtropical Anticyclone (Zachos et al., 2001; Strecker et al., 2007; Garziona et al., 2008). The elevation of the Andes to its current condition dates back to 15 Ma (Alpers and Brimhall, 1988) or 10 Ma (Gregory-Wodzicki, 2000), coinciding with the divergence times within the genus *Drimys*. These dates also coincide with the origin or divergence of other taxa associated with increased aridity: i) *Chuquiraga*, whose origin and diversification is associated with the elevation of the Andes at the end of the Tertiary and with the climatic fluctuations of the Pleistocene and Holocene (Ezcurra, 2002); ii) *Malesherbiaceae*, which originated in the late Miocene or early Pliocene (Gengler-Nowak, 2002), iii) *Chaetanthera*, whose ancestor lived in the lowlands, in the Miocene-Pliocene it took refuge in highlands (Hershkovitz et al., 2006a); iv) *Tropaeolum* section *Chilensia*, whose divergence is associated with its environment becoming more arid in the middle Miocene (Hershkovitz et al., 2006b); v) *Heliotropium* section *Cochranea*, which diverged between the Miocene and Pliocene (Luebert and Wen, 2008) and vi) *Schizanthus*, which diverged in the Pliocene (Pérez et al., 2006).

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