BRILL

IAWA Journal 41 (4), 2020: 720–739

iawa

Forensic identification of CITES Appendix I Cupressaceae using anatomy and mass spectrometry

Rene J. Carmona[1], **Michael C. Wiemann**[2], **Pieter Baas**[3], **Cristobal Barros**[4], **Gabriela D. Chavarria**[5], **Pamela J. McClure**[6], and **Edgard O. Espinoza**[6,*]

[1]University of Chile, Facultad de Ciencias Forestales y de la Conservación de la Naturaleza, Santa Rosa 11.315, Zip Code 8820808, Santiago, Chile

[2]Center for Wood Anatomy Research, Forest Products Lab, Madison, WI 53726, USA

[3]Naturalis Biodiversity Center, P.O. Box 9517, 2300 RA Leiden, The Netherlands

[4]International Technical Assistance, Department of the Interior. Los Españoles 2264, Santiago 7520249, Chile

[5]Denver Museum of Nature and Science, Denver, CO, USA

[6]National Fish and Wildlife Forensic Laboratory, Ashland, OR 97520, USA

*Corresponding author; email: Ed_Espinoza@fws.gov

Accepted for publication: 24 February 2020

ABSTRACT

Alerce (*Fitzroya cupressoides* (Mol.) Johnst.) and Guaitecas cypress (*Pilgerodendron uviferum* (Don) Florin) are two of the three closely-related species of conifers in the Cupressaceae that are endemic to southern Chile and Argentina. Both are listed in Appendix I of the Convention on International Trade in Endangered Species of Fauna and Flora (CITES). The presence or absence of nodular (conspicuously pitted) end walls in the parenchyma cells provide good diagnostic characters to separate the two species wood anatomically, but the latter is sometimes difficult to distinguish. Therefore, a collaborative project was designed to study the chemical-molecular expression of these species by analyzing the heartwood using DART TOFMS (Direct Analysis in Real-Time (DART) Time-of-Flight Mass Spectrometry (TOFMS). This study compares the anatomical features of heartwood for both species and demonstrates that anatomy in conjunction with chemistry can separate them. DART TOFMS analysis combined with PCA was able to unequivocally determine taxonomic source with a statistical certainty of 99%. The mass spectra results obtained from heartwood demonstrated that identification is feasible after a few seconds, using a very small sample. DART TOFMS is a robust tool for reliable species identification and is useful to identify the taxonomic source of finished products or timber that are suspected of being illegally harvested.

DOI 10.1163/22941932-bja10002

Keywords: *Fitzroya cupressoides*; *Pilgerodendron uviferum*; alerce; Guaitecas cypress; mass spectrometry.

INTRODUCTION

The southern tip of South America is home to three CITES (International Convention on Trade in Endangered Species of Wild Fauna and Flora; 1984) protected tree species: *Araucaria araucana* (Mol.) C. Koch (pehuén) in the Araucariaceae, and *Fitzroya cupressoides* (Mol.) Johnst. (alerce), and *Pilgerodendron uviferum* (Don) Florin (Guaitecas cypress) in the Cupressaceae. Because of harvesting and habitat loss, these trees are listed in CITES Appendix I, which bans them from international trade without special permits, and gives them the same protection as the elephant and rhinoceros. Despite these bans, the continued exploitation of these trees has been difficult to curtail. A study in 1997 (CONAF/PNUD/FAO 1997) showed a 50% decline in alerce forests. An important aspect of stopping the illegal trade of these species is to have effective and rapid tools for identifying the species source of suspected banned timber. Direct Analysis in Real-Time (DART) Time-of-Flight Mass Spectrometry (TOFMS) has been shown to be a great asset for species identification of heartwood. Evans *et al.* (2017) demonstrated that this approach was useful to determine the taxonomic source of *Araucaria* and *Agathis* heartwood. Additionally, the same approach using chemotype by mass spectrometry has been successfully used for *Aquilaria* (Lancaster & Espinoza 2012b), *Cedrela* (Paredes-Villanueva *et al.* 2018), *Dalbergia* (Lancaster & Espinoza 2012a; McClure *et al.* 2015; Espinoza *et al.* 2015), and *Pericopsis* (Deklerck *et al.* 2017). In this study, we evaluate if DART TOFMS can be used to differentiate between species source of heartwood from *F. cupressoides* and *P. uviferum*. Barros (2017) has described the historical protection of these taxa in Chile and provides a context for their current protection.

Guaitecas cypress and alerce are endemic to southern Chile and Argentina, and the morphology and anatomy of these two species are similar. They are easily separated from *Araucaria* because only genera of the Araucariaceae have alternate tracheid pitting. The goal of this paper is to describe the habitat and wood anatomy of Guaitecas cypress and alerce and to explore if the analysis of molecules found in their heartwood can be used to identify these species to ensure legal compliance.

Guaitecas cypress is an evergreen species having a straight trunk with reddish-brown bark. It can reach up to 1 m in diameter and 40 m in height. Juvenile trees display a variable crown, but fully mature trees have a crown that is wide and pyramidal, occupying only the upper third of the total height (Cruz & Lara 1981; Diaz-Vaz 1985). The distribution of Guaitecas cypress in the south of Chile is intermittent; it is the only conifer in the world to grow in these extreme southern latitudes. It is a slow-growing species, with a reported growth in diameter from 0.39 to 2.66 mm per year (Plaza 2001); 0.44 to 0.51 mm per year (Roig & Boninsegna 1991); 0.46 to 1.31 per year (Szeics *et al.* 2000); and 0.7 to 1.2 mm per year (Cruz & Lara 1981). The average annual growth in height is reported to be only 5.8 cm per year (Roig & Boninsegna 1991).

The heartwood of Guaitecas cypress is uniform pale brown with a yellowish tinge, and the sapwood has a yellowish-white color. The wood has a very characteristic and persistent odor, a fine and homogeneous texture, and fine to medium grain. It is very durable and easy to work and has a medium dry density (0.5 g/cm^3). The wavy growth rings are about one mm in width, well-defined, each with a narrow latewood band that is darker in color than the earlywood. Wood rays are narrow and barely visible, and the axial parenchyma is not visible to the naked eye. The tracheids are square or hexagonal in cross-section, with a tangential diameter range of 23 µm to somewhat greater than 30 µm, and an average tracheid length of 1.8 mm, with some up to 2.6 mm. Tracheid bordered pits are arranged in single longitudinal rows on the radial walls. The rays are homogeneous, and cross-field pits are cupressoid with between two and four, but sometimes up to seven, pits per cross-field (Diaz-Vaz 1985). Although Diaz-Vaz (1985) reports that axial parenchyma is diffuse and sparse, Phillips (1948) lists its parenchyma as abundant.

Due to its high resistance to decay and insect attack, the Guaitecas cypress heartwood has been much sought after for use in building construction, for utility poles and fences, and for use in marine construction (Cruz & Lara 1981; Plaza 2001). According to Cruz and Lara (1981), the indigenous Chonos and the Alacalufes communities logged large forest areas for firewood. When European settlers arrived, they burned cypress forests to make the land usable for agricultural and livestock purposes. Today the Guaitecas cypress is at risk due to the continued indiscriminate use of fire to expand farmland, the illegal logging in reserves and national parks, and poor harvesting practices that occur on private lands. The only current legal use of Guaitecas cypress is to harvest fallen trees for wood posts and poles. The species was registered as an endangered tree with CITES in 1975 with an Appendix I designation, which denotes that all international trade is prohibited (CITES 1984). Additionally, since 2013 it has been classified as vulnerable by the International Union for Conservation of Nature (IUCN) (Souto *et al.* 2013).

Alerce has a conical trunk that is sometimes more than two meters in diameter and up to 30 m tall (Diaz-Vaz 1983). Its reddish bark is thick, furrowed and fibrous, peeling off in strips, and in young and isolated trees the branches may reach the ground as they achieve greater diameter (Rodríguez *et al.* 1983). Alerce grows slowly in height and diameter and is long-lived, with some trees reaching 3600 years of age (Lara & Villalba 1993). It is endemic to sub-Antarctic South American conifer forests. Alerce populations grow in the coastal mountain range usually below 1000 m. In the Chilean valleys, Alerce may grow at around 200 m above the sea level, but in the Andes Mountains, it is found above 1000 m (Donoso 2006).

The heartwood of alerce is dark reddish-brown and its sapwood is thin and yellowish. The texture is fine, and the wood is without a distinctive smell or taste. The wood is very durable, has moderate mechanical strength, is easy to work, easy to dry, and has good dimensional stability. It has a dry density of about 0.5 g/cm^3. The wood has clearly visible, often wavy growth rings that are between 0.1 and 3.0 mm in width. Rays are homogeneous, with 1-5 cupressoid pits per cross-field. They have an average height of 90 µm and a maximum height of 250 µm, and their cell cavities contain dark reddish deposits. The tracheids are polygonal to rectangular in cross-section. The average tracheid diameter is 17 µm with

a maximum of 28 μm. Tracheid length ranges from 1.1 mm to 2.7 mm, with an average of 2.0 mm. Tracheid bordered pits are arranged in single longitudinal rows in the radial walls. Axial parenchyma is abundant, diffuse, contains red deposits, and is distributed throughout the rings (Diaz-Vaz 1983).

Logging of alerce began around the middle of the 17th century when its beautiful grain and high natural strength of its wood were discovered. Because of its color, texture, and durability, it is used both for interior and exterior applications such as utility poles, boats, window blinds, ornamental plates, and musical instruments (Diaz-Vaz 1983). Intense industrial logging began in the 18th and 19th centuries and dramatically reduced the populations of the species. By the beginning of the 1900s, one-third of the alerce forests had been decimated. Roads and motorized transportation developments of the 1930s expanded the harvest in the coastal and the high mountain ranges (Golte 1996). The lack of sustainable harvesting practices, combined with intensive logging and the use of fire to clear land, nearly eliminated the alerce forests in the Chilean lowlands (Lara *et al.* 2003; Wolodarsky-Franke & Lara 2005). In 1976 the high price of alerce timber and unsustainable logging practices led to a legal decree in Chile which bans all logging of alerce trees (CONAF 2016, Decreto Supremo No 490).

The traditional approach to the identification of a wooden object is to use its physical and anatomical features. Diagnostic characters were described and illustrated by Wheeler *et al.* (1989) for angiosperms, and by Richter *et al.* (2004) for gymnosperms. An online computerized wood identification database (InsideWood) for angiosperms is freely available (Wheeler 2011; InsideWood 2004 onwards), but no such online database is yet available for gymnosperms, although InsideWood contains many photographs of gymnosperms. The most comprehensive sources of gymnosperm wood identification are Phillips (1948), which illustrates the important anatomical and physical features and tabulates their presence and absence in individual species, and Greguss (1955), which describes most of the gymnosperms and illustrates their anatomy with micrographs and line drawings.

Peirce (1937) compared the anatomy of genera of Cupressaceae and constructed a key to their identification. He found that the walls of the parenchyma cells provided the most valuable features for genus separation, with nodular (distinctly pitted) end-walls found in *Fitzroya*, nodular or smooth end-walls found in *Libocedrus* (including *Pilgerodendron*), and indentures (abruptly thin portions in ray cells where their horizontal and vertical walls meet) absent in both. Boutelje (1955) reported nodular end-walls but no indentures in *Fitzroya*, and smooth end-walls without (or with inconspicuous) indentures in *Pilgerodendron*. Heinz (2004) reported smooth and nodular end-walls in *Fitzroya*, but only smooth end-walls in *Pilgerodendron*; he comments that indentures have no diagnostic significance because of their variability.

Greguss (1955) states that ray widths are uniseriate and rarely biseriate and up to 20 cells high in *P. uviferum*, and uniseriate and up to 19 cells high in *F. cupressoides*. Gasson *et al.* (2011) report that the rays of both species are exclusively uniseriate, but that those of *P. uviferum* are very low (up to four cells high) whereas those of *F. cupressoides* are medium (5–15 cells high). Diaz-Vaz (1985) describes the rays of *P. uviferum* as uniseriate and up to eight cells high (average three), and Diaz-Vaz (1983) describes the rays of *F. cupressoides* as

uniseriate and rarely biseriate and up to ten cells high (average three). Therefore, we are doubtful of the value of ray size to consistently separate these species.

According to Phillips (1948), the only differences between *Fitzroya* and *Pilgerodendron* pertain to heartwood color, odor, and indentures and nodular end walls in the ray cells. These characters are either variable and difficult to define (color), lost in manufactured products (odor), can only be used positively (indentures), or are difficult to discern clearly (nodules). Heinz (2020, personal communication) confirmed that nodular end walls in the ray cells of *Fitzroya* constitutes a clear difference from the smooth end walls in *Pilgerodendron*. Although the lack of clear physical and anatomical differences makes use of these features for the identification of finished products unreliable, the presence of odor differences in fresh material suggests that chemistry might be useful in species separation.

MATERIALS AND METHODS

Materials

Twenty-one samples of *P. uviferum* were collected by RJC of the Universidad de Chile. Thirty-five samples of *F. cupressoides* were obtained from various sources including the xylarium of the Universidad de Chile, Santiago; Burke Modern Museum, Washington, DC, USA; USDA Forest Service, Center for Wood Anatomy Research, Madison, WI, USA; and the Smithsonian Institution, Washington, DC, USA. For both species, some reference samples were held back and not used to develop the statistical models, so that they could be used as validation samples to assess the prediction quality of the model. Tables A1 and A2 in the Appendix show the origin of the samples and how each specimen was used. For wood anatomy analyses, transverse, radial and tangential sections were obtained from the slide collection of the Center for Wood Anatomy Research in Madison, WI, USA and the Naturalis Biodiversity Center, Leiden, The Netherlands.

Anatomy

Cross-sections were photographed at 40×, and tangential sections at 100×, using an Olympus DP27 digital camera mounted on an Olympus BX43 microscope. The IAWA microscopic features list (Richter *et al.* 2004) was used for anatomical descriptions. To determine whether nodules and indentures can be used reliably, prepared slides from the wood collections of the Forest Products Laboratory (Madison, WI, USA) and the Naturalis Biodiversity Center (Leiden, The Netherlands) were examined for these features using the Olympus microscope at 100× and 400×.

Chemistry methods

Mass spectra were acquired using a DART-SVP ion source (IonSense, Saugus, MA, USA) coupled to a JEOL AccuTOF 4G LC Plus Mass Spectrometer (JEOL USA, Peabody, MA, USA). With no further sample preparation, a sliver of wood was held in a stream of heated helium gas produced by the DART Ion Source. As compounds were emitted from the wood, they were drawn into the mass analyzer. Spectra were acquired in positive ion mode with

the DART. DART and mass spectrometry collection parameters have been reported elsewhere. A mass calibration standard of polyethylene glycol 600 (Ultra Scientific, Kingstown, RI, USA) was run between every fifth sample.

TSS Unity (Shrader Software Solutions, Grosse Pointe Park, MI, USA) data reduction software was used to export the text files of the mass-calibrated, centroided mass spectra for molecular formula determination and further analysis. Heat maps and principal component analysis (PCA were conducted using the Mass Mountaineer Mass Spectral Interpretation Tools software (RBC Software, Peabody, MA, USA) using a tolerance of 5 mDa and a 5% threshold. The classification algorithms of Mass Mountaineer (v5) were used to calculate the principal components of each data set. To assess model accuracy, leave-one-out cross-validation (LOOCV) was employed. The LOOCV is based on the distance from the cluster mean of each sample that is omitted. Essentially, each sample is successively omitted from the training set and placed as an unknown, thus subjecting each sample for comparison against the entire training set. In short, LOOCV is a metric of how well the model performs. When analyzing an unknown specimen, Mass Mountaineer can give a probability estimate to the classification of the spectrum.

RESULTS

Anatomy results

Figure 1 shows a cross-section (top, taken at 40×) and a tangential section (bottom, taken at 100×) of *P. uviferum*. The transition between latewood and earlywood is gradual to abrupt. Axial parenchyma is abundant and concentrated in the latewood/earlywood transition zone. Wood rays are uniseriate and occasionally biseriate in part and are 1–15 cells high. Figure 2 shows a cross-section (top, taken at 40×) and a tangential section (bottom, taken at 100×) of *F. cupressoides*. Just as in *P. uviferum*, the transition between latewood and earlywood is gradual to abrupt, and axial parenchyma is abundant. The rays are uniseriate and are 1–10 cells high. Radial sections of most *Fitzroya* samples showed distinct nodular secondary end walls (Fig. 3), and tangential sections showed nodular end walls in the axial parenchyma cells (Fig. 4). In *P. uviferum* the ray and axial parenchyma cell end walls were always smooth (Figs 5 and 6, respectively). Some cells of ray parenchyma in *P. uviferum* clearly showed indentures (Fig. 5, arrow), but this feature was variable in its distinctness in both genera. Of the six slides of *P. uviferum* (two from Leiden, four from Madison), all had smooth ray cell end-walls, and only one showed indentures. Of the ten slides of *Fitzroya* (two from Leiden, eight from Madison), nine had nodular end-walls. Only the eight Madison samples were examined for indentures, and of these five showed indentures. The data card for the lone sample of *Fitzroya* that did not have nodular end-walls contained only the information that it came from Chile, so it seemed possible that is a misidentified trade sample. However, DART-TOFMS analysis of a sliver from this sample indicated that it was correctly identified.

Chemistry results

Table A1 in the Appendix lists the sources and samples used in this study. Of the 35 samples of *F. cupressoides*, 28 were used for model development and seven were held back

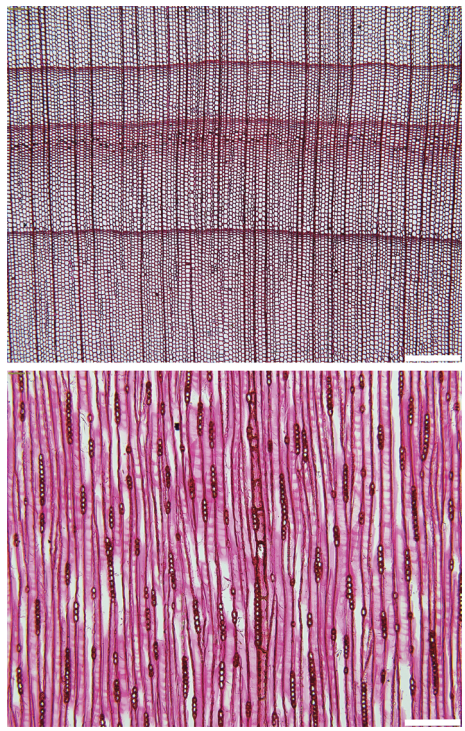


Figure 1. *Pilgerodendron uviferum*. Top, cross-section showing three growth rings and zonate axial parenchyma as black dots; scale bar is 500 µm. Bottom, tangential surface showing uniseriate rays, one partially biseriate ray, and a strand of axial parenchyma; scale bar is 200 µm.

to be used for validation. Of the 21 *P. uviferum* samples, 18 were used for the model and three were held back and used for validation.

For each species, the individual spectra from each sample were averaged (*F. cupressoides* n = 28; *P. uviferum* = 18), and the averaged spectrum for each class is shown in Figure 7. Not all the ions detected by the mass spectrometer could be characterized, but the tentative assignments of the most intense ions are listed in Table 1. Molecular assignments were made by comparing the high-resolution masses detected by DART TOFMS against the species-metabolite database curated by KNApSAcK (Web-1). The *F. cupressoides* spectrum is dominated by the base peak at a mass-to-charge ratio (m/z) of 219.105, which is characteristic of cinnamyl isovalerate ($C_{14}H_{18}O_2$ +H). Nakatsubo *et al.* (2003) and Umezawa (2003) have reported the presence of isotaxiresinol and cubebin, and the spectrum in Figure 7 shows these compounds respectively at m/z = 345.132 ($C_{19}H_{22}O_6$ –H) and m/z = 357.130 ($C_{20}H_{20}O_6$ +H).

The average spectrum for *P. uviferum* is significantly different from that of *F. cupressoides*, and it shows a base peak at m/z = 203.184, which is characteristic of copaenol. This agrees with the results of Oyarzun and Garbarino (1988).

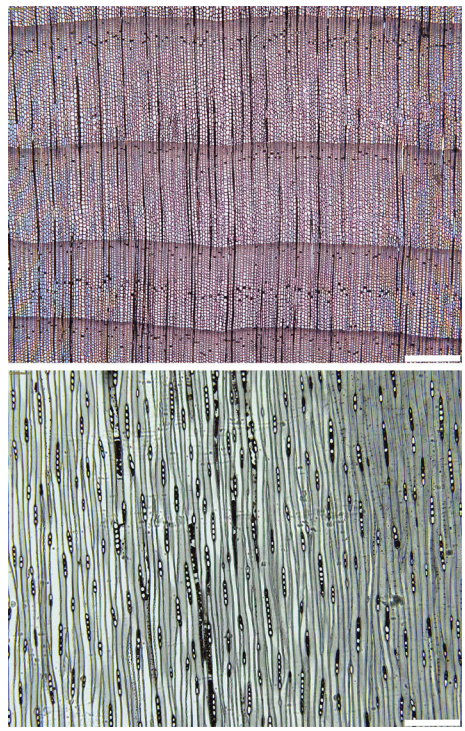
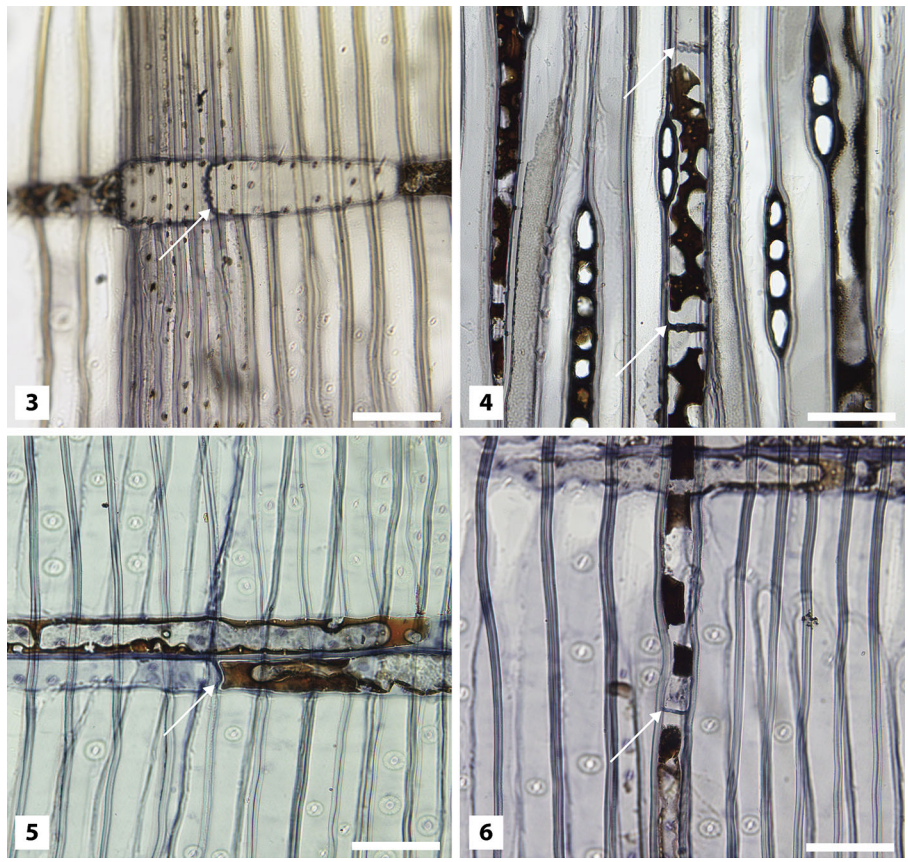


Figure 2. *Fitzroya cupressoides*. Top, cross-section showing four growth rings and zonate axial parenchyma as black dots; scale bar is 500 μm. Bottom, tangential surface showing uniseriate rays and strands of axial parenchyma; scale bar is 200 μm.

A heat map is a graphical representation of all the samples analyzed, and this is shown in Figure 8. The X coordinate is the m/z of a molecule, and the Y coordinate represents the sample analyzed. Therefore, each row is indicative of all the ions found in that specific sample. The color intensity is an indication of the concentration of an ion present in a specimen. This graph shows that the chemotypes for the species are different from each other and that the chemical profile is reproducible for each individual tested.

The results of a covariance PCA are plotted in Figure 9. The first three principal components (PC1, PC2, and PC3) are plotted. The first principal component accounts for 73.4% of the variability in the data, the second accounts 7.4%, and the third accounts for 4.7%. Overall the PCA analysis covers 85.6% of the variance, and it is sufficient to separate the data into interpretable groups. PCA results showed that all *F. cupressoides* samples for PC1 have values of 70 or greater, whereas the *P. uviferum* samples all have PC1 values of less than −90. The LOOCV of the PCA model was 99.9%. None of the 46 spectra used to create the model was misclassified.

Using the validation samples, the PCA model correctly assigned the species in every case. The results of the assignments and the associated probability with each assignment are shown in Table A3 in the Appendix. The correct assignment for each of the validation



Figures 3–6. (3) Nodular end walls (white arrow) in ray parenchyma of *F. cupressoides*; scale bar is 50 µm. (4) Nodular end walls (white arrow) in axial parenchyma of *F. cupressoides*; scale bar is 50 µm. (5) Smooth end walls and indentures (white arrow) in ray parenchyma of *P. uviferum*; scale bar is 50 µm. (6) Smooth end walls (white arrow) in axial parenchyma of *P. uviferum*; scale bar is 50 µm.

samples gives confidence that the PCA model can accurately predict the species source of a wood spectrum.

DISCUSSION

Traditionally, taxon determination is done when the geographical source is known, and diagnostic characters are present (leaves, cones, flowers, bark). However, determining the taxon of a log or board, or identifying the species of wood in a musical instrument, is a much more challenging endeavor. Although we initially considered that *Fitzroya cupressoides* and *Pilgerodendron uviferum* could not be separated wood anatomically with any certainty, a closer examination of the presence or absence of nodular end walls in the ray parenchyma cells (IAWA Softwood List feature 86, Richter *et al.* 2004) provided a reliable character to tell them apart, confirming Philips (1948). The anatomical separation of the

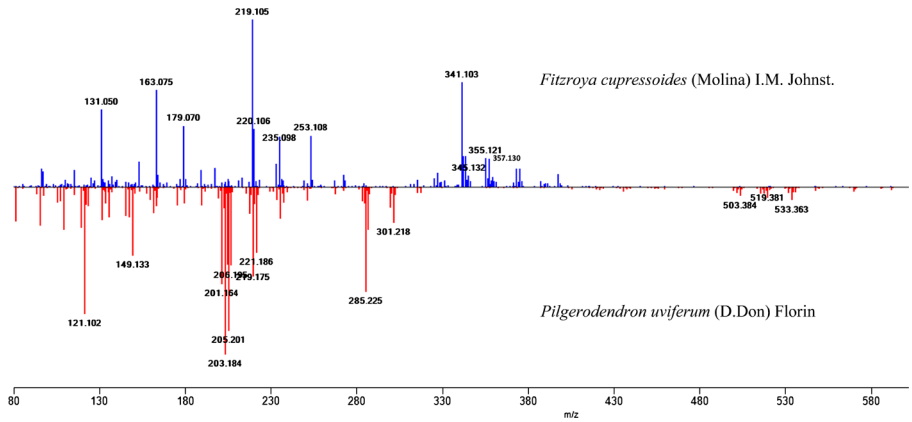


Figure 7. Average spectra of 28 individual samples of *F. cupressoides* and 18 individual samples of *P. uviferum* showing the m/z of the most prominent ions.

two species considered here depends on the observation of difficult-to-discern nodular ray cell end-walls, which are present in *F. cupressoides* but absent in *P. uviferum*. Analysis of *F. cupressoides* and *P. uviferum* using DART TOFMS demonstrated that this approach can unequivocally separate these two protected species. The results shown in the heat map graph (Fig. 8) demonstrated that the chemotypes for these species are different from each other and that the chemical profile is reproducible for each individual tested. The LOOCV validation (99.9%), as well as the correct identification of ten validation samples, demonstrates that this approach will not yield spurious results.

The level of reproducibility seen on the heat map infers genetic control of the ions. García-Flores *et al.* (2015) demonstrated the molecular compounds detected by mass spectrometry in bean leaves were predominantly under genetic control. Rendón-Anaya *et al.* (2017) extended this observation and showed a high correlation between genome and chemotype profile. Paredes-Villanueva *et al.* (2018) showed that DART TOFMS chemical profiles of *Cedrela odorata* L. heartwood, collected from three distinct habitats in Bolivia, did not show environmental influences that could lead to incorrect species identification. Deklerck *et al.* (2017) showed that DART TOFMS analysis of *Pericopsis laxiflora* (Benth.) Van Meeuwen, a tree indigenous to West Africa, had indistinguishable chemotypes from *Pericopsis mooniana* Thw., a tree indigenous to South East Asia (from the Philippines to Papua New Guinea). The logical conclusion that can be arrived from these experiments is that the chemotypes detected by DART TOFMS are products of a transcribed genetic expression of the tree's genome and that the environmental effect, if any, is minor.

CONCLUSION

Today all trade in *Fitzroya cupressoides* and *Pilgerodendron uviferum* is banned, but despite these bans, the illegal logging continues. One of the challenges stopping illegal logging has been the species identification of the wood once the trees are turned into lumber. DART TOFMS has been shown to be a reliable tool for taxonomic determination of heartwood.

Table 1.
Tentative assignments of ions detected by DART TOFMS.

m/z	Detected in:	Formula	Tentative Assignment
121.102	*P. uviferum*	Unknown	Unknown
131.050	*F. cupressoides* *P. uviferum*	$C_9H_8O_2$ –OH	4-Hydroxycinnamaldehyde
149.133	*P. uviferum*	Unknown	Unknown
153.055	*F. cupressoides*	$C_8H_8O_3$ +H	Vanillin
163.075	*F. cupressoides*	$C_{10}H_{10}O_2$ +H	beta-Dolabrin
179.070	*F. cupressoides* *P. uviferum*	$C_{10}H_{10}O_3$ +H	alpha-Dolabrinol
201.164	*P. uviferum*	$C_{15}H_{22}$ –H	(R)-1-Methyl-4-(1,2,2-trimethylcyclopentyl)-benzene
203.184	*P. uviferum*	$C_{15}H_{24}O$ –OH	Copaenol*
205.201	*P. uviferum*	$C_{15}H_{24}$ +H	(E)-Caryophyllene
219.105	*F. cupressoides*	$C_{14}H_{18}O_2$ +H	Cinnamyl isovalerate
219.175	*P. uviferum*	$C_{15}H_{24}O$ –H	Copaenol*
221.186	*P. uviferum*	$C_{15}H_{24}O$ +H	Copaenol*
235.098	*F. cupressoides*	Unknown	Unknown
253.108	*F. cupressoides*	Unknown	Unknown
285.225	*P. uviferum*	$C_{20}H_{28}O$ +H	Pisiferin
301.218	*P. uviferum*	$C_{20}H_{28}O_2$ +H	Sugiol
341.103	*F. cupressoides*	$C_{19}H_{18}O_7$ –OH	Retusin
345.132	*F. cupressoides*	$C_{19}H_{22}O_6$ –H	Isotaxiresinol**
355.121	*F. cupressoides*	$C_{10}H_{10}O_3$ 2 –H	alpha-Dolabrinol
357.130	*F. cupressoides*	$C_{20}H_{20}O_6$ +H	(-)-Cubebin**
503.384	*P. uviferum*	Unknown	Unknown
519.381	*P. uviferum*	$C_{35}H_{50}O_3$ +H	Sugikurojin I
533.363	*P. uviferum*	Unknown	Unknown

* Oyarzun & Garbarino (1988).
** Nakatsubo *et al.* (2003); Umezawa (2003).

When illegal trade is suspected, verifying the identity of a sample of Alerce or Guaitecas cypress is easy, the analysis is inexpensive, it requires minimal sample preparation, and can be done in minutes. It is reassuring that classical wood anatomy is also capable of separating the two endangered softwoods, although there is probably overlap with other Cupressaceae not included in this study.

ACKNOWLEDGEMENTS

The authors thank Dr. Immo Heinz (Hamburg) for checking his extensive collection of wood anatomical slides of *Fitzroya* and *Pilgerodendron* for the presence or absence of indentures and nodular end walls. Additionally, we thank Ms. Bonnie Yates and Mr. Barry Baker for their editorial review and suggestions, to Aiden Espinoza for preparing wood samples for analysis, and to Karen Nelson of the Forest Products Laboratory for preparing the wood anatomy images.

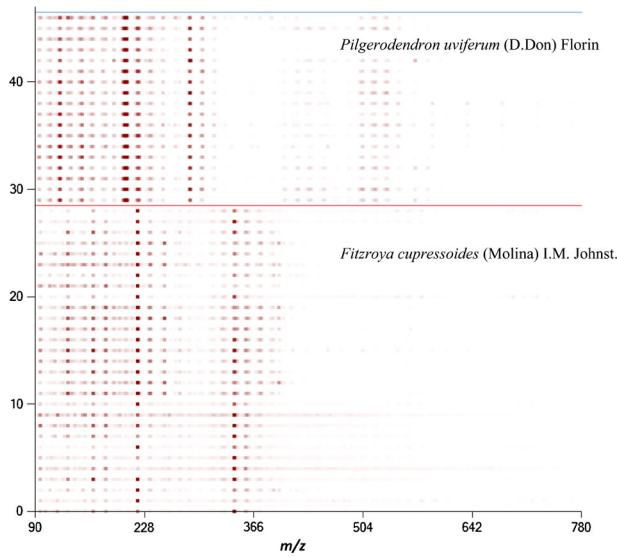


Figure 8. Heat map showing all the ions detected in the *F. cupressoides* and *P. uviferum* samples. The X-axis shows the mass-to-charge ratio (m/z) of the molecules detected, while the Y-axis shows the spectrum for each sample.

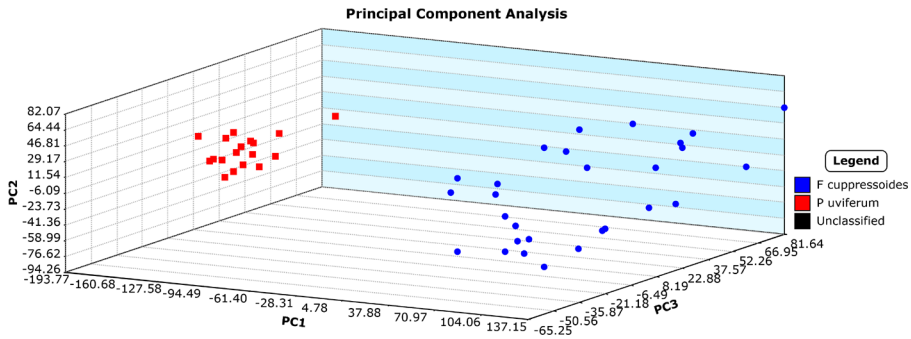


Figure 9. Graphical representation of the PCA analysis of the 46 samples. PCA was calculated using 202 variables (ions) which were selected for their power to discriminate between the two species. The LOOCV was calculated to be 99.9%.

REFERENCES

Barros C. 2017. Análisis de la protección legal y comercio internacional de las especies araucaria, alerce y ciprés de la Guaitecas. Internal Report: "International Technical Assistance Program, U.S. Department of the Interior", Chile. Unpublished.

Boutelje JB. 1955. The wood anatomy of *Libocedrus* Endl., s. lat., and *Fitzroya* J.D. Hook. Acta Horti Bergiani 17: 177–216 + 8 plates.

CITES. 1984. Appendices I, II and III of the convention on international trade in endangered species of wild fauna and flora. UNEP-WCMC. http://www.cites.org/eng/app/appendices.php.

CONAF. 2016. Normativa Forestal. Corporación Nacional Forestal, Santiago, Chile. http://www.conaf.cl/wp-content/files_mf/1465240427NormativaForestal.pdf (accessed 6 January 2019).

CONAF/PNUD/FAO. 1997. Catastro Vegetacional de Chile. Programa de Investigación y Desarrollo Forestal, Documento de Trabajo N° 38. 78 p.

Cruz G, Lara A. 1981. Tipificación, cambio de estructura y normas de manejo para ciprés de las Guaitecas (*Pilgerodendron uviferum* (D. Don) Florin) en la Isla Grande de Chiloé. Tesis Ingeniero Forestal. Facultad de Ciencias Agrarias y Veterinarias, Universidad de Chile. Santiago, Chile. 215 p.

Deklerck V, Finch K, Gasson P, Van den Bulcke J, Van Acker J, Beeckman H, Espinoza E. 2017. Comparison of species classification models of mass spectrometry data: kernel discriminant analysis vs random forest; a case study of afrormosia (*Pericopsis elata* (Harms) Meeuwen). Rapid Commun. Mass Sp. 31: 1582–1588. DOI: 10.1002/rcm.7939.

Diaz-Vaz JE. 1983. *Fitzroya cupressoides*. Descripción anatómica. Bosque 5: 47–49.

Diaz-Vaz JE. 1985. *Pilgerodendron uvifera*. Descripción anatómica. Bosque 6: 123–124.

Donoso C. 1993. Bosques templados de Chile y Argentina. Editorial Universitaria, Santiago. 438 pp.

Donoso C. 2006. Las especies arbóreas de los bosques templados de Chile y Argentina: Autoecología. 678 p. ISBN 9789567173273. https://lib.ugent.be/catalog/rug01:001357175.

Espinoza EO, Wiemann MC, Barajas-Morales J, Chavarria GD, McClure PJ. 2015. Forensic analysis of CITES protected *Dalbergia* timber from the Americas. IAWA J. 36: 311–325.

Evans P, Mundo I, Wiemann M, Chavarría G, McClure P, Voin D, Espinoza E. 2017. Identification of selected CITES-protected Araucariaceae using DART TOFMS. IAWA J. 38: 266–281.

García-Flores M, Juarez-Colunga S, García-Casarrubias A, Trachsel S, Winkler R, Tiessen A. 2015. Metabolic profiling of plant extracts using direct-injection electrospray ionization mass spectrometry allows for high throughput phenotypic characterization according to genetic and environmental effects. J. Ag. Food Chem. 63: 1042–1052.

Gasson P, Baas P, Wheeler E. 2011. Wood anatomy of CITES-listed tree species. IAWA J. 32: 155–198.

Golte W. 1996. Exploitation and conservation of *Fitzroya cupressoides* in southern Chile. In: Hunt D (ed.), Temperate trees under threat. International Dendrology Society, Great Britain. http://www.unep-wcmc.org/species/tree_study/americas/esp/232.htm.

Greguss P. 1955. Identification of living gymnosperms on the basis of xylotomy. Budapest. 263 pages + micrographs.

Heinz I. 2004. Systematische Erfassung und Dokumentation der mikroanatomischen Merkmale der Nadelhölzer aus der Klasse der Pinatae. Thesis, Munich.

InsideWood. 2004-onwards. Published on the Internet.

Lancaster CA, Espinoza EO. 2012a. Analysis of select *Dalbergia* and trade timber using direct analysis in real time and time-of-flight mass spectrometry for CITES enforcement. Rapid Commun. Mass Sp. 26: 1147–1156.

Lancaster CA, Espinoza EO. 2012b. Evaluating agarwood products for 2-(2-phenylethyl)-chromones using direct analysis in real time time-of-flight mass spectrometry. Rapid Commun. Mass Sp. 26: 2649–2656.

Lara A, Soto D, Armesto JJ, Donoso PJ, Wernli C, Nahuelhual L, Squeo F. 2003. Key scientific components for a national policy regarding the uses, services and conservation of native forests in Chile. Reunión Científica sobre Bosques Nativos. Valdivia, Chile. Universidad Austral de Chile, Iniciativa Científica Milenio de Mideplan. 134 p.

Lara A, Villalba R. 1993. A 3,620-year temperature reconstruction from *Fitzroya cupressoides* tree rings in southern South America. Science 260: 1104–1106.

McClure PJ, Chavarria GD, Espinoza EO. 2015. Metabolome chemotypes of CITES protected *Dalbergia* timbers from Africa, Madagascar and Asia. Rapid Commun. Mass Sp. 29: 783–788.

Nakatsubo T, Li L, Chiang VL, Hattori T, Shimada M, Umezawa T. 2003. Basic studies towards elucidation of heartwood formation mechanisms. Wood Res. 90: 5–6.

Oyarzun ML, Garbarino JA. 1988. Sesquiterpenoids from *Pilgerodendron uvifera*. Phytochem. 27: 1121–1123.

Paredes-Villanueva K, Espinoza E, Ottenburghs J, Sterken MG, Bongers F, Zuidema PA. 2018. Chemical differentiation of Bolivian *Cedrela* species as a tool to trace illegal timber trade. Forestry 91: 603–613. DOI: 10.1093/forestry/cpy019.

Peirce AS. 1937. Systematic anatomy of the woods of the Cupressaceae. Tropical Woods 49: 5–21.

Phillips EWJ. 1948. Identification of softwoods by their microscopic structure. London, 56 p.

Plaza J. 2001. Dinámica de renovales de Ciprés de las Guaitecas (*Pilgerodendron uviferum* (D.Don Florin) en la zona de Lago Vargas Provincia de Capitán Prat, XI Región. Tesis Facultad de Cs. Forestales, Universidad Austral de Chile, VaIdivia. 78 p.

Rendón-Anaya M, Montero-Vargas JM, Saburido-Álvarez S, Vlasova A, Capella-Gutierrez S, Ordaz-Ortiz JJ, Aguilar OM, Vianello-Brondani RP, Santalla M, Delaye L, Gabaldón T, Gepts P, Winkler R, Guigó R, Delgado-Salinas A, Herrera-Estrella A. 2017. Genomic history of the origin and domestication of common bean unveils its closest sister species. Genome Biol. 18: 1–17.

Richter HG, Grosser D, Heinz I, Gasson PE. (Editors, IAWA Committee) 2004. IAWA list of microscopic features for softwood identification. IAWA J. 25: 1–70. DOI: 10.1163/22941932-90000349.

Rodríguez R, Matthei O, Quezada M. 1983. Flora Arbórea de Chile. Ed. Univ. Concepción. Chile. 408 p.

Roig FA, Boninsegna JA. 1991. Estudio sobre el crecimiento radial, basal, en altura y de las condiciones climáticas que afectan el desarrollo de *Pilgerodendron uviferum*. Revista Chilena de Historia Natural 64: 53–63.

Souto C, Premoli A, Gardner M. 2013. *Pilgerodendron uviferum*. The IUCN red list of threatened species 2013: e.T32052A2809552. DOI:10.2305/IUCN.UK.2013-1.RLTS.T32052A2809552.en (accessed 27 June 2018).

Szeics J, Lara A, Díaz S, Aravena JC. 2000. Dendrochronological studies of *Pilgerodendron uviferum* in southwestern South America. In: Roig FA (ed.), Dendrocronología en América Latina. EDIUNC, Mendoza: 245–269.

Umezawa T. 2003. Diversity in lignin biosynthesis. Phytochem. Rev. 2: 371–390.

Wheeler EA. 2011. InsideWood — a web resource for hardwood anatomy. IAWA J. 32: 199–211.

Wheeler EA, Baas P, Gasson PE, (IAWA Committee). 1989. IAWA list of microscopic features for hardwood identification. IAWA Bull. n.s. 10: 219–332.

Wolodarsky-Franke A, Lara A. 2005. The role of "forensic" dendrochronology in the conservation of alerce (*Fitzroya cupressoides* (Mol.) Johnst.) forests in Chile. Dendrochrologia 22: 235–240. DOI: 10.1016/j.dendro.2005.05.008.

WEB SITES

Web-1: http://kanaya.naist.jp/knapsack_jsp/top.html (accessed 11 June 2018).

Web-2: InsideWood. 2004-onwards. http://insidewood.lib.ncsu.edu (accessed 11 June 2018).

Edited by Lloyd Donaldson

Table A1.
Chemotype reference specimens used in this study.

Species	Accession No.	Sample	Institutional source	Geographic provenance notes
Fitzroya cupressoides	WD162596	BM10E05-3530	Burke Modern Museum, Seattle, WA, USA	Chile
Fitzroya cupressoides	WD162667	BM10E05-3530–2	Burke Modern Museum, Seattle, WA, USA	Chile
Fitzroya cupressoides	WD162597	BM10E06-3531	Burke Modern Museum, Seattle, WA, USA	Chile
Fitzroya cupressoides	WD162735	MADw20931	Forest Products Laboratory, Madison, WI, USA	Chile
Fitzroya cupressoides	WD162736	MADw25180	Forest Products Laboratory, Madison, WI, USA	Chile
Fitzroya cupressoides	WD162737	MADw3670	Forest Products Laboratory, Madison, WI, USA	Chile
Fitzroya cupressoides	WD162738	MADw3726	Forest Products Laboratory, Madison, WI, USA	Chile
Fitzroya cupressoides	WD162739	MADw3735	Forest Products Laboratory, Madison, WI, USA	Chile
Fitzroya cupressoides	WD162740	MADw3739	Forest Products Laboratory, Madison, WI, USA	Chile
Fitzroya cupressoides	WD162741	MADw8596	Forest Products Laboratory, Madison, WI, USA	Valdivia, Chile
Fitzroya cupressoides	WD166299	USw20036	Smithsonian Institute, Washington, DC, USA	Angol, Chile
Fitzroya patagonica	WD165467	USw4426	Smithsonian Institute, Washington, DC, USA	Chile
Fitzroya patagonica	WD165566	USw6605	Smithsonian Institute, Washington, DC, USA	Llanquihue, Chile
Fitzroya cupressoides	WD165199	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile

Table A1.
(Continued.)

Species	Accession No.	Sample	Institutional source	Geographic provenance notes
Fitzroya cupressoides	WD165200	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile
Fitzroya cupressoides	WD165202	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile
Fitzroya cupressoides	WD165203	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile
Fitzroya cupressoides	WD165204	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile
Fitzroya cupressoides	WD165205	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile
Fitzroya cupressoides	WD165206	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile
Fitzroya cupressoides	WD165207	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile
Fitzroya cupressoides	WD165208	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile
Fitzroya cupressoides	WD170098	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile
Fitzroya cupressoides	WD170099	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile
Fitzroya cupressoides	WD170100	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile
Fitzroya cupressoides	WD170102	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile

Table A1.
(Continued.)

Species	Accession No.	Sample	Institutional source	Geographic provenance notes
Fitzroya cupressoides	WD170103	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile
Fitzroya cupressoides	WD170104	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile
Pilgerodendron uviferum	WD165215	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	WD165216	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	WD165217	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	WD165218	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	WD165219	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	WD165220	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	WD165221	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	WD165223	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	WD165224	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile

Table A1.
(Continued.)

Species	Accession No.	Sample	Institutional source	Geographic provenance notes
Pilgerodendron uviferum	WD165225	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	WD165226	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	WD165227	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	WD170107	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	WD170108	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	WD170109	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	WD170110	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	WD170111	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	WD170112	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile

Table A2.
Anatomy reference slides used in this study.

Species	Slides	Institutional source	Geographic provenance notes
Fitzroya cupressoides	MADw20931	Forest Products Laboratory, Madison, WI, USA	Chile
Fitzroya cupressoides	OCCT 28′, 1	Forest Products Laboratory, Madison, WI, USA	Chile
Fitzroya cupressoides	OCCT 28′, 2	Forest Products Laboratory, Madison, WI, USA	Chile
Fitzroya patagonica	21065	Forest Products Laboratory, Madison, WI, USA	Chile
Fitzroya cupressoides	Y 3747	Forest Products Laboratory, Madison, WI, USA	Chile
Fitzroya patagonica	Y 3762	Forest Products Laboratory, Madison, WI, USA	Chile
Fitzroya patagonica	Y 9544	Forest Products Laboratory, Madison, WI, USA	Chile
Fitzroya patagonica	E.T.S.I.M	Forest Products Laboratory, Madison, WI, USA	Chile
Fitzroya patagonica	SJR 3762	Forest Products Laboratory, Madison, WI, USA	Llanquihue, Chile
Fitzroya cupressoides	R48–81	Naturalis Biodiversity Center, Leiden, The Netherlands	Chile
Pilgerodendron uviferum	OCCT 10′	Forest Products Laboratory, Madison, WI, USA	Chile
Pilgerodendron uviferum	OCCT 10″	Forest Products Laboratory, Madison, WI, USA	Chile
Pilgerodendron uviferum	Y 3768	Forest Products Laboratory, Madison, WI, USA	Chile
Pilgerodendron uviferum	E.T.S.I.M	Forest Products Laboratory, Madison, WI, USA	Chile
Pilgerodendron uviferum	R34–75	Naturalis Biodiversity Center, Leiden, The Netherlands	Chile
Pilgerodendron uviferum	R57–46	Naturalis Biodiversity Center, Leiden, The Netherlands	Chile

Table A3.
Taxonomic assignment of validation samples.

Species	Classified by PCA as:	Assignment probability	Accession No.	Sample	Institutional Source	Geographic Provenance notes
Fitsroya patagonica	*F. cupressoides*	98.4%	WD162599	BM10E10-0563	Burke Modern Museum, Seattle, WA, USA	Chile
Fitzroya cupressoides	*F. cupressoides*	99.9%	WD162733	MADw13715	Forest Products Laboratory, Madison, WI, USA	Chubut, Rio Negro, Neuquen, Argentina
Fitzroya cupressoides	*F. cupressoides*	97.9%	WD162734	MADw19184	Forest Products Laboratory, Madison, WI, USA	Argentina
Fitsroya patagonica	*F. cupressoides*	94.7%	WD168044	USw22689	Smithsonian Institute, Washington, DC, USA	Chile
Fitzroya cupressoides	*F. cupressoides*	99.9%	WD168148	USw25911	Smithsonian Institute, Washington, DC, USA	Chile
Fitzroya cupressoides	*F. cupressoides*	99.9%	WD165201	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile
Fitzroya cupressoides	*F. cupressoides*	99.9%	WD170101	–	Universidad de Chile, Santiago, Chile	Sector de Valdivia, Provincia de Valdivia, XIV Region de los Rios, Chile
Pilgerodendron uviferum	*P. uviferum*	90.1%	WD165222	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	*P. uviferum*	96.8%	WD165228	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile
Pilgerodendron uviferum	*P. uviferum*	97.4%	WD170113	–	Universidad de Chile, Santiago, Chile	Sector de Tortel, Provincia Capitan Prat, XIII Region Aysen, Chile