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DO ENVIRONMENTAL FACTORS AFFECT THE TAXONOMIC RELIABILITY OF LEAF CUTICULAR MICROMORPHOLOGICAL CHARACTERS? A CASE STUDY IN PODOCARPACEAE

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Leaf cuticle micromorphology has been cited as an important set of taxonomic characters in gymnosperms, but previous studies have largely been based on small sample sizes. The premise of this study was to understand whether external factors affect cuticular micromorphology of Podocarpaceae. Two example species, *Prumnopitys andina* and *Podocarpus salignus*, were studied. Of 21 sampled characters, nine (c.43% of the total) were visually assessed as being moderately reliable or highly reliable for taxonomic discrimination for both species, with an additional six (c.29%) being moderately reliable or highly reliable for only one or other of the example species, and six characters (c.29%) unreliable for both. Seven of the most variable stomatal characters were selected for further analysis to establish whether environmental factors affect them. The relationship between these seven stomatal characters, the environment and climate was analysed using the R 'vegan' package and climate data gathered from WorldClim. Our results showed that both species had larger stomata in moist and shady conditions, and a higher density of (smaller) stomata in sunny and drier conditions. An additional novel finding was the presence of stomata on the adaxial leaf surface in 46% of samples of *Prumnopitys andina*: the first record of adaxial stomata in this species, highlighting the necessity of studying multiple samples of a given species. In conclusion, these results indicate that larger sample sizes than have hitherto been employed in cuticle micromorphological studies are necessary to fully document the amount of phenotypic variation that exists.

Keywords. Climate, cultivation, cuticular micromorphology, environment, *Podocarpus*, principal components analysis, *Prumnopitys*, R, sample size, stomata, variation.

Introduction

Ever since Alvin & Boulter (1974) perfected the technique of isolating leaf cuticles using so-called 'chromic acid' for examination by scanning electron microscopy (SEM), the technique has been routinely used to examine the cuticles of various plant taxa and often to infer taxonomic conclusions. These taxa include many gymnosperm genera (Xiang & Fu, 1998; Barone Lumaga *et al.*, 1999; Kim *et al.*, 1999; Ickert-Bond, 2000;

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Whang *et al.*, 2001, 2004; Ma *et al.*, 2009; Mickle *et al.*, 2011), as well as angiosperm groups (e.g. Proteaceae: Carpenter, 1994; Carpenter *et al.*, 2005; *Cercis*, Fabaceae: Zou *et al.*, 2008; *Cornus*, Cornaceae: Hardin & Murrell, 1997; Sapindaceae: Pole, 2010).

Among the two principal southern hemisphere conifer families, Araucariaceae have been studied by Stockey & Ko (1986: *Araucaria* Juss.) and Stockey & Atkinson (1993: *Agathis* Salisb.), while genera of Podocarpaceae (the second-largest conifer family, with about 200 species) have been the subject of much research, beginning with a small-scale survey of some New Caledonian species (Stockey & Ko, 1988) and continuing with studies of individual genera or parts thereof [e.g. Stockey & Ko (1990: New Caledonian species of *Dacrydium* Sol. ex Lamb.); Hill & Carpenter (1991: *Acmopyle* Pilg. and *Dacrycarpus* (Endl.) de Laub.); Stockey *et al.* (1992: *Falcatifolium* de Laub.); Stockey *et al.* (1995: *Parasitaxus* de Laub.); Stockey & Frevel (1997: *Prumnopitys* Phil.); Stockey *et al.* (1998: *Podocarpus* L'Hér. ex Pers. sect. *Scytopodium* de Laub.); Mill & Stark Schilling (2009: *Saxegothaea* Lindl.); and Stark Schilling & Mill (2011: Caribbean and Central American species of *Podocarpus*)].

The use of leaf cuticle micromorphological techniques has revealed important taxonomic characters of the cuticle and stomata that have been used to recognise sections within various gymnosperm genera (Stockey & Ko, 1988; Hill & Carpenter, 1991; Kim *et al.*, 1999; Ickert-Bond, 2000; Whang *et al.*, 2001, 2004; Mill & Stark Schilling, 2009; Whiting, 2009). Many studies have indicated that leaf cuticle micromorphology in combination with SEM has a systematic importance, capable of being used to differentiate species within genera (Stockey *et al.*, 1992; Stockey & Frevel, 1997; Xiang & Farjon, 2003; Stark Schilling & Mill, 2011).

However, nearly all the studies that have employed cuticular micromorphology as a taxonomic tool have used very few samples per individual species, typically one to three. This has been particularly true of large-scale taxonomic surveys such as those by Pole (2010: c.160 species of Australasian Sapindaceae), Whiting (2009: 69 species of *Podocarpus*), Hardin & Murrell (1997: 52 species of *Cornus*) and Carpenter (1994: 41 species of Proteaceae) but has also been the case in smaller-scale studies such as those by Stockey & Atkinson (1993: 21 species), Stockey & Ko (1986: 20 taxa), Stark Schilling & Mill (2011: 13 species), Stockey & Frevel (1997: 11 taxa), Stockey *et al.* (1998: 10 taxa of seven species) and Stockey *et al.* (1992: five species), and even of research dealing with a single species (Stockey *et al.*, 1995: three samples; Mill & Stark Schilling, 2009: one sample for SEM, although this was compared using light microscopy with 29 others). There are many possible reasons for the almost universal low sampling per species, including availability of material. Probably the most important factor, however, is the slowness of the technique; in gymnosperms, at least, the standard protocol involves a pretreatment in 'chromic acid' lasting 96 h under a fume hood. This, together with the cost of time on an SEM, automatically limits the number of samples that can be prepared in each batch and also the total that can be prepared within the budget allocated for the study.

From samples of typically one or two, less commonly three to five specimens, assumptions have frequently been made that the cuticle micromorphology is virtually

invariable and is therefore a good taxonomic tool. Inferences have been made concerning taxonomic placement and rank, and there have been many studies that have used the cuticle of living species in comparison with fossil taxa to deduce nearest living relatives of the fossils. However, this small-scale sampling has meant that virtually nothing is known about the possible effects that abiotic factors such as altitude, latitude and climate might have on the cuticular micromorphology of a given species.

One study that did attempt to assess intraspecific variation in cuticular characters is that by Leng *et al.* (2001), who studied 16 samples of the cuticle of *Metasequoia glyptostroboides* Hu & W.C.Cheng (Cupressaceae) from nine native populations as well as four cultivated specimens and two grafts; they found that, with one exception from an isolated locality, the samples were uniform at the micromorphological level. However, they provided no information about the climate or altitude of the various localities sampled.

A study that gave a very different result is that by McNeilly *et al.* (1987), who studied the effects of exposure to salt spray on the cuticle morphology of three species of Poaceae. All of them showed more or less marked differences in cuticle characters when exposed to salt spray, with the differences in the external cuticle micromorphology being so pronounced in *Agrostis stolonifera* L. that the two samples could by the uninitiated be mistaken as belonging to two different species. Characters of the internal surfaces of the cuticle were not studied. Nevertheless, this study indicates that cuticular micromorphology is not always as constant as frequently assumed, and that the potential effects of abiotic factors need to be taken into consideration when using cuticular micromorphology as a taxonomic tool.

The aim of this case study, therefore, was to establish the degree, if any, to which cuticular micromorphological characters used in taxonomic research vary according to geographical or environmental parameters such as latitude, altitude or climate. The study focused on two species, *Prumnopitys andina* (Poepp. ex Endl.) de Laub. and *Podocarpus salignus* D.Don. These are both members of the gymnosperm family Podocarpaceae and both are native to Chile. These two taxa were chosen because: 1) the cuticle micromorphology of the family is well known; 2) both selected species had been examined previously (*Prumnopitys andina*: Stockey & Frevel, 1997; *Podocarpus salignus*: Whiting, 2009); 3) sets of herbarium specimens of each species had been collected across a range of different climatic conditions in the wild specifically for the purpose of examining cuticular variation in them; 4) both species are also cultivated in certain gardens in the UK, allowing assessment of the effects, if any, of cultivation on cuticular micromorphology.

The principal objectives were to test: 1) the intraspecific variability of the leaf cuticle as a taxonomic character across a wider sample size than previously used in other studies; 2) the hypothesis that leaf cuticle characters are affected by environmental factors, for example does the size and structure of stomata vary in different environmental conditions; and 3) whether there are any differences in the leaf cuticle micromorphology between plants of the same species growing in the wild in Chile and in cultivation in Scotland.

TABLE 1. Number of accessions sampled for each species

Species	Origin	Location	No. of accessions
Prumnopitys andina	Cultivation	Benmore Botanic Garden	1
		Kilmun Arboretum	5
		Royal Botanic Garden Edinburgh, Nursery	52
	Wild	VII Maule	1
		VIII Bío Bío	30
		IX Araucanía	16
Podocarpus salignus	Cultivation	Benmore Botanic Garden	18
		Kilmun Arboretum	15
	Wild	VII Maule	23
		VIII Bío Bío	8
		IX Araucanía	5
		XIV Los Ríos	2

Because the generic names of both genera studied begin with the letter '*P*', they are, when appropriate, abbreviated as *Po.* (for *Podocarpus*) and *Pr.* (for *Prumnopitys*).

MATERIALS AND METHODS

Sample collection

In total, 176 accessions were sampled (105 for *Prumnopitys andina* and 71 for *Podocarpus salignus*) (Table 1). Approximately half (58 for *Prumnopitys andina* and 33 for *Podocarpus salignus*) were collected from plants of known wild origin in cultivation from three sites: 1) the living collection of one of the Royal Botanic Garden Edinburgh (RBGE) regional gardens, Benmore Botanic Garden (Dunoon, Argyll, Scotland); 2) the International Conifer Conservation Programme safe site at Kilmun Arboretum, Argyll, Scotland; and 3) a known wild origin *Prumnopitys andina* 'conservation hedge' in the RBGE living collection (Gardner, 2013). Each single accession was sampled from both shaded and non-shaded branches. Further samples of both species were collected from herbarium material conserved at E (47 specimens of *Prumnopitys andina*, 38 of *Podocarpus salignus*). These specimens encompass almost the entire known geographical range of both species: for *Prumnopitys andina* from the Chilean region Maule in the north (35°54′S) to La Araucanía in the south (38°51′S); and for *Podocarpus salignus* from Maule in the north (35°19′S) to Los Ríos in the south (39°42′S). Some of the specimens (eight of *Prumnopitys andina* and 20 of *Podocarpus salignus*) had been collected by M. F. Gardner, C. Morter and G. Ovstebo in 2011 in Linares and Ñuble provinces specifically for cuticle analysis from full-sun and shaded branches. For a detailed list of accessions and collections, see Appendix table 1 (for *Prumnopitys andina*) and Appendix table 2 (for *Podocarpus salignus*). In addition, to further explore the environmental tolerances of *Podocarpus salignus*, all 245 occurrence records of the species in the Global Biodiversity Information Facility (GBIF) were

downloaded. As detailed in the *Acknowledgements* and *References*, these comprised specimens from the following herbaria: B, CHR, E (also Living Collection), FR, K, L (Naturalis dataset), MA, MEL (Australia's Virtual Herbarium dataset), MO, NAM, NY, P, S, SANT, SI, U (Naturalis dataset) and US, as well as living material at RBGE and University of California Botanical Garden, Berkeley.

Sample preparation

Following the methods proposed by Alvin & Boulter (1974) for gymnosperm leaf cuticle analysis, with modification to the techniques proposed by Stockey & Frevel (1997), Stockey *et al.* (1998), Mill & Stark Schilling (2009) and Stark Schilling & Mill (2011) to better suit Podocarpaceae, a 20/120% (16.66%) solution of dihydroxidodioxidochromium (H_2CrO_4, 'chromic acid') was prepared. Leaves were removed from each specimen of *Podocarpus salignus* and *Prumnopitys andina*, and stainless steel punches were used to remove six to eight circular leaf cuticle discs of 4 mm diameter for *Po. salignus*, but 3 mm diameter for the smaller leaves of *Pr. andina.* Leaf discs were placed into chromic acid for 96 h at room temperature. After this pretreatment process, the isolated leaf cuticles were washed with deionised water, placed on filter paper and dried for a minimum of 4 h in Petri dishes.

Selected samples were mounted onto 0.5″ pin-type aluminium specimen stubs using carbon adhesive tabs and sputter-coated with 100% platinum for 1 min using an Emitech K575X sputter coater. Scanning electron microscopy was performed on a Zeiss Leo Supra 55 VP instrument. Four images for each specimen were made. Both the adaxial and abaxial leaf surfaces were examined for both species. For the adaxial surface of *Podocarpus salignus*, magnifications of × 300 and × 1200 were used. The abaxial surface was photographed at magnifications of × 550 and × 1500, owing to diagnostic characters being smaller. The adaxial surface epidermal cells of *Prumnopitys andina* were smaller than in *Podocarpus salignus*, yet the cells of the abaxial surface were slightly larger. Consequently, for the adaxial surface of *Prumnopitys andina*, magnifications of × 350 and × 1300 were used, whereas for the abaxial surface magnifications of × 450 and × 1400 were used. Lower magnifications of × 450 (*Prumnopitys andina*) and × 550 (*Podocarpus salignus*) and higher magnifications of × 1400 (*Pr. andina*) and × 1500 (*Po. salignus*) were used to count the total number of stomata on the abaxial surface for both species.

Assessment of taxonomic reliability

Twenty-one leaf cuticle characters were visually assessed for their taxonomic reliability: seven each of the stomata, epidermal cells and subsidiary cells, as enumerated in Table 2 (see *Results*). Some were found to be taxonomically informative, whereas others show high variability and are therefore less informative. A three-level ranking system was employed to assess taxonomic reliability.

**, *Highly reliable*. Character invariable or some slight variation, not directly or partially affected by abiotic factors. Subject to little interspecific variation. These

TABLE 2. Taxonomic reliability of leaf cuticle characters in *Prumnopitys andina* and *Podocarpus salignus*, based on visual assessment

Character no.	Character	*Prumnopitys andina*	*Podocarpus salignus*	Used in main study
	Stomata			
1	Linkage of stomata within rows parallel to leaf axis	U	U	
2	Number of stomata (wide)	U	U	Table 3, character 1
3	Size and shape of stomatal apparatus	U	U	Size: Table 3, character 3
4	Size and shape of inner stomatal apparatus	U	U	Size: Table 3, character 4
5	Presence or absence of polar extensions	U	U	
6	Size of polar extensions	U	U	
7	Shape of polar extensions	U	**	
	Epidermal cells			
8	Degree of undulation of epidermal cell walls	**	*	
9	Inner texture of epidermal cells	**	*	
10	Presence or absence of pit-like protuberances in epidermal cells	*	**	
11	Margin of epidermal cells	*	*	
12	Uniformity of adaxial epidermal cell rows	*	**	
13	Visibility of epidermal cells	*	U	
14	Shape and size of epidermal cells	*	*	
	Subsidiary cells			
15	Margin of lateral subsidiary cells	U	*	
16	Number of subsidiary cells	**	*	
17	Texture of subsidiary cells	*	*	
18	Shape and size of polar subsidiary cells	U	*	Lengths: 'upper', Table 3, character 6; 'lower', Table 3 character 7
19	Shape of lateral subsidiary cells	*	*	
20	Size of lateral subsidiary cells	*	U	Right cell length: Table 3, character 5
21	Number of divisions in subsidiary cells	U	**	

**, Highly reliable; *, moderately reliable; U, unreliable.

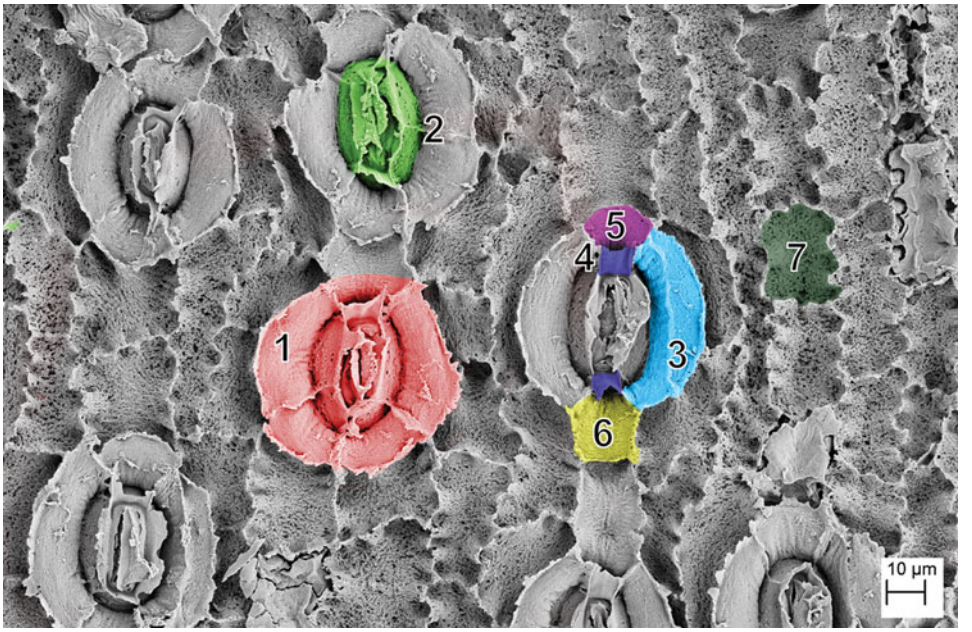

FIG. 1. Distinguishing stomatal and epidermal characters from the abaxial inner cuticle surface of *Prumnopitys andina*. 1, Stomatal apparatus; 2, inner stomatal apparatus with guard cells; 3, right lateral subsidiary cell; 4, polar extensions; 5, upper polar subsidiary cell; 6, lower polar subsidiary cell; 7, abaxial epidermal cell. Note: the terms 'right lateral subsidiary cell', 'upper polar subsidiary cell' and 'lower polar subsidiary cell' refer to *the image axis as viewed*, *not* the axis of the leaf itself.

characters are considered highly reliable in taxonomy, even when a small sample size is used.

*, *Moderately reliable*. Character somewhat variable; use with caution. Considered somewhat unreliable for use in taxonomic studies.

U, *Unreliable*. Highly variable, very unreliable and consequently of little use for taxonomic purposes.

Seven of the characters that were assessed to be the least reliable for taxonomic purposes were subjected to further measurement and analysis to try to establish what abiotic factors, if any, might be responsible for their unreliability. These seven characters were as follows.

1. *No. stom. wide*. Number of whole stomata visible per field at lower magnification (wide field of view: see Figs 3B, 4B).
2. *No. stom*. Number of whole stomata visible per field at higher magnification (see Figs 3E,F, 4E,F).
3. *Stom. size*. Size (area) of entire stomatal apparatus (including subsidiary cells) (μm^2) – coloured red and numbered '1' in Figs 1 and 2.

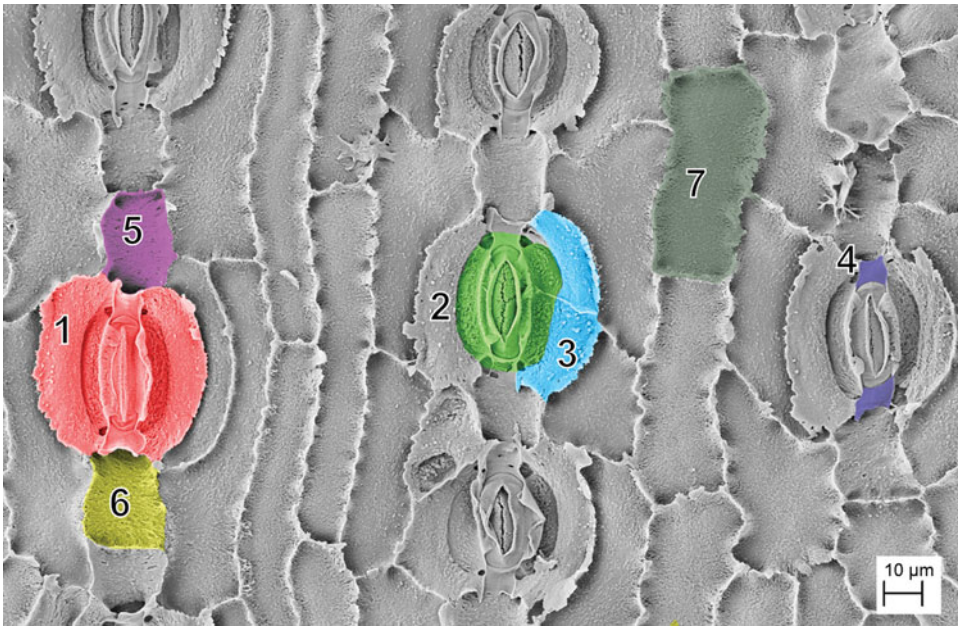

FIG. 2. Distinguishing stomatal and epidermal characters from the abaxial inner cuticle surface of *Podocarpus salignus*. 1, Stomatal apparatus; 2, inner stomatal apparatus with guard cells; 3, right lateral subsidiary cell; 4, polar extensions; 5, upper polar subsidiary cell; 6, lower polar subsidiary cell; 7, abaxial epidermal cell. Note: the terms 'right lateral subsidiary cell', 'upper polar subsidiary cell' and 'lower polar subsidiary cell' refer to *the image axis as viewed*, *not* the axis of the leaf itself.

4. *Inner app. size*. Size (area) of 'inner' stomatal apparatus (i.e. stoma and guard cells only) (μm^2) – coloured light green and numbered '2' in Figs 1 and 2.
5. *Right lateral cells*. Length of right-hand subsidiary cells (µm) – coloured pale blue and numbered '3' in Figs 1 and 2.
6. *Upper pol. cells*. Length (µm) of 'upper' polar subsidiary cells, when present – coloured purple and numbered '5' in Figs 1 and 2.
7. *Lower pol. cells*. Length (µm) of 'lower' polar subsidiary cells, when present – coloured yellowish and numbered '6' in Figs 1 and 2.

These characters are illustrated for *Prumnopitys andina* in Fig. 1 and for *Podocarpus salignus* in Fig. 2. Also shown in these figures are the polar extensions (purple, numbered '4') and abaxial epidermal cells (dark green, numbered '7').

Image analysis

Measurements and analysis of SEM images were carried out using ImageJ version 1.47u (Rasband, 1997–2012). A maximum of three stomata were measured from

TABLE 3. Seven stomatal characters selected for measurement and analysis in *Prumnopitys andina* and *Podocarpus salignus*

Character no.	Character abbreviation	Character definition	Character no. in Table 2
1	No. stom. wide	Number of whole stomata visible per field at lower magnification (wide field of view)	2
2	No. stom.	Number of whole stomata visible per field at higher magnification	Not applicable
3	Stom. size	Size (area) of entire stomatal apparatus (including subsidiary cells) (μm^2)	3 pro parte (size)
4	Inner app. size	Size (area) of 'inner' stomatal apparatus (i.e. stoma and guard cells only) (μm^2)	4 pro parte (size)
5	Right lateral cells	Length of right-hand subsidiary cells (μm)	20
6	Upper pol. cells	Length of 'upper' polar subsidiary cells, when present (μm)	18 pro parte
7	Lower pol. cells	Length of 'lower' polar subsidiary cells, when present (μm)	18 pro parte

the abaxial surface of each sample for both species at magnifications of × 1500 (*Podocarpus salignus*) or × 1400 (*Prumnopitys andina*). These characters are illustrated for *Prumnopitys andina* in Fig. 1 and for *Podocarpus salignus* in Fig. 2. The subsequent analyses focused on seven stomatal characters, summarised in Table 3.

Environmental data

Data gathering, statistical analysis and visualisations were all carried out within R 3.2.1 (R Core Team, 2013). Shape files of the Chile and UK country boundaries were obtained from the *GADM database of Global Administrative Areas* (Global Administrative Areas, 2012), using the R library 'raster' 2.1-37 (Hijmans & Etten, 2013). Climate data were also gathered through the R library 'raster' from WorldClim (Hijmans *et al.*, 2005). Two areas ('tiles') were downloaded for Chile (area 33 lon = –90, lat = 0 and area 43 lon = –90, lat = –30). A single tile was downloaded for Scotland (area 15 lon = –30, lat = 60). For each species' occurrence records (recorded using a global positioning system device), climate and elevation data were extracted from the downloaded layers and then appended to the species dataset file. The climate variables encompassed temperature (mean annual, minimum, maximum and range)

and precipitation (annual total, precipitation in the wettest month, and precipitation in the driest month).

Statistical analysis

The stomatal characters were analysed using both univariate and multivariate statistics. As a first step, descriptive statistics of central tendency and spread were produced for each of seven stomatal characters in both species. Subsequently, Welch two sample *t*-tests were used to test for statistically significant differences in these characters between the species. Finally, all characters were analysed simultaneously within the framework of an ordination using the 'vegan' community ecology package in R version 2.0-7 (Oksanen *et al.*, 2013). The analysis involved three major steps.

1. To explore visually whether there are differences in the sets of stomatal characters (in two-dimensional 'character space') between the two species, a principal components analysis (PCA) was conducted of all characters of both species. The PCA was centred and scaled to unit variance.
2. To explore whether the characters vary with environmental factors, we first explored the effect of sun exposure (visually in box plots).
3. To test whether in addition to sunlight the characters vary with climate, we conducted two separate PCAs for each species for sunlit and shaded conditions, respectively, and used the function 'envfit' from the R 'vegan' package to assess the strength of the correlation between the climatic variables and the ordination axes.

Adaxial leaf surface

Forty samples (26 of *Prumnopitys andina* and 14 of *Podocarpus salignus*) were examined using light microscopy to check for the presence of adaxial stomata. Fourteen of the 26 samples of *Prumnopitys andina* and nine of the 14 samples of *Podocarpus salignus* were of wild origin, the remaining samples of each species being of cultivated material. All specimens of *Podocarpus salignus* were exclusively hypostomatic, i.e. stomata were never present on the adaxial leaf surface. However, in *Prumnopitys andina* the character was found to be variable, some specimens being hypostomatic and some differentially amphistomatic with a few stomata on the adaxial surface. Therefore, a general linear model with binomial error distribution was used to test whether the presence of adaxial stomata in *Prumnopitys andina* varied with environmental factors.

Results

Species leaf cuticle micromorphological descriptions

Prumnopitys andina (Fig. 3). Leaves hypostomatic or differentially amphistomatic (Fig. 3A,B), with fewer or no stomata on the adaxial surface (Fig. 3A). *Abaxial surface*:

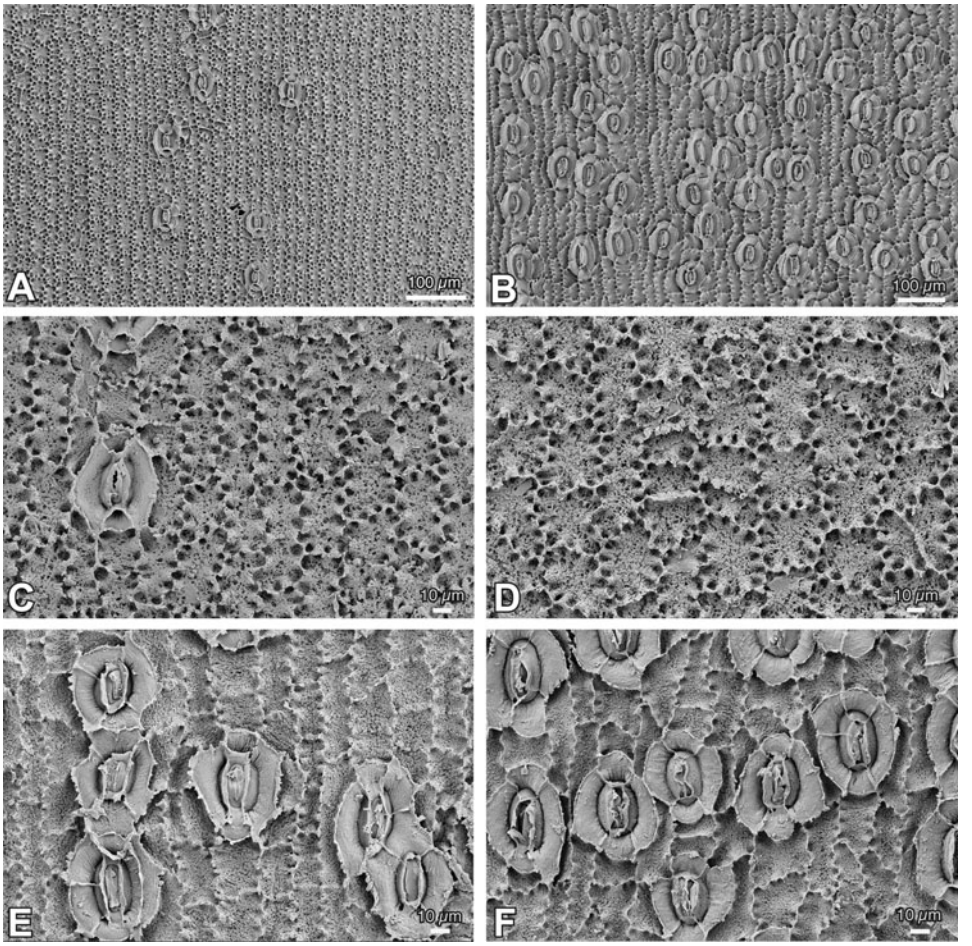

FIG. 3. Leaf cuticle (inner surface) anatomy of *Prumnopitys andina*. A, Adaxial normally non-stomatal surface yet with stomata present, × 350. B, Abaxial stomatal surface of the leaf cuticle, × 450. C, Adaxial normally non-stomatal surface, showing the presence of stomata, × 1300. D, Adaxial non-stomatal surface of the leaf cuticle, with distinctive darkened protuberances that appear like pits surrounding cuticle cells, × 1300. E and F, Abaxial stomatal surface of the leaf cuticle, showing the variation in stomatal density. × 1400.

stomata in distinct rows oriented parallel to the long axis of the leaf, sometimes irregularly clustered (Fig. 3B). Stomatal apparatus greatly varying in size and shape among observed samples, but often irregularly rounded to oval in shape (Fig. 3B). Distal and proximal polar cells present, often distinct (Fig. 3E,F). Lateral subsidiary cells two to three, sometimes four; flanges between subsidiary and guard cells broad, with irregular edges and either smooth or striated on their inner surface (Fig. 3E,F). Polar subsidiary cells present, sometimes greatly reduced, rectangular to trapezoid in shape. *Adaxial surface* (Fig. 3C,D): epidermal cells rectangular, arranged in parallel

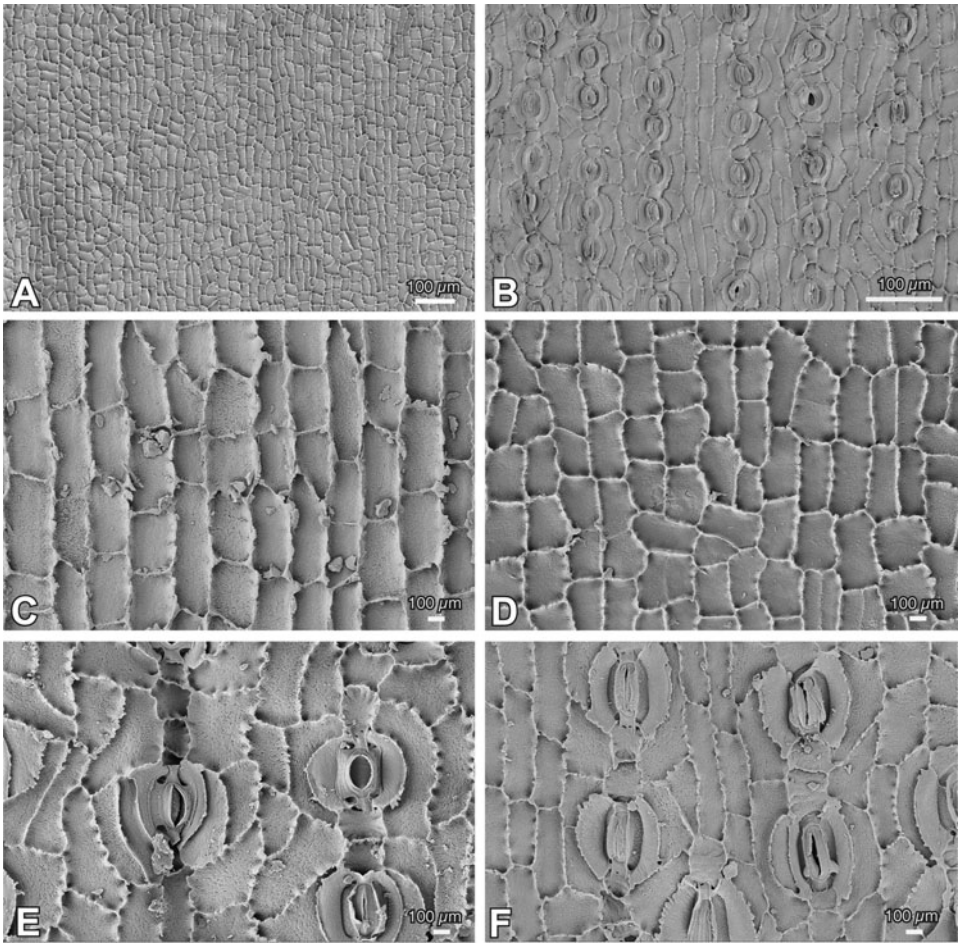

FIG. 4. Leaf cuticle (inner surface) anatomy of *Podocarpus salignus*. A, Adaxial non-stomatal surface, × 300. B, Abaxial stomatal surface of the leaf cuticle, showing the prominent parallel rows of stomata, × 550. C and D, Adaxial non-stomatal surface of the leaf cuticle, also showing the variation in adaxial leaf cuticle patterning between the samples, × 1200. E and F, Abaxial cuticle surface, showing variation in leaf cuticle structure and differences between open and closed stomatal apertures, × 1500.

rows oriented along the long axis of the leaf. Margins of cells undulating to crenate with distinct protuberances that appear like 'hollowed pits' that surround the undulating surface of the epidermal cells. Stomata present fairly frequently.

Podocarpus salignus (Fig. 4). Leaves hypostomatic (Fig. 4A,B). *Abaxial surface*: stomata arranged in distinct rows parallel with the long axis of the leaf, often tending to be opposite to subopposite in adjacent rows and sometimes clustered in groups of three or four (Fig. 4B). Stomatal apparatus variable in size and shape (Fig. 4B), irregularly

rounded to oval in shape (Fig. 4E,F). Lateral subsidiary cells very distinct and enlarged, variable in size and structure; flanges between subsidiary cell and guard cell often wing-like in appearance, with rounded to irregular edges (Fig. 4E,F). Polar subsidiary cells distinct but sometimes greatly reduced, rectangular and uneven in shape (Fig. 4E,F). *Adaxial surface* (Fig. 4C,D): epidermal cells closely packed, rectangular to irregularly rectangular, arranged in distinct lines or irregularly spaced rows parallel with the long axis of the leaf; outer margin of epidermal cells slightly undulating or nearly straight, irregular. Stomata not observed in any sample.

Variability and reliability of cuticular characters as a taxonomic tool

Of the 21 characters assessed for their taxonomic reliability in each species (Table 2), for *Prumnopitys andina* three characters were considered highly reliable, eight moderately reliable and 10 unreliable. In *Podocarpus salignus*, four characters were considered highly reliable, nine moderately reliable and eight unreliable. Characters of the epidermal cells were the most reliable for taxonomic purposes; in *Prumnopitys andina*, all seven epidermal cell characters (Table 2, nos. 8–14) were either highly reliable (two: nos. 8 and 9) or moderately reliable (the other five), whereas in *Podocarpus salignus* six out of the seven were highly reliable (nos. 10 and 12) or moderately reliable, with only one character (no. 13, visibility of epidermal cells) being unreliable. Characters of the subsidiary cells (Table 2, nos. 15–21) were almost as reliable as those of the epidermal cells, especially in *Podocarpus salignus*. In *Prumnopitys andina*, one subsidiary cell character was highly reliable (no. 16, number of subsidiary cells), three (nos. 16, 19 and 20: texture, shape and size) were moderately reliable and three (nos. 15, 18 and 21) were unreliable. For *Podocarpus salignus*, one character (no. 21, number of divisions) was considered highly reliable, five (nos. 15–19) moderately reliable and only one (no. 20) unreliable. The least reliable category of characters for taxonomic purposes, for these two species, was that relating to the stomata (Table 2, nos. 1–7). Of these, all were unreliable in *Prumnopitys andina* and six out of seven were unreliable in *Podocarpus salignus*. In that species, however, the shape of the polar extensions was assessed as highly reliable. The data indicated that a given character, in any of the three categories (stomata, epidermal cells and subsidiary cells), may be highly reliable in one species but unreliable in another. However, overall, in these two species, characters of the epidermal cells and to a lesser extent the subsidiary cells were more reliable than those of the stomata for taxonomic purposes, especially when dealing with small sample sizes.

Differences in stomatal characters between species

Descriptive statistics for the stomatal characteristics in each species are summarised in Tables 4 and 5 and Fig. 5. *Prumnopitys andina* had a greater density and number of stomata than *Podocarpus salignus. Prumnopitys andina* also had the larger stomatal apparatus of the two species. However, the inner stomatal apparatus was slightly larger

TABLE 4. Summary of measurements for seven leaf micromorphological characters in 29 samples of *Prumnopitys andina*, showing values for minimum, first quartile, median, mean, third quartile and maximum range of stomatal size[a]

Value	1. No. stom. wide	2. No. stom.	3. Stom. size (μm^2)	4. Inner app. size (μm^2)	5. Right lateral cells (μm)	6. Upper pol. cells (μm)	7. Lower pol. cells (μm)
Minimum	17.00	4.00	1455	353.9	36.08	1.73	2.69
First quartile	49.75	6.00	1757	464.1	40.20	3.27	3.66
Median	57.00	6.00	1918	490.3	43.18	4.10	4.66
Mean	56.25	6.38	1957	505.1	42.98	4.17	4.96
Third quartile	64.75	7.00	2127	563.3	44.39	4.71	5.85
Maximum	80.00	10.00	2508	699.0	55.52	9.13	8.32

[a] For explanation of character abbreviations, please see the main text.

TABLE 5. Summary of measurements for seven leaf micromorphological characters in 25 samples of *Podocarpus salignus*, showing values for minimum, first quartile, median, mean, third quartile and maximum range of stomatal size[a]

Value	1 No. stom. wide	2 No. stom.	3 Stom. size (μm^2)	4 Inner app. size (μm^2)	5 Right lateral cells (μm)	6 Upper pol. cells (μm)	7 Lower pol. cells (μm)
Minimum	9.00	1.00	1177	298.6	34.90	3.40	3.87
First quartile	29.00	4.00	1590	610.3	40.95	5.37	6.21
Median	34.50	4.00	1714	664.8	45.16	7.32	7.18
Mean	33.58	4.28	1758	658.4	45.00	7.13	7.23
Third quartile	40.25	6.00	1873	726.7	48.65	8.64	8.15
Maximum	52.00	7.00	2421	865.1	56.40	12.09	13.02

[a] For explanation of character abbreviations, please see the main text.

in *Podocarpus salignus* and was subject to much greater variability than *Prumnopitys andina*. The right lateral subsidiary cells showed little variation in length between species. The upper and lower polar extensions were larger in *Podocarpus salignus* than in *Prumnopitys andina*.

The *t*-tests showed that the character differences between *Prumnopitys andina* and *Podocarpus salignus* were all statistically significant, with the exception of the size of the right lateral subsidiary cells, which was, however, marginally significant ($P \leq 0.1$) (Table 6).

The multivariate analyses of all characters (Fig. 6) corroborated the previous findings (greater density of stomata and smaller inner stomatal apparatus in *Prumnopitys andina* than in *Podocarpus salignus*), but also showed that these characters are somewhat variable and can overlap between the two species.

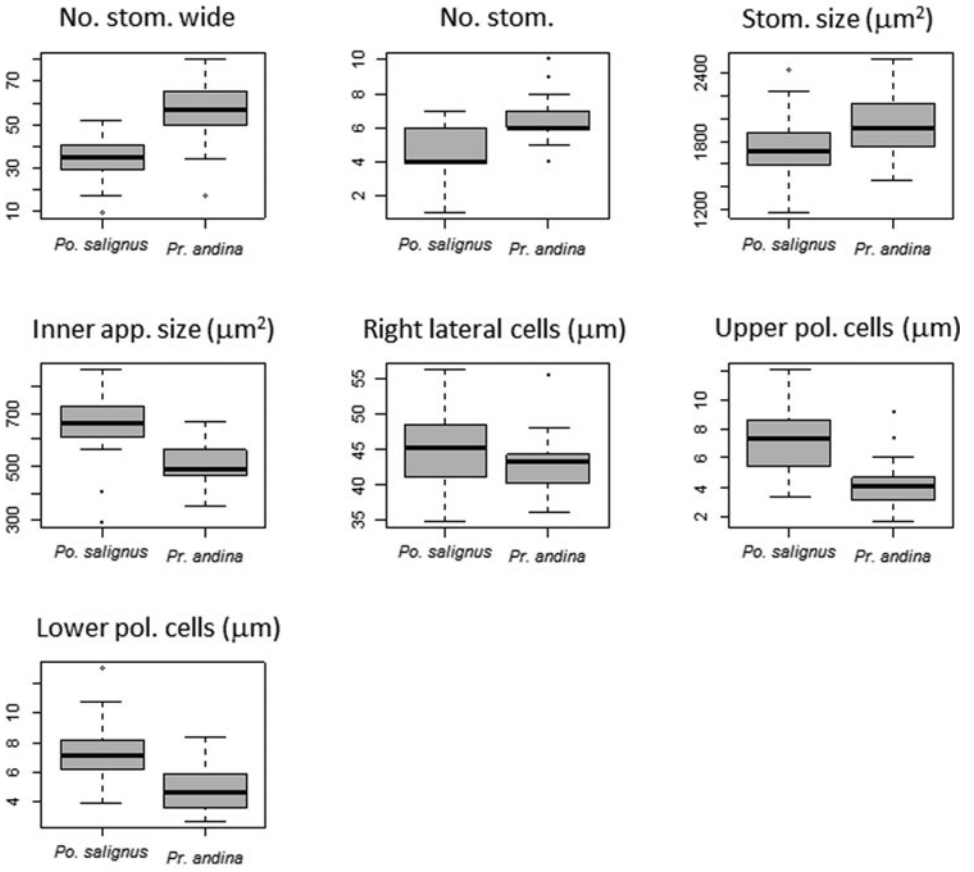

FIG. 5. Box plots of character differentiation between *Prumnopitys andina* and *Podocarpus salignus*. Each box plot visualises the characters shown in Tables 4 and 5; each shows the mean (black line), interquartile range (grey box), and minimum and maximum of each character (whiskers). The box plots allow an easy direct comparison between each of the characters for both species presented in Tables 4 and 5. For explanation of character abbreviations, please see the main text.

Effect of environmental factors on stomatal characters

Climate for Prumnopitys andina. The wild occurrence records for this species encompassed the following environments (Appendix table 3): elevations from 722 m (Ñuble province) to 1127 m (Malleco province); minimum temperatures from –0.8°C to maximum temperatures of > 27°C (both Ñuble province), and mean temperatures from 7°C (Malleco) to 11.2°C (Ñuble). The total annual precipitation ranged from 1060 mm (Linares) to 1943 mm (Malleco), precipitation in the driest month from 18 mm to 46 mm, and precipitation in the wettest month from 219 mm to 333 mm (with these extremes being present in the same provinces as total annual rainfall).

TABLE 6. Measurement of significance between seven characters for *Prumnopitys andina* and *Podocarpus salignus* using Welch two-sample *t*-test

Character no.	Character	*t*	Degrees of freedom	*P*
1	No. of stomata at lower magnification (wide field of view)	6.71	48.56	$\leq 0.001^{***}$
2	No. of stomata at higher magnification	4.85	51.56	$\leq 0.001^{***}$
3	Size of entire stomatal apparatus	2.50	50.09	$\leq 0.05^{*}$
4	Size of inner stomatal apparatus	–5.16	39.99	$\leq 0.001^{***}$
5	Length of right-hand subsidiary cells	–1.49	44.00	≤ 0.1
6	Length of upper polar subsidiary cells	–5.59	42.59	$\leq 0.001^{***}$
7	Length of lower polar subsidiary cells	–4.67	43.77	$\leq 0.001^{***}$

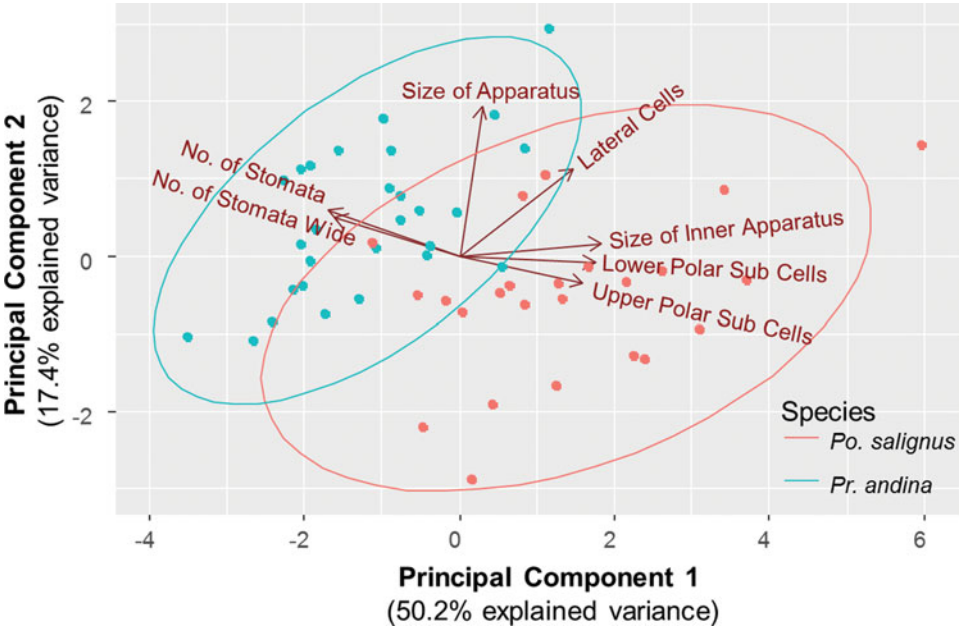

FIG. 6. Principal component analysis ordination diagram showing character differentiation between *Prumnopitys andina* and *Podocarpus salignus*. The species ellipses have a normal probability size of 0.95. The plot was drawn using the R library 'ggbiplot' (Vu, 2011). For explanation of character abbreviations, please see the main text.

In Scotland, the species is cultivated at lower elevations (33–50 m) than it occurs in its natural range. The temperature at the cultivation sites was within the temperature range experienced in the wild, as was the minimum rainfall in the driest month. However, overall the species encountered a substantially drier climate in Edinburgh, with only 676 mm total annual rainfall.

Climate for Podocarpus salignus. The records used for this species in the ordination analyses all came from the Chilean province of Linares and encompassed the following environments (Appendix table 3): elevations from 740 m to 755 m; minimum temperatures from 0.3°C to maximum temperatures of 26.8°C, and mean temperatures from 10.9°C to 14.2°C. The total annual precipitation ranged from 833 mm to 1301 mm, precipitation in the driest month from 7 mm to 19 mm, and precipitation in the wettest month from 199 mm to 274 mm. Therefore, even though these records all stemmed from the same province and a similar altitude, they encompassed a wide range of climates. The cultivated plants in Scotland encountered lower temperatures (annual mean temperature 8.6°C) and more rainfall (annual precipitation > 1700 mm) than the wild populations they originate from. However, a download from the GBIF (for datasets used, see *Materials and Methods*, *Acknowledgements* and *References*) of all available records for *Podocarpus salignus*, in combination with WorldClim data (Hijmans *et al.*, 2005), showed that in other parts of its range the species encounters even more extreme conditions. The species tolerates temperatures ranging from a minimum of –3.8°C to a maximum of 29.7°C, and rainfall from 7 mm in the driest month to 434 mm in the wettest month, and a total annual rainfall of up to 2409 mm.

Analysis of the influence of environmental factors. Figure 7 shows that in both species shaded leaves tended to have fewer and larger stomata (e.g. the size of the inner apparatus). In addition, greater moisture availability (total annual rainfall) appeared to have a positive effect on stomata size and a negative effect on stomata density: *Prumnopitys andina* encountered an overall drier climate in cultivation than in the wild, and the cultivated trees were characterised by a greater density of smaller stomata for a given level of sun exposure. This effect was particularly pronounced in shaded leaves (Fig. 7). Unfortunately, no data were available on sun exposure for *Podocarpus salignus* in cultivation, but in the wild shaded leaves had larger and fewer stomata.

Figure 8 shows the output of the PCA for stomatal characters of each species separately, standardised by sun exposure and overlaid with climatic variables. The analysis corroborated that in both species greater annual moisture availability positively correlated with the size of the stomata (apparatus, inner apparatus and/or lateral and polar cells) and negatively correlated with the density of stomata. The effect of temperature was less clear, potentially because rainfall and temperature were positively associated for the samples of *Prumnopitys andina* but negatively associated in the samples for *Podocarpus salignus.* The same is true for precipitation in the driest month, which positively correlated with total annual rainfall for *Podocarpus salignus* and negatively for *Prumnopitys andina.*

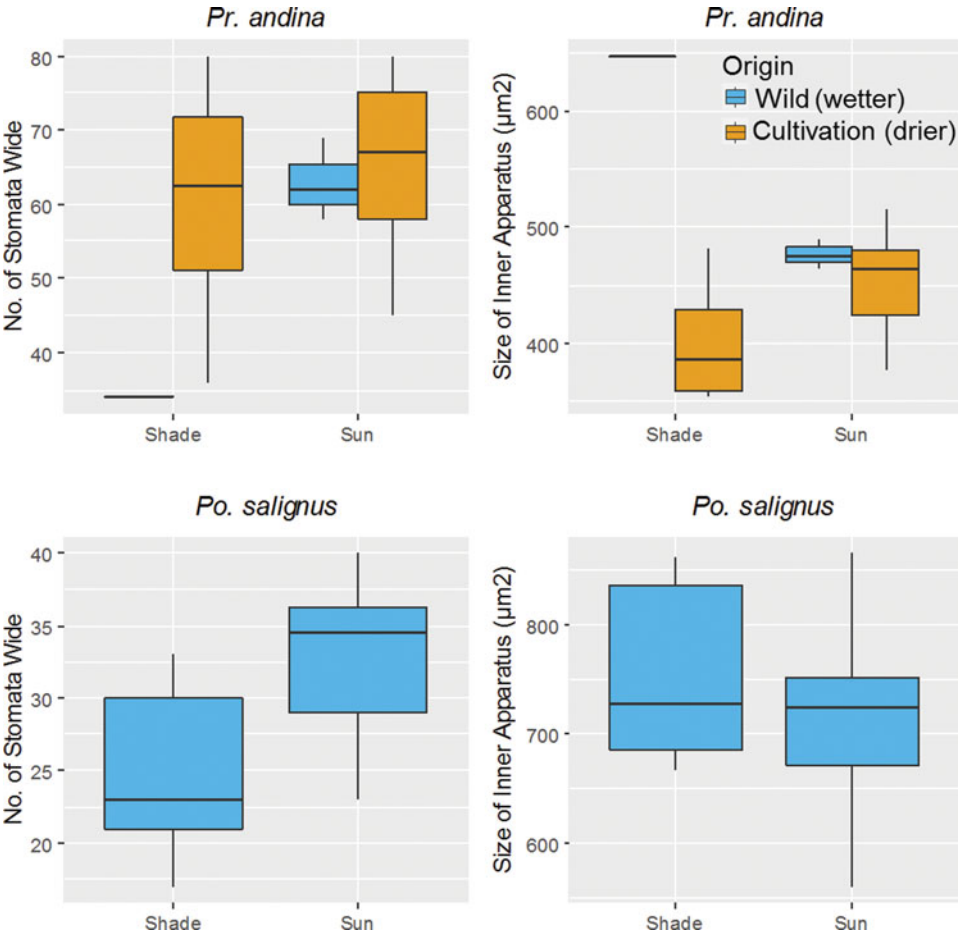

FIG. 7. Changes in the number of stomata and size of the inner apparatus in *Prumnopitys andina* and *Podocarpus salignus* with light conditions. In shady environments, there are fewer and larger stomata. In addition, greater moisture availability appears to influence these characters. *Prumnopitys andina* encounters a significantly drier climate in cultivation than in the wild, and developed more and smaller stomata. For explanation of character abbreviations, please see the main text.

Presence of stomata on the adaxial leaf surface

Stomata were found on the adaxial leaf surface in 12 of the 26 samples (46.2%) in *Prumnopitys andina* (Fig. 3): six out of 14 samples from Chile (42.8%) and six out of 12 samples from Scotland (50%). Six environmental factors were tested for their possible effects on this character, namely minimum temperature, maximum temperature, temperature range, annual precipitation, precipitation in the wettest month and precipitation in the driest month. However, none of these factors was a statistically significant predictor for the presence of stomata on the adaxial surface,

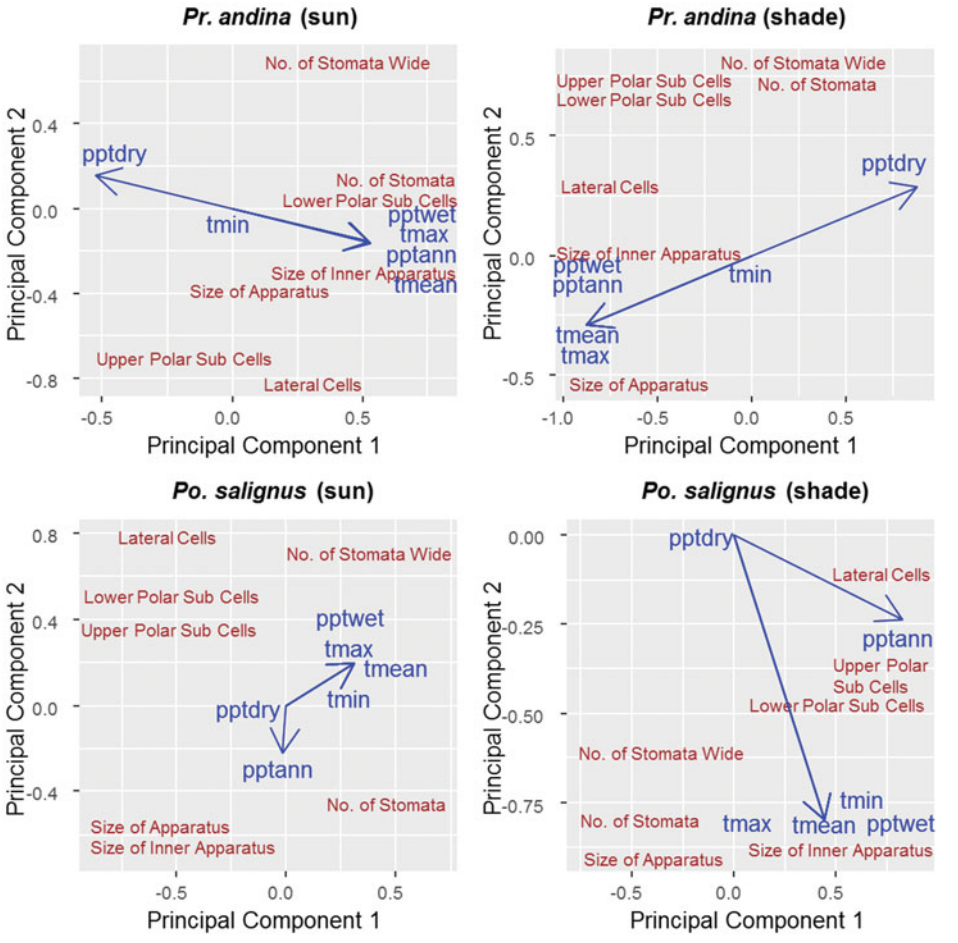

FIG. 8. Principal component analysis for stomatal characters of *Prumnopitys andina* and *Podocarpus salignus* (in sunny and shade environments), overlaid with climatic data. Moisture availability tends to increase the size of the inner apparatus and/or lateral cells and polar cells, and to decrease the number of stomata. pptann, annual precipitation; pptdry, precipitation in the driest month; pptwet, precipitation in the wettest month; tmax, temperature in the warmest month; tmean, annual mean temperature; tmin, temperature in the coldest month. For explanation of character abbreviations, please see the main text.

although there was a small positive association between the presence of adaxial stomata and total annual precipitation.

DISCUSSION

This study analysed whether cuticle micromorphology produces reliable taxonomic characters in two example species of Podocarpaceae, as well as whether these

characters are influenced by environmental factors. Overall, there were significant differences in the micromorphological cuticular characters between the two species. The stomatal apparatus of *Prumnopitys andina* was larger than in *Podocarpus salignus*, and *Pr. andina* also had the higher density of stomata of the two species. The inner apparatus, on the other hand, was larger in *Podocarpus salignus* (Tables 4 and 5).

However, these characters also varied with environmental factors. In both species, higher levels of precipitation and shade correlated with an increase in the size of stomata (apparatus, inner apparatus, guard cells and/or polar cells), and with a decrease in the density of stomata. Therefore, depending on the environment, there was some overlap between these characters in the two species. The effect of drought on both shaded and sun-exposed leaves was particularly visible in *Prumnopitys andina*, which experienced substantially drier conditions in cultivation than in the wild. With respect to *Podocarpus salignus*, although the cultivated samples in Scotland encounter a colder and wetter environment than the wild populations from which they originate, the species range in its entirety comprises even more extreme conditions, as noted in the *Results*.

Having said this, a number of leaf cuticle characters remain constant and show little or no variation regardless of climate. These more constant characters may be genetically controlled in the phenotype of the species (Zhang *et al.*, 2012) and may be regarded as taxonomically the most reliable. For these two species of Podocarpaceae, such constant characters include most epidermal cell and subsidiary cell characters, such as wall undulation; texture of the inner surface; presence of pit-like structures; uniformity of epidermal cell rows; and number, texture and shape of subsidiary cells (as indicated by * or ** in Table 2).

Adaxial stomata

Stockey & Frevel (1997) found that the leaves of three species of *Prumnopitys* [*Pr. ferruginoides* (Compton) de Laub. and *Pr. ladei* (F.M.Bailey) de Laub. from New Caledonia and Australia, and *Pr. standleyi* (J.Buchholz & N.E.Gray) de Laub. from Central America] were amphistomatic. However, all the other species studied by them, including *Prumnopitys andina*, were thus far thought to be strictly hypostomatic. As with other studies of the Podocarpaceae (Stockey & Ko, 1990; Stockey *et al.*, 1992, 1998; Mill & Stark Schilling, 2009; Whiting, 2009), only a small sample size was used per species (only one specimen per species, except for *Prumnopitys ladei*, of which two were sampled). By contrast, we examined the cuticles of 26 samples of *Prumnopitys andina* of both cultivated and wild origin and found adaxial stomata in 12 of them. This is a newly recorded character for this species. Although the presence or absence of adaxial stomata showed no statistically significant relationship with environmental conditions, there was some indication that their presence was increased by higher precipitation and lower minimum temperature.

Despite the large sample size of *Podocarpus salignus* examined here, no stomata were observed on the adaxial leaf surface, supporting the description of the leaf cuticle

of the species presented by Whiting (2009). These results also support the findings of Stockey & Ko (1988), Stockey *et al.* (1998) and Stark Schilling & Mill (2011) that members of the genus *Podocarpus* lack stomata on the adaxial surface of the leaves, except in a few rare cases such as *Podocarpus elongatus* (Sol.) L'Hér. ex Pers. (Stockey *et al.*, 1998).

Importance of a large sample size

Many studies of leaf cuticle micromorphological characters in gymnosperms generally, and Podocarpaceae in particular, have used small sample numbers, typically one to three per species. Although this may allow a more rapid description of stomatal characters and a larger range of species to be studied, it will not account for either phenotypic or genetic variability within taxa. Hill & Carpenter (1991) suggested that the single sample of the cuticle of *Acmopyle pancheri* (Brongn. & Gris) Pilg. (Podocarpaceae) that was used by Stockey & Ko (1988) was an extreme morphological example for that species, and that the cuticle was not as distinctive as implied in the earlier description by Stockey & Ko (1988).

When studying the genus *Araucaria* Juss., Stockey & Ko (1986) sampled all accepted species in the genus and made strong assumptions about the leaf cuticle micromorphology in the genus. However, in most cases they used only a single sample accession for each species. Hence this would not account for any intraspecific variability, and possibly lead to inaccurate, or at least incomplete, descriptions of species.

The larger sample size used in this study uncovered greater intraspecific variability within both species than has been apparent in previous studies in the Podocarpaceae. One of the most interesting findings was the presence of stomata on the adaxial surface of the leaf cuticle in some samples of *Prumnopitys andina*. Nevertheless, the sample size we used still did not yield enough data to provide statistical significance for the presence of stomata on the adaxial surface in relation to climatic conditions. Therefore, our study shows that many leaf cuticle micromorphological characters are subject to a great deal of intraspecific variation, and emphasises the importance of using greater sample sizes than has been hitherto considered acceptable for the purpose of using leaf cuticle micromorphological characters in taxonomic research. Ideally, future studies of leaf cuticles should focus on utilising a larger sample size. Doing this for *Prumnopitys* would allow one to test whether all species within the genus potentially have adaxial stomata and if their presence is correlated with decreased temperatures, shade and/or higher levels of precipitation, as suggested by the results of this study.

Adaptive significance in differences in leaf cuticle micromorphology in relation to environmental factors

The results suggest that the variation in the size of the stomatal apparatus and inner stomatal apparatus in both species may not be heavily constrained by the genetic make-up of the species, but that they are controlled by environmental conditions. This is supported by several other studies, which found differences in stomatal morphology in

relation to different environments, for example Sharma & Dunn (1968) in *Kalanchoe fedtschenkoi* Raym.-Hamet & H.Perrier; Schulze *et al.* (1980) in desert species including *Artemisia herba-alba* Asso, *Hammada scoparia* (Pomel) Iljin, *Prunus armeniaca* L., *Reaumuria negevensis* Zohary & Danin and *Zygophyllum dumosum* Boiss.; Bakker (1991) in glasshouse-grown vegetables including sweet pepper (*Capsicum annuum* L.), cucumber (*Cucumis sativus* L.), tomato (*Lycopersicon esculentum* Mill.) and eggplant (*Solanum melongena* L.); Poole *et al.* (1996) in the angiosperm tree *Alnus glutinosa* (L.) Gaertn. (Betulaceae); and Mitton *et al.* (1998) in *Pinus edulis* Engelm.

A possible explanation for the effect of precipitation and sun exposure is provided by Aasamaa *et al.* (2001), who showed that there is a negative relationship between the size of stomata and their response time to drought (closure). Consequently, a small number of large stomata could lead to stress and hydraulic dysfunction in dry conditions. A higher density of smaller stomata in sunny and/or dry environments may also allow for greater temperature control through increased evaporation. In humid and shady conditions, the slower dynamic behaviour of large stomata is less disadvantageous (Hetherington & Woodward, 2003). Allen & Pearcy (2000) have shown that shade-tolerant species retain open stomata through parts of the day to minimise the impact of slow response times during the few sunlit hours.

The differentially amphistomatic leaves found in some samples of *Prumnopitys andina* may also have adaptive significance in allowing the species to increase its rate of transpiration in shady and wetter environments. Mott *et al.* (1982) inferred that the presence of amphistomatic leaves was somewhat correlated with a shaded environment and a constantly available supply of soil water. The adaptive advantage of amphistomatic leaves means that such plants are able to maintain a higher maximum leaf conductance and a higher rate of transpiration than those with epistomatic or hypostomatic leaves (Mott *et al.*, 1982). This indicates that species of *Prumnopitys* with the ability to produce stomata on the adaxial leaf surface, such as *Prumnopitys andina*, may be capable of a higher rate of transpiration when growing in areas of higher precipitation, by increasing their water uptake, thereby increasing their photosynthetic capacity.

Suggestions for future research

Our research showed that there is variation in leaf cuticle characters in different environmental conditions in the two example species. Further research should also focus on other members of the Podocarpaceae, and more widely gymnosperms, to test whether environmental factors play a role in leaf cuticle micromorphology in other groups too.

In addition, variation in leaf cuticle micromorphology has still not really been assessed at the population level. How does variation in leaf cuticle micromorphology relate across an entire population, and across multiple populations within the Podocarpaceae and within conifers as a whole?

More specifically, the presence of adaxial stomata, found in some samples of *Prumnopitys andina*, highlighted the importance of the use of a large sample size.

However, in this study the sample size was still not great enough to determine the statistical significance of stomata on the adaxial surface of the leaf cuticle in relation to climatic factors.

Finally, it would be interesting to gain an understanding of the developmental responses to increased precipitation and other climatic factors. The genetic responses to climatic conditions in the Podocarpaceae could also provide useful insights into the development of stomata in the gymnosperms.

Conclusions

The results from this study support the hypothesis that external factors can have an effect on cuticular micromorphology of Podocarpaceae. Both species showed phenotypic plasticity, and in cultivation were able to adapt to environments that are quite different to their native habitat. This raises the general question of how variable species really are, and how the effects of climate change may be offset by phenotypic plasticity, which ultimately could be the key to their survival.

In *Prumnopitys andina*, stomata were found on the adaxial surface of the leaf cuticle, which was a newly recorded character for the species. Although no statistical significance was found with the presence of adaxial stomata and climatic factors, there was some indication that the presence of this character is linked to increased precipitation, lower temperature and leaves that were growing in the shade. Many other of the descriptive characters used in leaf cuticle micromorphology studies appeared to be subject to a great deal of intraspecific variation too. In both species, the size of the stomatal apparatus and inner stomatal apparatus and the density of stomata were highly variable and influenced by environmental factors.

However, a number of leaf cuticle characters can be considered reliable in both species: degree of undulation of epidermal cell walls, inner texture of epidermal cells, presence or absence of pit-like protuberances in epidermal cells, margin of epidermal cells, uniformity of adaxial epidermal cell rows, shape and size of epidermal cells, and number of subsidiary cells. Because of the degree of variation in leaf cuticle characters in both species, this study has indicated the importance in using a large sample size. Using a greater range of samples means that it becomes possible to assess the degree of variability in the leaf cuticle characters and to establish how useful they may be as a tool for taxonomic research. Because much of the work in the Podocarpaceae has focused on smaller samples, interspecific variation may not have been accounted for, leading to inaccurate descriptions.

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GBIF is hereby acknowledged in terms of the relevant licences: Allan Herbarium, Christchurch (Wilton, 2017); Australia's Virtual Herbarium (Council of Heads of Australasian Herbaria, 2017); Berkeley Natural History Museums (2017); Herbarium Berolinense (2017); Herbarium Senckenbergianum (2017); Herbarium of Namur (Martin & Heughebaert, 2017); Instituto de Botánica Darwinion, Buenos Aires (SI) (Instituto de Botánica Darwinion, 2017); Missouri Botanical Garden Tropicos Specimen Database (Magill *et al.*, 2016); Muséum national d'Histoire naturelle, Paris (Chagnoux, 2017); National Museum of Natural History, Smithsonian Institution (Orrell & Hollowell, 2017); Naturalis Biodiversity Center (Creuwels, 2017); New York Botanical Garden Herbarium (Ramirez & Tulig, 2015); Phanerogamic Botanical Collections, Stockholm (Telenius & Shah, 2016); Real Jardín Botánico de Madrid (2016); RBGE (2017a, Herbarium; 2017b, Living Collection); Royal Botanic Gardens, Kew (2016); Herbario SANT (García San León, 2017); Universidad Nacional de La Plata (Williams, 2011); and University of California, Davis Center for Plant Diversity (Dean & Starbuck, 2016).The RBGE is supported by the the Scottish Government's Rural and Environment Science and Analytical Services Division. The helpful comments of two anonymous reviewers are gratefully acknowledged.

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APPENDIX TABLE 1. List of accessions and collections for *Prumnopitys andina*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
E00028541	W	Chile	IX La Araucánia	Cautín	Melipeuco: 3 km E of Melipeuco	38°51′S	71°42′W	600	M.F. Gardner & S.G. Knees	4700	E	JC146
E00078122	W	Chile	IX La Araucánia	Cautín	Melipeuco: Cordillera de los Andes near Melipeuco	38°45′57″S	71°35′05″W	900	K.L. Matthews & D.B. White	46	E	JC145
E00078123	W	Chile	IX La Araucánia	Cautín	Melipeuco: Cordilllera de los Andes near Melipeuco	38°50′51″S	71°40′34″W	520	K.L. Matthews & D.B. White	55	E	JC147
E00158482	W	Chile	VIII Biobío	Ñuble	San Fabián: Sector El Ingles	36°39′56.2″S	71°16′26.6″W	696	M.F. Gardner, P. Hechenleitner, C. Martínez & P. Thomas	DCI 394	E	JC158
E00158576	W	Chile	VIII Biobío	Ñuble	San Fabián: Sector El Ingles	36°39′08.3″S	71°16′12.4″W	696	M.F. Gardner, P. Hechenleitner, C. Martínez & P. Thomas	DCI 390	E	JC166
E00158590	W	Chile	VIII Biobío	Ñuble	San Fabián:Sector El Ingles	36°40′16.4″S	71°16′58.6″W	699	M.F. Gardner, P. Hechenleitner, C. Martínez & P. Thomas	DCI 416	E	JC164
E00158595	W	Chile	VIII Biobío	Ñuble	San Fabián: Sector El Ingles	36°40′11.3″S	71°18′17.9″W	670	M.F. Gardner, P. Hechenleitner, C. Martínez & P. Thomas	DCI 403	E	JC165
E00158607	W	Chile	VIII Biobío	Ñuble	San Fabián: Sector El Ingles	36°39′13.8″S	71°16′08.8″W	696	M.F. Gardner, P. Hechenleitner, C. Martínez & P. Thomas	DCI 391	E	JC159

APPENDIX TABLE 1. *(Continued)*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
E00158620	W	Chile	VIII Biobío	Ñuble	San Fabián: Sector El Ingles	36°40′11.2″S	71°18′17.9″W	669	M.F. Gardner, P. Hechenleitner, C. Martínez & P. Thomas	DCI 398	E	JC160
E00158726	W	Chile	IX La Araucánia	Malleco	Angol: Sector Cerro Pelado	37°50′14.5″S	72°50′02″W	1127	M.F. Gardner, P. Hechenleitner, C. Martínez & P. Thomas	DCI 606	E	JC171
E00158738	W	Chile	IX La Araucánia	Malleco	Angol: Sector Cerro Pelado	37°50′06.4″S	72°50′30.9″W	1078	M.F. Gardner, P. Hechenleitner, C. Martínez & P. Thomas	DCI 609	E	JC170
E00158739	W	Chile	IX La Araucánia	Malleco	Angol: Sector Cerro Pelado	37°49′38.6″S	72°49′35.3″W	976	M.F. Gardner, P. Hechenleitner, C. Martínez & P. Thomas	DCI 627	E	JC168
E00158742	W	Chile	IX La Araucánia	Malleco	Angol: Sector Cerro Pelado	37°49′42.9″S	72°49′36.5″W	989	M.F. Gardner, P. Hechenleitner, C. Martínez & P. Thomas	DCI 632	E	JC169
E00158743	W	Chile	IX La Araucánia	Malleco	Angol: Sector Cerro Pelado, on road from Angol to Rosario	37°49′52.6″S	72°49′54.7″W	1039	M.F. Gardner, P. Hechenleitner, C. Martínez & P. Thomas	DCI 619	E	JC161
E00182342	W	Chile	VIII Biobío	Ñuble	Chillán: Cordillera de los Andes, road from El Cerrillo to Parecela Los Lleuques	36°51′32.7″S	71°35′53.2″W	1007	M.F. Gardner & S.G. Knees	6795	E	JC144

APPENDIX TABLE 1. *(Continued)*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
E00182717	W	Chile	VII Maule	Linares	Colbún: Cordillera de los Andes, Sector Quebrada Ballical	35°53′32.9″S	70°59′47.9″W	871	P. Brownless, M.F. Gardner, P. Hechenleitner & P. Thomas	DCI 1150	E	JC142
E00183571	W	Chile	VIII Biobío	Biobío	Alto Biobío: 5 km west of Nitrao	37°41′S	71°18′W	900	M.F. Gardner & S.G. Knees	5556	E	JC143
E00189660	W	Chile	IX La Araucánia	Malleco	Curacautín: Cordillera de los Andes, Parque Nacional Conguillio	38°41′10″S	71°37′16.4″W	1002	P. Brownless, M.F. Gardner, P. Hechenleitner, P. Hollingsworth, M. Hollingsworth & P. Thomas	DCI 920	E	JC174
E00189661	W	Chile	IX La Araucánia	Malleco	Curacautín: Cordillera de los Andes, Parque Nacional Conguillio	38°41′09.9″S	71°37′13.6″W	1022	P. Brownless, M.F. Gardner, P. Hechenleitner, P. Hollingsworth, M. Hollingsworth & P. Thomas	DCI 928	E	JC156
E00189662	W	Chile	VIII Biobío	Biobío	Antuco: Cordillera de los Andes, Fundo Los Ciervos	37°13′52.6″S	71°26′40.9″W	859	P. Brownless, M.F. Gardner, P. Hechenleitner & P. Thomas	DCI 1027	E	JC154
E00189663	W	Chile	VIII Biobío	Biobío	Antuco: Cordillera de los Andes, Fundo Los Ciervos	37°12′03.6″S	71°26′54″W	858	P. Brownless, M.F. Gardner, P. Hechenleitner & P. Thomas	DCI 1015	E	JC149
E00189665	W	Chile	VIII Biobío	Ñuble	Pinto: Cordillera de los Andes, Termas de Chillán	36°51′41.7″S	71°34′41.9″W	1240	P. Brownless, M.F. Gardner, P. Hechenleitner & P. Thomas	DCI 1066	E	JC140

APPENDIX TABLE 1. *(Continued)*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
E00189667	W	Chile	IX La Araucánia	Malleco	Curacautín: Cordillera de los Andes, Parque Nacional Conguillio	38°41′11.5″S	71°37′05″W	1015	P. Brownless, M.F. Gardner, P. Hechenleitner, P. Hollingsworth, M. Hollingsworth & P. Thomas	DCI 915	E	JC153
E00196365	W	Chile	VIII Biobío	Biobío	Antuco: Cordillera de los Andes, Fundo Los Ciervos	37°12′48.9″S	71°26′19.3″W	877	P. Brownless, M.F. Gardner, P. Hechenleitner & P. Thomas	DCI 1020	E	JC157
E00196366	W	Chile	VIII Biobío	Ñuble	Pinto: Cordillera de los Andes, Termas de Chillán	36°51′51.5″S	71°35′55″W	1005	P. Brownless, M.F. Gardner, P. Hechenleitner & P. Thomas	DCI 1084	E	JC150
E00196367	W	Chile	VIII Biobío	Biobío	Santa Barbara: Cordillera de los Andes, Alto Bio-Bío Sector, south bank of Rio Trapatrapa	37°43′21.7″S	71°14′48″W	963	P. Brownless, M.F. Gardner, P. Hechenleitner & P. Thomas	DCI 708	E	JC152
E00196368	W	Chile	VIII Biobío	Biobío	Santa Barbara: Cordillera de los Andes, Alto Bio-Bío Sector, south bank of Rio Trapatrapa	37°43′33.8″S	71°13′46.6″W	983	P. Brownless, M.F. Gardner, P. Hechenleitner & P. Thomas	DCI 707	E	JC151
E00196369	W	Chile	VIII Biobío	Biobío	Santa Barbara: Cordillera de los Andes, Alto Bio-Bío Sector, between Lepoy and Ralco	38°03′46″S	71°25′22.4″W	782	P. Brownless, M.F. Gardner, P. Hechenleitner & P. Thomas	DCI 776	E	JC148

APPENDIX TABLE 1. *(Continued)*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
E00196370	W	Chile	VIII Biobío	Linares	Colbún: Cordillera de los Andes, Sector Quebrada Ballical	35°53′32.9″S	70°59′37.9″W	871	P. Brownless, M.F. Gardner, P. Hechenleitner & P. Thomas	DCI 1140	E	JC138
E00196371	W	Chile	VIII Biobío	Biobío	Antuco: Cordillera de los Andes, Fundo Los Ciervos	37°11′18″S	71°26′29.9″W	868	P. Brownless, M.F. Gardner, P. Hechenleitner & P. Thomas	DCI 1008	E	JC137
E00196372	W	Chile	VIII Biobío	Biobío	Santa Barbara: Cordillera de los Andes, Alto Bio-Bío Sector, south bank of Rio Trapatrapa	37°43′21.7″S	71°14′48″W	963	P. Brownless, M.F. Gardner, P. Hechenleitner & P. Thomas	DCI 709	E	JC136
E00196373	W	Chile	IX La Araucánia	Malleco	Curacautín: Cordillera de los Andes, Parque Nacional Conguillio	38°41′10″S	71°37′16.4″W	1000	P. Brownless, M.F. Gardner, P. Hechenleitner, P. Hollingsworth, M. Hollingsworth & P. Thomas	DCI 921	E	JC141
E00196374	W	Chile	IX La Araucánia	Malleco	Curacautín: Cordillera de los Andes, Parque Nacional Conguillio	38°41′09.4″S	71°36′55.7″W	980	P. Brownless, M.F. Gardner, P. Hechenleitner, P. Hollingsworth, M. Hollingsworth & P. Thomas	DCI 906	E	JC139
E00196375	W	Chile	IX La Araucánia	Malleco	Curacautín: Cordillera de los Andes, Parque Nacional Conguillio	38°41′09.4″S	71°36′55.7″W	980	P. Brownless, M.F. Gardner, P. Hechenleitner, P. Hollingsworth, M. Hollingsworth & P. Thomas	DCI 946	E	JC173

APPENDIX TABLE 1. *(Continued)*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
E00196376	W	Chile	VIII Biobío	Biobío	Santa Barbara: Cordillera de Los Andes, Alto Bio-Bío Sector, Río Trapatrapa near junction with Río Quenco	37°41′43″S	71°17′35.2″W	859	P. Brownless, M.F. Gardner, P. Hechenleitner & P. Thomas	DCI 713	E	JC155
E00196377	W	Chile	VIII Biobío	Biobío	Antuco: Cordillera de los Andes, Fundo Los Ciervos	37°11′28.1″S	71°26′18.2″W	874	P. Brownless, M.F. Gardner, P. Hechenleitner & P. Thomas	DCI 1010	E	JC172
E00236118	W	Chile	IX La Araucánia	Malleco	Victoria: 3 km de pua hacia Selva Negra	38°20′30″S	72°19′49.6″W	312	P. Hechenleitner, M.F. Gardner & D. Weber	PHV 375	E	JC167
E00420513	W	Chile	VIII Biobío	Ñuble	San Fabián: Sector El Ingles	36°39′33.2″S	71°16′15″W	723	M.F. Gardner, C. Morter & G. Ovstebo	76A	E	JC134
E00420514	W	Chile	VIII Biobío	Ñuble	San Fabián: Sector El Ingles	36°39′33.2″S	71°16′15″W	723	M.F. Gardner, C. Morter & G. Ovstebo	76B	E	JC135
E00420517	W	Chile	VIII Biobío	Ñuble	San Fabián: Sector El Ingles	36°39′20.7″S	71°16′11.9″W	722	M.F. Gardner, C. Morter & G. Ovstebo	73A	E	JC95
E00420518	W	Chile	VIII Biobío	Ñuble	San Fabián: Sector El Ingles	36°39′20.7″S	71°16′11.9″W	722	M.F. Gardner, C. Morter & G. Ovstebo	73B	E	JC96
E00420519	W	Chile	VIII Biobío	Ñuble	San Fabián: Sector El Ingles	36°39′08.3″S	71°16′12.6″W	737	M.F. Gardner, C. Morter & G. Ovstebo	74A	E	JC97
E00420520	W	Chile	VIII Biobío	Ñuble	San Fabián: Sector El Ingles	36°39′08.3″S	71°16′12.6″W	737	M.F. Gardner, C. Morter & G. Ovstebo	74B	E	JC98
E00420532	W	Chile	VIII Biobío	Ñuble	San Fabián: Sector El Ingles	36°39′7.4″S	71°16′12.6″W	737	M.F. Gardner, C. Morter & G. Ovstebo	75B	E	JC100
E00420533	W	Chile	VIII Biobío	Ñuble	San Fabián: Sector El Ingles	36°39′7.4″S	71°16′12.6″W	737	M.F. Gardner, C. Morter & G. Ovstebo	75A	E	JC99

APPENDIX TABLE 1. *(Continued)*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
E00593092	W	Chile	VIII Biobío	Chillian	Pinto: Fundo Los Lleuques	36°51′53.7″S	71°35′42.6″W	965	P.R. Baxter & M.F. Gardner	146	E	JC163
E00593097	W	Chile	IX La Araucánia	Angol	Victoria: east of Púa close to Santa Eugenia on road to Selva Oscura	38°20′34.1″S	72°19′44.8″W	321	P.R. Baxter & M.F. Gardner	79	E	JC162
19921591*L	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU2	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC16	19921591*L	JC26
19921603*B	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC30	19921603*B	JC49
19921604*A	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC34	19921604*A	JC47
19921604*B	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC27	19921604*B	JC50
19921610*B	C	Uk	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC29	19921610*B	JC46
19921611*C	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC26	19921611*C	JC58
20030096*B	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU307	E	JC131
20040045*G	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU305	E	JC129
20040045*H	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU304	E	JC128
20040045*I	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU303	E	JC127
20040045*J	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU302	E	JC126
20040053*F	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU301	E	JC125
20040053*G	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU300	E	JC124

APPENDIX TABLE 1. *(Continued)*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
20040053*H	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU299	E	JC123
20040053*I	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU298	E	JC122
20040053*J	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU297	E	JC121A
20040097*F	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU296	E	JC120
20040097*G	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU295	E	JC119
20040097*H	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU294	E	JC118
20040097*I	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU293	E	JC117
20040097*J	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU292	E	JC134
20040098*F	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU291	E	JC116A
20040098*G	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU290	E	JC115
20040098*H	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU289	E	JC133A
20040098*H	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU289	E	JC133B
20040098*I	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU288	E	JC113
20040098*J	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU287	E	JC112
20040099*F	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU286	E	JC114
20040099*G	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU285	E	JC110A
20040099*G	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU285	E	JC110B
20040099*H	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU284	E	JC101
20040099*I	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU283	E	JC108
20040099*J	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU282	E	JC107
20040100*F	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU281	E	JC106
20040100*G	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU280	E	JC105
20040100*H	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU279	E	JC104
20040100*J	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU278	E	JC103
20040113*F	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU277	E	JC102
20040113*G	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU276	E	JC109

APPENDIX TABLE 1. *(Continued)*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
20040113*H	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU275	E	JC132
20040113*I	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU274	20040113*I	JC78
20040113*J	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU273	20040113*J	JC77
20040139*F	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU272	20040139*F	JC76
20040139*G	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU271	20040139*G	JC75A
20040139*G	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU271	20040139*G	JC75B
20040139*H	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU270	20040139*H	JC74
20040139*I	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU269	20040139*I	JC73
20040139*J	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU268	20040139*J	JC72
20040140*H	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU267	20040140*H	JC71
20040140*J	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU265	20040140*J	JC69
20040140*L	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU266	20040140*L	JC70
20040141*F	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU264	20040141*F	JC68
20040141*G	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU263	20040141*G	JC67
20040141*H	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU262	20040141*H	JC65
20040141*I	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU261	20040141*I	JC64
20040141*J	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU260	20040141*J	JC63
20070033*AE	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU306	E	JC130B
20071033*AE	C	UK	Scotland	Edinburgh	RBGE, Nursery	55°58′11.3″N	3°12′43.3″W		J.A.R. Clugston	JCLU306	E	JC130A

APPENDIX TABLE 2. List of accessions and collections: *Podocarpus salignus*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
E00011196	W	Chile	IX La Araucánia	Malleco	Angol: 12 km west of Angol on road to Piedra del Aguila	37°47′S	72°49′W	800	M.F. Gardner & C.N. Page	4970	E	JC89
E00011203	W	Chile	VIII Biobío	Ñuble	Pinto: Los Lleuques	36°51′S	71°38′W	850	M.F. Gardner, S.G. Knees & M.L. De Vore	4667	E	JC88
E00011204	W	Chile	VIII Biobío	Biobío	Alto Biobío: 3 km from Ralco towards Laguna Barco	37°5′S	71°35′W	500	M.F. Gardner & S.G. Knees	5579	E	JC87
E00011205	W	Chile	IX La Araucánia	Malleco	Angol: 12 km west of Angol on road to Piedra del Aguila	37°47′S	72°49′W	800	M.F. Gardner & C.N. Page	4970	E	JC81
E00089351	W	Chile	IX La Araucánia	Malleco	Traiguén, Cordillera de la Costa	38°14′57″S	72°30′36″W	100	P. Baxter, P. Brownless, M. Bustos, M. Gardner, K. Matthews, H.S. Maxwell & D. Rae	UCEXC 439	E	JC90
E00089357	W	Chile	VIII Biobío	Arauco	Lebu: Cordillera de Nahuelbuta, Rio Trongol	37°36′54″S	73°16′58″W	170	P. Baxter, P. Brownless, M. Bustos, M. Gardner, K. Matthews, H.S. Maxwell & D. Rae	UCEXC 518	E	JC82
E00089358	W	Chile	IX La Araucánia	Malleco	Angol: Cordillera de Nahuelbuta	37°48′07″S	72°51′37″W	490	P. Baxter, P. Brownless, M. Bustos, M. Gardner, K. Matthews, H.S. Maxwell & D. Rae	UCEXC 483	E	JC83

APPENDIX TABLE 2. *(Continued)*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
E00158541	W	Chile	VII Maule	Cauquenes	Pelluhue: Reserva Nacional Los Queules	35°58′46″S	72°41′21.2″W	340	M.F. Gardner, P. Hechenleitner V, C. Martínez A & P. I. Thomas	DCI 211	E	JC86
E00158588	W	Chile	VIII Biobío	Penco	Penco: along side of road to San José	36°41′35.1″S	72°54′57.7″W	246	M.F. Gardner, P. Hechenleitner V, C. Martínez A & P. I. Thomas	DCI 491	E	JC61
E00158594	W	Chile	VIII Biobío	Concepción	Penco: Fundo Quebrada Honda	36°41′35.1″S	72°54′57.7″W	246	M.F. Gardner, P. Hechenleitner V, C. Martínez A & P. I. Thomas	DCI 490	E	JC92
E00158608	W	Chile	VII Maule	Linares	Parral: Road to leading to Reserva Nacional Los Bellotos del Melado	35°51′25.6″S	71°09′6.2″W	768	M.F. Gardner, P. Hechenleitner V, C. Martínez A & P. I. Thomas	DCI 305	E	JC80
E00158682	W	Chile	VII Maule	Linares	Parral: Bullileo, East of Laguna El Amargo	36°19′29.8″S	71°24′13.6″W	700	M.F. Gardner, P. Hechenleitner V, C. Martínez A & P. I. Thomas	DCI 350	E	JC85
E00158741	W	Chile	IX La Araucánia	Malleco	Angol: sector Cerro Pelado, on road from Angol to Rosario	37°49′53.8″S	72°50′13.4″W	997	M.F. Gardner, P. Hechenleitner V, C. Martínez A & P. I. Thomas	DCI 631	E	JC79
E00167239	W	Chile	XIV Los Ríos	Valdivia	Valdivia: Cordillera de la Costa, Parque Oncol	39°42′00″S	73°18′58″W	500	M.F. Gardner	6462	E	JC91

APPENDIX TABLE 2. *(Continued)*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
E00182341	W	Chile	VIII Biobío	Ñuble	Chillán: Cordillera de Los Andes: Puente Atacalco close to the Río Diguillon	36°53′23.8″S	71°37′41.1″W	628	M.F. Gardner & S.G. Knees	GAK 6793	E	JC93
E00182642	W	Chile	VIII Biobío	Ñuble	Chillán: Cordillera de Los Andes: Puente Atacalco close to the Río Diguillon	36°53′23.8″S	71°37′41.1″W	628	M.F. Gardner & S.G. Knees	6794	E	JC94
E00194593	W	Chile	XIV Los Ríos	Valdivia	Paillaco: 30 km south of Valdivia	n/a	n/a	150	Z. Debreczy, G. Biró & I. Rácz	47900	E	JC60
E00420539	W	Chile	VII Maule	Linares	Parrall: Eastern banks of Laguna de Amargo	36°18′55.7″S	71°24′16″W	740	M.F. Gardner, C. Morter & G. Ovstebo	105B	E	JC4
E00420540	W	Chile	VII Maule	Linares	Parrall: Eastern banks of Laguna de Amargo	36°18′55.7″S	71°24′16″W	740	M.F. Gardner, C. Morter & G. Ovstebo	105A	E	JC3
E00420541	W	Chile	VII Maule	Linares	Parrall: Eastern banks of Laguna de Amargo	35°18′45.7″S	71°24′11.8″W	755	M.F. Gardner, C. Morter & G. Ovstebo	104A	E	JC1
E00420542	W	Chile	VII Maule	Linares	Parrall: Eastern banks of Laguna de Amargo	35°18′45.7″S	71°24′11.8″W	755	M.F. Gardner, C. Morter & G. Ovstebo	104B	E	JC2
E00420543	W	Chile	VII Maule	Linares	Parrall: Southern end of Laguna de Amargo	36°18′17.8″S	71°24′21.4″W	740	M.F. Gardner, C. Morter & G. Ovstebo	112A	E	JC17
E00420544	W	Chile	VII Maule	Linares	Parrall: Southern end of Laguna de Amargo	36°18′17.8″S	71°24′21.4″W	740	M.F. Gardner, C. Morter & G. Ovstebo	112B	E	JC18

APPENDIX TABLE 2. *(Continued)*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
E00420545	W	Chile	VII Maule	Linares	Parrall: Eastern banks of Laguna de Amargo	36°18′57.4″S	71°24′17.4″W	733	M.F. Gardner, C. Morter & G. Ovstebo	107A	E	JC7
E00420546	W	Chile	VII Maule	Linares	Parrall: Eastern banks of Laguna de Amargo	36°18′57.4″S	71°24′17.4″W	733	M.F. Gardner, C. Morter & G. Ovstebo	107B	E	JC8
E00420547	W	Chile	VII Maule	Linares	Parrall: Southern end of Laguna de Amargo	36°19′16.0″S	71°24′20.6″W	740	M.F. Gardner, C. Morter & G. Ovstebo	110A	E	JC13
E00420548	W	Chile	VII Maule	Linares	Parrall: Southern end of Laguna de Amargo	36°19′16.0″S	71°24′20.6″W	740	M.F. Gardner, C. Morter & G. Ovstebo	110B	E	JC14
E00420549	W	Chile	VII Maule	Linares	Parrall: Southern end of Laguna de Amargo	36°19′15.9″S	71°24′20.7″W	740	M.F. Gardner, C. Morter & G. Ovstebo	111A	E	JC15
E00420550	W	Chile	VII Maule	Linares	Parrall: Southern end of Laguna de Amargo	36°19′15.9″S	71°24′20.7″W	740	M.F. Gardner, C. Morter & G. Ovstebo	111B	E	JC16
E00420551	W	Chile	VII Maule	Linares	Parrall: Eastern banks of Laguna de Amargo	36°18′56.2″S	71°24′16.8″W	736	M.F. Gardner, C. Morter & G. Ovstebo	106A	E	JC5
E00420552	W	Chile	VII Maule	Linares	Parrall: Eastern banks of Laguna de Amargo	36°18′56.2″S	71°24′16.8″W	736	M.F. Gardner, C. Morter & G. Ovstebo	106B	E	JC6
E00420553	W	Chile	VII Maule	Linares	Parrall: Southern end of Laguna de Amargo	36°19′17.1″S	71°24′21.2″W	740	M.F. Gardner, C. Morter & G. Ovstebo	113A	E	JC19
E00420554	W	Chile	VII Maule	Linares	Parrall: Southern end of Laguna de Amargo	36°19′17.1″S	71°24′21.2″W	740	M.F. Gardner, C. Morter & G. Ovstebo	113B	E	JC20

APPENDIX TABLE 2. *(Continued)*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
E00420558	W	Chile	VII Maule	Linares	Parrall: Southern end of Laguna de Amargo	36°19′00.7″S	71°24′13.9″W	749	M.F. Gardner, C. Morter & G. Ovstebo	108A	E	JC9
E00420560	W	Chile	VII Maule	Linares	Parrall: Southern end of Laguna de Amargo	36°19′00.7″S	71°24′13.9″W	749	M.F. Gardner, C. Morter & G. Ovstebo	108B	E	JC10
E00420561	W	Chile	VII Maule	Linares	Parrall: Southern end of Laguna de Amargo	36°19′14.9″S	71°24′19.7″W	746	M.F. Gardner, C. Morter & G. Ovstebo	109A	E	JC11
E00420562	W	Chile	VII Maule	Linares	Parrall: Southern end of Laguna de Amargo	36°19′14.9″S	71°24′19.7″W	746	M.F. Gardner, C. Morter & G. Ovstebo	109B	E	JC12
E00593099	W	Chile	VIII Biobío	Ñuble	Chillán: Comuna de Pinto	36°53′26.8″S	71°37′34.5″W	627	P.R. Baxter & M.F. Gardner	141	E	JC84
19870712*A	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU2	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC10	19870712*A	JC24
19870712*B	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU2	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC41	19870712*B	JC39
19870712*F	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU1	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC2	19870712*F	JC31
19870834*A	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YS5	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC1	19870834*A	JC29
19920683*AQ	C	Uk	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU2	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC9	19920683*AQ	JC43

APPENDIX TABLE 2. *(Continued)*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
19921585*AL	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU2	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC18	19921585*AL	JC38
19921585*AM	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU1	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC3	19921585*AM	JC30
19921585*AN	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU2	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC17	19921585*AN	JC21
19921588*B	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU2	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC11	19921588*B	JC23
19921590*I	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU2	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC14	19921590*I	JC36
19921591*C	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU2	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC12	19921591*C	JC22
19921591*K	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU2	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC40	19921591*K	JC25
19921660*AC	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU2	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC15	19921660*AC	JC27
19931647*W	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC24	19931647*W	JC56
19931647*X	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC21	19931647*X	JC34
19931647*Y	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC35	19931647*Y	JC44

APPENDIX TABLE 2. *(Continued)*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
19931648*C	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC28	19931648*C	JC59
19931648*I	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC33	19931648*I	JC54
19931648*L	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC39	19931648*L	JC45
19931648*T	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC37	19931848*T	JC51
19931648*V	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC25	19931648*V	JC57
19931648*W	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC32	19931648*W	JC52
19931649*M	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC24	19931649*M	JC32
19931649*N	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC31	19931649*N	JC55
19931649*O	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC38	19931649*O	JC48
19931649*P	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC36	19931649*P	JC53

APPENDIX TABLE 2. *(Continued)*

Barcode/ accession no.	Wild (W) or cultivated (C)	Country	Region	Province	Locality	Latitude	Longitude	Altitude (m)	Collector(s)	Collection no.	Herbarium	Sample no.
19931649*T	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC22	19931649*T	JC33
19931660*AE	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU1	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC4	19921960*AE	JC28
19931660*Y	C	UK	Scotland	Argyll	Dunoon: Kilmun Arboretum	55°59′31.43″N	4°55′57.54″W		J.A.R. Clugston & T. Christian	JTC20	19931660*Y	JC35
19941902*A	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU1	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC6	19941902*A	JC40
19961040*I	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU1	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC8	19961040*I	JC42
19961040*J	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU1	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC7	19961040*J	JC41
19980654*L	C	UK	Scotland	Argyll	Dunoon: Benmore Botanic Garden, YU2	56°01′27.6″N	4°59′51.1″W		J.A.R. Clugston & T. Christian	JTC19	19980654*L	JC37

APPENDIX TABLE 3. Effect of climate on leaf cuticle micromorphology. The table shows the localities of gathered material for specimens of *Prumnopitys andina* and *Podocarpus salignus*, with climatic data obtained from WorldClim (Hijmans *et al.*, 2005); for *Pr. andina*, pink shading indicates the highest value, and blue the lowest value, and for *Po. salignus*, yellow shading indicates the highest value, and green the lowest value

Species	County or province	Altitude (m)	Longitude (°)	Latitude (°)	Temperature (°C)				Precipitation (mm)		
					Mean	Minimum	Maximum	Range	Annual	Driest month	Wettest month
Scotland, UK											
Po. salignus/ *Pr. andina*	Argyll	50	–4.93265	55.99207	7.8	-0.6	17.8	18.4	1756	93	208
Po. salignus/ *Pr. andina*	Argyll	45	–4.99752	56.02434	8.6	0.1	18.4	18.3	1651	87	191
Pr. andina	Edinburgh	33	–3.21203	55.96981	8.7	1.0	19.3	18.2	676	41	68
Chile											
Pr. andina	Biobío	874	–71.4384	–37.1911	9.9	0.0	25.7	25.7	1439	29	267
Pr. andina	Ñuble	965	–71.5952	–36.8649	9.1	–0.8	24.5	25.3	1353	24	267
Po. salignus	Linares	755	–71.4033	–35.3127	14.2	3.0	30.4	27.4	833	7	199
Po. salignus	Linares	740	–71.4059	–36.3214	10.9	0.3	26.7	26.4	1301	19	274
Pr. andina	Linares	871	–70.9939	–35.8925	10.7	–0.6	26.9	27.5	1060	18	219
Pr. andina	Malleco	1000	–71.6212	–38.6861	9.0	0.4	24.2	23.8	1925	45	331
Pr. andina	Malleco	1078	–72.8419	–37.8351	7.0	–0.7	20.1	20.8	1594	30	303
Pr. andina	Malleco	1127	–72.8339	–37.8374	7.4	–0.3	20.5	20.8	1577	30	300
Pr. andina	Malleco	1002	–71.6212	–38.6861	9.0	0.4	24.2	23.8	1925	45	331
Pr. andina	Malleco	980	–71.6155	–38.6859	9.1	0.5	24.4	23.9	1943	46	333
Pr. andina	Ñuble	737	–71.2702	–36.6521	10.9	0.1	26.9	26.8	1230	21	246
Pr. andina	Ñuble	723	–71.2708	–36.6592	10.9	0.1	27.0	26.9	1233	21	246
Pr. andina	Ñuble	699	–71.2829	–36.6712	11.2	0.4	27.3	26.9	1253	21	250
Pr. andina	Ñuble	722	–71.27	–36.6558	10.9	0.1	26.9	26.8	1230	21	246