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Identification of selected CITES-protected Araucariaceae using DART TOFMS

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ABSTRACT

Determining the species source of logs and planks suspected of being *Araucaria araucana* (Molina) K. Koch (CITES Appendix I) using traditional wood anatomy has been difficult, because its anatomical features are not diagnostic. Additionally, anatomical studies of *Araucaria angustifolia* (Bertol.) Kuntze, *Araucaria heterophylla* (Salisb.) Franco, *Agathis australis* (D. Don) Lindl., and *Wollemia nobilis* W.G. Jones, K.D. Hill & J.M. Allen have reported that these taxa have similar and indistinguishable anatomical characters from *A. araucana*. Transnational shipments of illegal timber obscure their geographic provenance, and therefore identification using wood anatomy alone is insufficient in a criminal proceeding. In this study we examine the macroscopic appearance of selected members of the Araucariaceae and investigate whether analysis of heartwood chemotypes using Direct Analysis in Real Time (DART) Time-of-Flight Mass Spectrometry (TOFMS) is useful for making species determinations. DART TOFMS data were collected from 5 species (n = 75 spectra). The spectra were analyzsed statistically using supervised and unsupervised classification algorithms. Results indicate that *A. araucana* can be distinguished from the look-alike taxa. Another statistical inference of the data suggests that *Wollemia nobilis* is more similar and within the same clade as *Agathis australis*. We conclude that DART TOFMS spectra can help in making species determination of the Araucariaceae even when the geographic provenance is unknown.

Keywords: Forensics, illegal logging, *Araucaria*, *Agathis*, *Wollemia*, multivariate statistics.

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INTRODUCTION

The development of ways of identifying woods that are similar in appearance, but have different properties, has long been encouraged by the international trade in timber (Record 1932). For example, the first Chief of the Forest Products Laboratory in Melbourne, Australia, I.H. Boas stated that "it is obvious that to develop overseas trade in certain species of our timber it is essential that these should be readily distinguished from other timbers superficially similar, but very different in properties" (Dadswell & Burnell 1932). The need to identify wood for the international timber trade has become even more pressing because of the introduction of legislation that bans the trade of certain timbers (Blundell 2007; Gasson 2011; Gasson *et al*. 2011). This legislation has led to increasing interest in new, more precise methods of identifying woods that are banned from international trade from very similar ones belonging to the same family or genus (Kite *et al*. 2010; Braga *et al*. 2011; Höltken *et al*. 2012; Lancaster & Espinoza 2012a,b; Bergo *et al*. 2016). A case in point is the need to develop a method of identifying *Araucaria araucana* (monkey puzzle, pehuén or Chilean pine) which is listed by CITES Appendix I, that can separate it from *A. angustifolia* (Paraná pine or curiy), the other South American member of the Araucariaceae, which is not banned from trade. Members of the Araucariaceae are restricted to South America and the Southwest Asia-Western Pacific region. The family contains three genera, *Araucaria* (19 species), *Agathis* (21 species), and *Wollemia,* which contains the single relictual species *W. nobilis* (Wollemi pine) (Whitmore 1977; Farjon 2008). *Wollemia nobilis* is protected under Australian laws, and although the remaining taxa, apart from *A. araucana*, have no trade restrictions, some are listed by the International Union for Conservation of Nature as being critically endangered or vulnerable.

Agathis and *Araucaria* possess wood with desirable commercial properties, and they have been heavily exploited in the past (Swain 1928; Tortorelli 1942; Record & Hess 1943). For example, both *Araucaria araucana* and *A. angustifolia* have suffered non-sustainable exploitation and a marked reduction in the total area they occupy as a result of land clearance (Lara *et al*. 1999; González *et al*. 2006; Ribeiro *et al*. 2009). Today remnant populations of *A. araucana* in Chile and Argentina cover approximately half the area they occupied when Europeans arrived at the beginning of the 16th century (Lara *et al*. 1999; González *et al*. 2006). The wood of *A. araucana* is 'pale yellowish, fine textured and is of good quality' according to Record and Hess (1943). It was harvested during the first half of the 20th century for construction, millwork, furniture timber, boxes, plywood, paper (pulpwood), turned objects, and small specialty wood items (Record & Hess 1943; Tortorelli 1956). In the case of *A. angustifolia*, remnant populations cover only 5 to 12% of the area they occupied when Europeans arrived at the beginning of the 16th century (Ribeiro *et al*. 2009). Europeans arriving in Brazil immediately recognized *A. angustifolia* as a valuable timber tree, and forest land grants began in 1511. By the mid-18th century, the *A. angustifolia* forests were being cleared to provide timber for shipbuilding, construction and related uses. The most destructive and extensive deforestation of the *A. angustifolia* forests, however, took place between 1870 and 1940. Unfortunately, the remnant populations are found in areas with high

levels of degradation and fragmentation, and only 0.62 % of the area the species once occupied is conserved (Vibrans *et al*. 2011; Reis *et al*. 2014). *Araucaria angustifolia* sawn wood and laminated wood remains one of Brazil's leading timber exports, although increasingly the wood is derived from plantations (Carvalho 2002).

The difficulty of separating *Araucaria araucana* from *A. angustifolia* is readily apparent when literature descriptions of the two species are compared. Brown (1978) describes *A. araucana* as "pale brown in colour, very similar in all respects to *A. angustifolia*, but lacking the bright red streaks common to that timber, and showing small brown flecks to a much greater degree than those appearing in Paraná pine (*A. angustifolia*)." His description of the timber of *A. angustifolia* is "pale brown, with a central core of darker brown coloured wood; it may be streaked with red, but this is sometimes absent." The descriptions by Record and Hess (1943) are of no more help. They describe *A. araucana* as "pale yellowish, fine-textured," and *A. angustifolia* as "various shades of brown, sometimes with bright red streaks." Record and Hess (1943) then go on to include one anatomical description that applies to both species. Thus, because *A. angustifolia* does not always possess red streaks, there is no reliable way of separating the two species based on their macroscopic appearance.

The microscopic features of *A. araucana* and *A. angustifolia* are not very helpful either. The microscopic anatomy of the two species is described in detail by Greguss (1955). Differences in his descriptions include growth ring characteristics, but these are variable and more related to growth conditions than to species. Longitudinal tracheid and ray structure descriptions overlap. Tracheid pitting is given as 1–3 interrupted rows in *A. angustifolia*, but as uniseriate or rarely biseriate in *A. araucana*. Rays are 1–8 cells high in both species; uniseriate, or rarely biseriate in *A. angustifolia,* but no width is given for *A. araucana*. Neither species has longitudinal parenchyma. Comparison of the species is complicated by the fact that his description of *A. angustifolia* is based on wood from a tree (presumably the stem), but the description of *A. araucana* is based on a branch (Greguss 1955). It is probable that the within-species variability exceeds between species variability, and no consistent anatomical characters can be used to separate the species. Tortorelli (1956) gave almost the same description for both species based on stem wood. However, in his final wood identification key based on their anatomical features, he stated that the "number of tracheids per mm^2 in *A. angustifolia* is between 350 and 500, but this number increases to 1300–1500 in *A. araucana*" allowing possible differentiation of the two species (Tortorelli 1956). Kukachka (1960) described the wood of *A. angustifolia*, but not that of *A. araucana*. Phillips (1941) did likewise.

Phillips (1941) mentions that longitudinal tracheids of *Araucaria* and *Agathis* contain alternate bordered pits, which "separates Araucariaceae from other gymnosperm families." The same is true of *Wollemia* (Heady *et al*. 2002). All three genera contain "araucarioid" cross-field pitting (Greguss 1955), but the genera cannot be reliably separated further using any other microscopic features (Phillips 1941; Heady *et al*. 2002; Esteban *et al*. 2004; Heinz 2004; Richter *et al*. 2004). Separation of species within the genera is even more difficult and has mainly relied in the past on physico-chemical differences in the woods. For example, Welch (1927) devised a chemical

test to separate four species of *Agathis* that were once commercially important in Australia, and a similar test was developed by Cohen (1933) to separate *Araucaria bidwillii* Hook. (bunya pine) from *Araucaria cunninghamii* Aiton *ex* D. Don (hoop pine). These two species can also be separated using the 'burning splinter test' (Swain 1928); a match-sized splinter of *A. cunninghamii* burns moderately well (with an occasional crackling sound) leaving a thick greyish ash, whereas *A. bidwillii* burns to form a thin russet-colored ash. *Araucaria cunninghamii* has wood that is identical to that of *A. hunsteinii* K. Schum. (Klinki pine), but the two species can be separated using a simple chemical test; a droplet of concentrated hydrochloric acid produces an intense green color on *A. hunsteinii*, whereas no such color is produced on *A. cunninghamii* (Bootle 1983).

The success of these early chemical tests to identify and separate individual members of the Araucariaceae suggests that more contemporary methods of chemical analysis might achieve the same desirable outcome. One such method is Direct Analysis in Real Time (DART) Time-of-Flight Mass Spectrometry (TOFMS). DART TOFMS has been shown to assist in the identification of timber species that are difficult to identify using macroscopic and microscopic (anatomical) features, for example, *Dalbergia* spp. (Lancaster & Espinoza 2012a; Espinoza *et al*. 2015; McClure *et al*. 2015), *Quercus* spp. (Cody *et al*. 2012) and *Aquilaria* spp. (Lancaster & Espinoza, 2012b; Espinoza *et al*. 2014). Here we hypothesize that DART TOFMS will be able to identify selected Araucariaceae wood samples including the wood of the critically endangered rare "living fossil" *Wollemia nobilis*.

MATERIALS AND METHODS

Wood samples

Heartwood from three species of *Araucaria* from South America and Australasia, one species of *Agathis* and samples of mature *Wollemia nobilis* heartwood and juvenile sapwood were collected from curated xylaria collections and/or commercial wood sources (see Table 1 and, in Supplementary data, Table 2). Species identity was verified by using curated xylarium specimens and comparing their chemotypes with the samples analyzed in the study. The species were chosen because of their CITES protection or rarity (in the case of *W. nobilis*), their similarity in appearance to a protected species, or their commercial importance. In total, 75 specimens representing 5 species were obtained. We acknowledge that our selection of *Araucaria* and *Agathis* species only represents ~10 % of the species in the two genera. Hence, we point out in the discussion section below the need for the analysis of additional species to strengthen the practical applications of our findings. Table 1 shows the source of the specimens, which included USDA, Center for Wood Anatomy Research, Forest Product Laboratory (SJRw**,** Lynch & Gasson 2010); Laboratorio de Dendrocronologia e Historia Ambiental IANIGLA-CONICET, CCT CONICET Mendoza, Mendoza, Argentina (RAH, RUC); Fenner School of Environment & Society, The Australian National University (ANU) (Dargavel *et al*. 2014; Dadswell *et al*. 2015); Gary Green, Syracuse, IN, USA (GG); Carlton McLendon Inc., Atlanta, GA, USA (CMI); and Cook Woods, Klamath Falls, OR, USA (CW). The sample of mature *Wollemia nobilis* heartwood was the same one

Table 1. Specimens studied and their source.

Species	CITES Appendix	Number of specimens	Country of origin	Collection*
Araucaria araucana	I	21	Argentina Argentina U.S.A. (Oregon)	RAH (10) RUC (10) CW (1)
Araucaria angustifolia	–	23	Argentina Brazil	SJR (6) SJR (17)
Araucaria heterophylla	–	10	U.S.A. (Florida)	GG (10)
Agathis australis	–	20	New Zealand	GG (10) CMI (10)
Wollemia nobilis	–	2†	Australia	ANU (2)

* Number of specimens from each collection in parentheses. Abbreviations are described in Materials and Methods. † One sample of mature heartwood (for DART TOFMS) and one larger sample of juvenile sapwood for macrophotography.

used by Heady *et al*. (2002) when they described the wood anatomy of the species. This heartwood sample is representative of the wood from the single population of c. 100 mature *W. nobilis* trees that exist in the wild.

Macroscopic appearance

Transverse surfaces of mature *Agathis* and *Araucaria* heartwood were prepared by sanding the specimens on a WS3000 sander (Work Sharp, Ashland, OR, USA). Each specimen was sanded with successively finer grit sandpaper in the progression of 80, 220, 400, 1000, 3600 and 6000 grit. A sliver of mature *Wollemia nobilis* heartwood was available for analysis by DART TOFMS, but a larger sample of mature heartwood was not available. Therefore, a transverse surface of *W. nobilis* wood from a young planted tree was prepared for macrophotography, as described above. Macroscopic photos were taken with a VSC 8000 imaging workstation (Foster + Freeman, Evesham, Worcestershire, UK).

DART TOFMS & mathematical post-processing of data

Mass spectra of heartwood 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). A sliver of heartwood, with no further sample preparation, was held in a stream of heated helium gas produced by a DART ion source. As compounds were emitted from wood, they were drawn into the mass analyzer. Spectra were acquired in positive ion mode with the DART ion source parameters set as: electrode 1 voltage, 150V; electrode 2 voltage, 250V; gas heater temperature at 350 °C. The mass spectrometer settings were: Orifice 1, 120 °C, voltage 30V; ring lens voltage 5V; Orifice 2 voltage 5V; ion guide RF voltage 600V; ion guide bias voltage 33V. The focus voltage was 10V, quad voltage was 20V, focus lens voltage was -120V, push bias voltage was -0.43V. Spectra were obtained over the mass range of m/z 60 to 1000 with a sampling interval of 0.25ns and recording interval at 0.80s. Accumulation time was

0.797s, wait time 0.003s. Data was acquired for up to 30 minutes. The helium flow rate for the DART source was 2.0 mL s^{-1}. The resolving power of the mass spectrometer, as stated by the manufacturer, is > 6000 (FWHM, full width at half maximum). A mass calibration standard of poly(ethylene glycol) 600 (Ultra, Kingstown, RI, USA) was run between every fifth sample.

TSS Unity data reduction software (Shrader Software Solutions, Inc., Grosse Pointe Park, MI, USA) was used to export the text files of the mass-calibrated, centroided mass spectra for molecular formula determination and further analysis. Heat maps, principal component analysis (PCA) and kernel discriminate analysis (KDA) were conducted using the Mass Mountaineer Spectral Interpretation Tools software (RBC Software, Peabody, MA, USA) using a tolerance of 5 mDa and a 1% threshold. When other statistical programs were used, the data was exported from the Mass Mountaineer heat map using a tolerance of 250 mmu and a 1% threshold, and saved as a Microsoft Excel spreadsheet. The classification algorithms of Mass Mountaineer and PAST 3.12 (http://folk.uio.no/ohammer/past/) were used to calculate the principal components of each data set (Hammer *et al*. 2001). 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 software is capable of assigning a probability estimate to the classification of the spectrum.

Kernel Discriminant Analysis (KDA) is a supervised learning algorithm that relies on *a priori* assignment of a class membership to achieve the greatest separation between classes in a training set by using a nonlinear function. This allows points that cannot be linearly separated in a two-dimensional space to be separated in higher dimensions. Estimated probabilities are based on Z scores (distance divided by standard deviation) based on a normal distribution (Baudat & Anouar 2000).

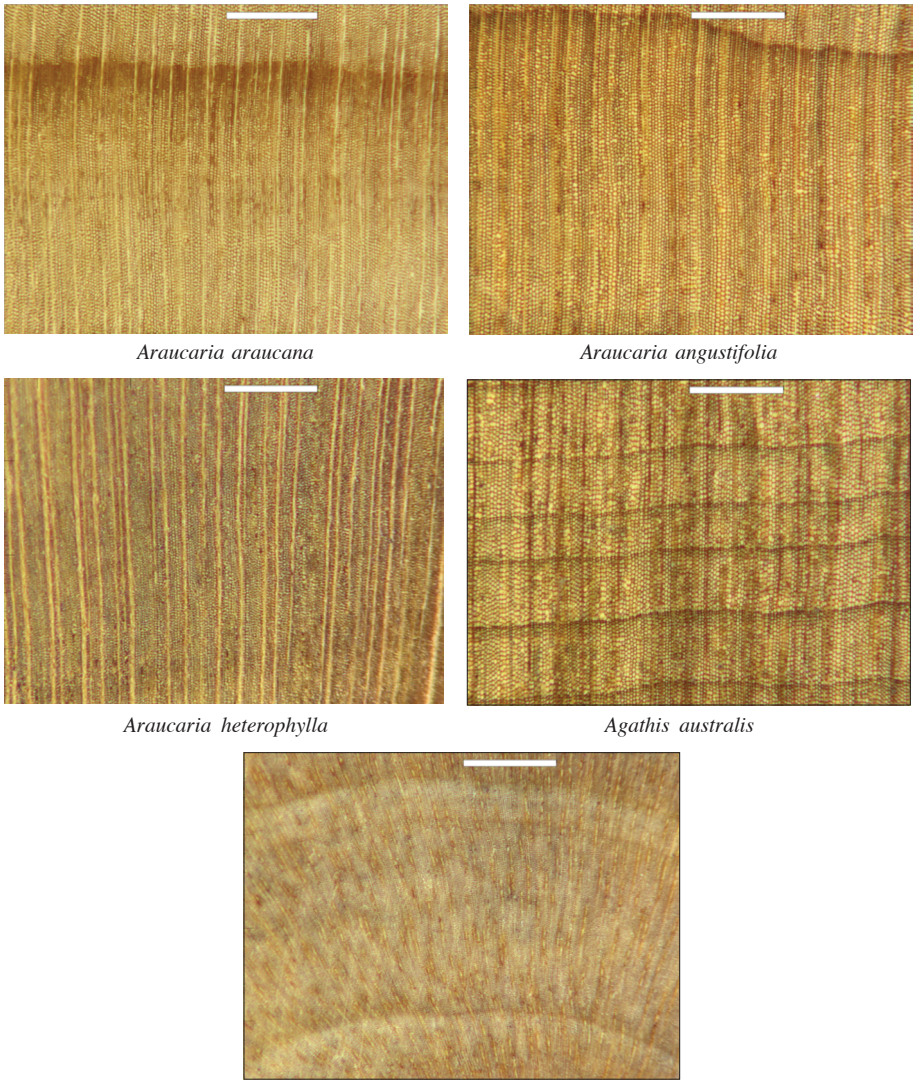
Wards cluster analysis was performed with PAST (v.3.12) (Hammer *et al*. 2001). Clusters were fitted (boot = 100) and joined such that increase in within-group variance is minimized.

RESULTS

The anatomy of the Araucariaceae analyzed here, and the difficulty of identifying and separating them based on their wood anatomy has been described by Phillips (1941), Greguss (1955), Tortorelli (1956), Kukachka (1960), Heady *et al*. (2002), Esteban *et al*. (2004), Heinz (2004) and Richter *et al*. (2004), as noted above. Images of transverse surface of each species show some differences in distinctiveness of annual rings and early-latewood transitions, but in accord with the observations of Greguss (1955) such differences are not sufficient to identify the different species (Fig. 1).

Figure 2 is a heat map of the data collected using DART TOFMS. A heat map is a graphical representation of the mass spectra results for every sample; the X coordinate is the mass to charge ratio (m/z) associated with a molecule and the Y coordinate represents the chemotype of each sample analyzed. The intensity of the color for each

compound is directly related to the total abundance of that ion in the chemotype. An advantage of the heat map is that a visual inspection of the data allows for immediate assessment of the difference in chemotypes associated with a data set. In the case of the Araucariaceae, Figure 2 shows: 1) that the chemotype of each species is reproducible, and 2) the chemotypes associated with *Araucaria araucana*, *Agathis australis* and *Wollemia nobilis* are each unique, whereas the chemotypes associated with *Araucaria angustifolia* and *A. heterophylla* are similar.



Araucaria araucana *Araucaria angustifolia*

Araucaria heterophylla *Agathis australis*

Wollemia nobilis

Figure 1. Macroscopic morphological photographs (×65) of the transverse surface of each of the five wood species (*Araucaria araucana*, *A. angustifolia*, *A. heterophylla*, *Agathis australis*, and *Wollemia nobilis*). Scale bar = 1 mm.

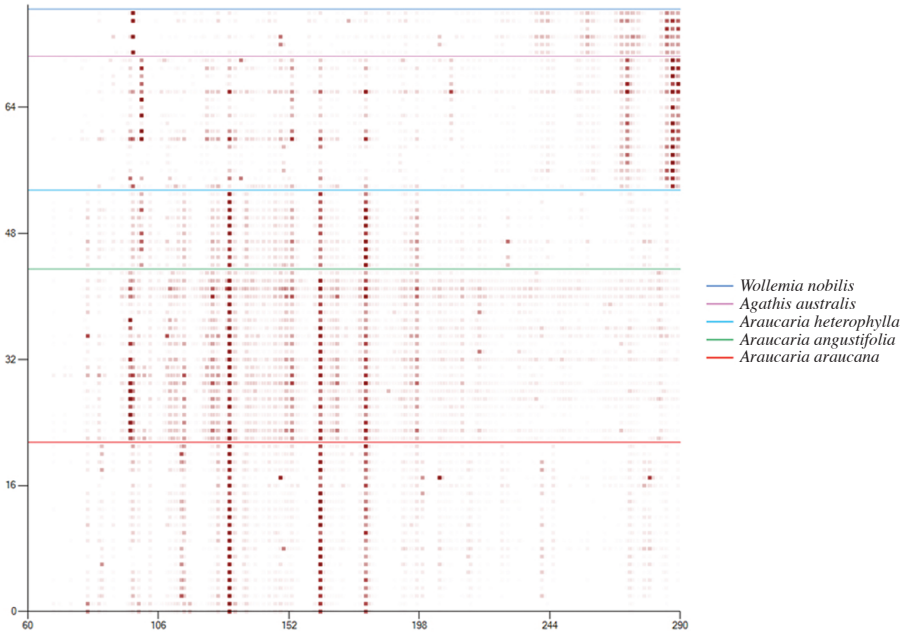


Figure 2. Heat map of the specimens analyzed. The X coordinate is the mass (m/z) associated with a molecule and the Y coordinate represents the chemotype for each sample.

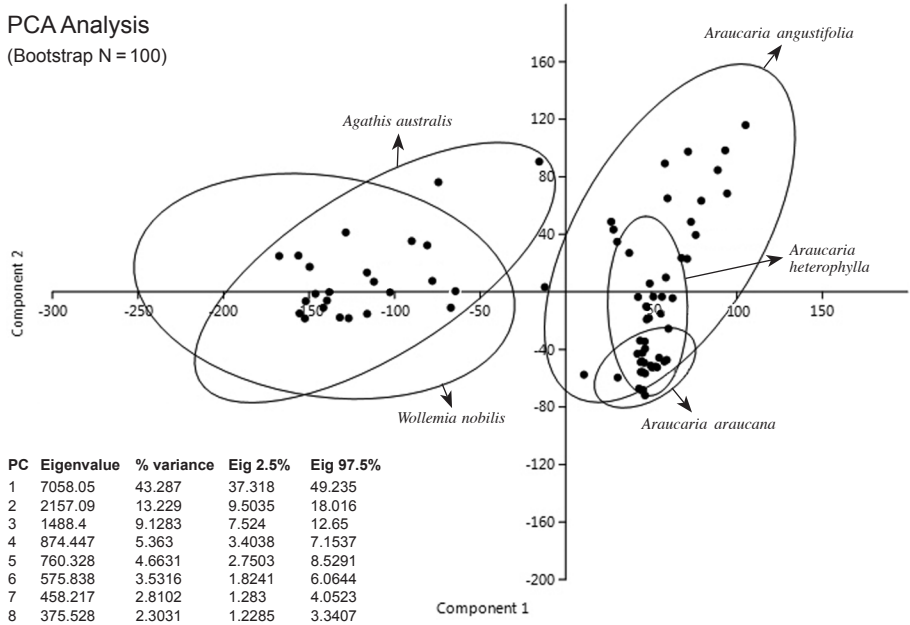
Principal component analysis (PCA) of the data (bootstrap n = 100) tells a slightly different story; *Araucaria heterophylla*, *A. araucana* and *A. angustifolia* are grouped together whereas *Agathis australis* and *W. nobilis* overlap in a second cluster (Fig. 3). Therefore, PCA analysis is of limited value in separating each of the sampled species.

The results associated with the kernel discriminate analysis (KDA) are shown in Figure 4. KDA is a supervised classification algorithm based on the concept of support vector machines (Christianni & Shawe-Taylor 2000) and kernel transformation (Boser *et al*. 1992). For a detailed description of these functions see Filzmoser and Varmuza (2014). The KDA shows separation of each taxon analyzed, and implies that the *W. nobilis* sample is closer to *Agathis australis* than the *Araucaria* spp. Leave-one-out cross validation is 94.8 % for the Araucariaceae. This indicates a good model fit for this taxon and a high discrimination ability among the species tested.

The DART TOFMS spectra of each species were also analyzed using Ward's method for hierarchical cluster analysis (boot n = 100). This unsupervised method joins clusters by minimizing within-group variance (Ward 1963; Hammer *et al*. 2001). Figure 5 shows the resulting dendrogram (correlation coefficient = 0.8). For purposes of clarity, only ten spectra per species are shown, except for *Wollemia nobilis* where the single specimen available was analyzed six times. The dendrogram (Fig. 5) shows two clades, one composed of *W. nobilis* and *Agathis australis* and a second which includes *Araucaria angustifolia*, *A. heterophylla* and *A. araucana*. Within the *Araucaria* clade, two samples of *A. angustifolia* are placed within the *A. araucana* cluster, but these samples grouped as expected in the heat map (Fig. 2) and the kernel discriminate analysis (Fig. 4).

The overall inferences from these analyses are:

– *Araucaria angustifolia*, *A. heterophylla* and *A. araucana* have chemotypes very similar to each other (see Fig. 2) and the statistical results of PCA (Fig. 3), and hierarchical cluster analysis (Fig. 5) support this interpretation.
– *Wollemia nobilis* and *Agathis australis* have chemotypes very similar to each other, but different from the *Araucaria* spp.; the PCA, and hierarchical cluster analysis support this conclusion (Fig. 3 & 5).
– KDA produces a model that separates all the species analyzed (Fig. 4).



PC	Eigenvalue	% variance	Eig 2.5%	Eig 97.5%
1	7058.05	43.287	37.318	49.235
2	2157.09	13.229	9.5035	18.016
3	1488.4	9.1283	7.524	12.65
4	874.447	5.363	3.4038	7.1537
5	760.328	4.6631	2.7503	8.5291
6	575.838	3.5316	1.8241	6.0644
7	458.217	2.8102	1.283	4.0523
8	375.528	2.3031	1.2285	3.3407
9	324.488	1.9901	1.0873	3.7564
10	278.638	1.7089	0.013039	2.7601

Figure 3. Graphical representation of the principal components analysis (PCA) (bootstrap n = 100); *Araucaria heterophylla*, *A. araucana* and *A. angustifolia* are grouped in one cluster whereas *Agathis australis* and *Wollemia nobilis* group in a second cluster.

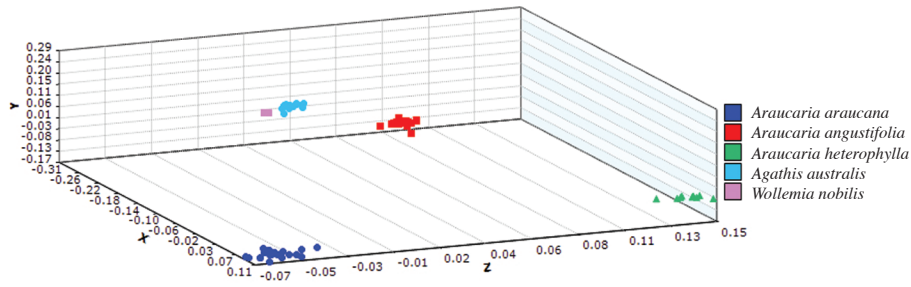


Figure 4. Graphical representation of the kernel discriminant analysis (KDA) showing that the species segregate distinctly (Leave-one-out cross-validation (LOOCV) is 94.8%).

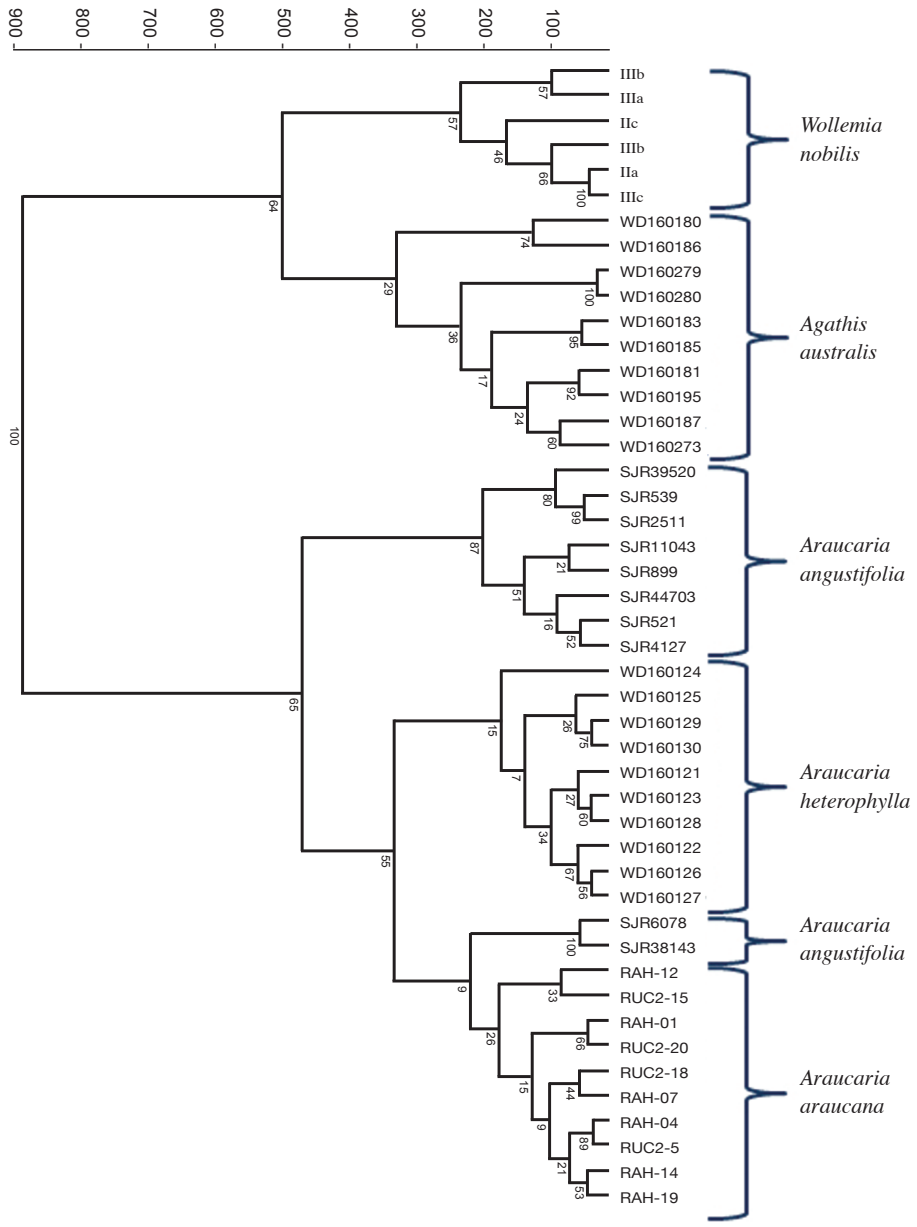


Figure 5. Hierarchical cluster analysis (Ward's method; boot n = 100).

DISCUSSION

The main aim of this study was to develop a method of identifying and separating the CITES-listed (Appendix I) species *Araucaria araucana* from the unlisted and more commonly traded species *A. angustifolia*. We achieved our aim by combining an atmospheric ionization method (DART) to liberate ions from wood surfaces, analysis of those ions using time-of-flight mass spectrometry (TOFMS) with (most importantly) sophisticated mathematical post-processing of spectra to capture differences and similarities in the complex mass spectra generated from analysis of *A. araucana* and *A. angustifolia*. One of the mathematical techniques used to analyze spectra, the supervised learning method, kernel discriminate analysis, clearly separated *A. araucana* from *A. angustifolia*. It is not possible to assign differences in the mass spectra of *A. araucana* and *A. angustifolia* to specific compounds because DART TOFMS of wood generates numerous adducts and mass fragments of multiple compounds (Pavlovich *et al*. 2016). For example, both *A. araucana* and *A. angustifolia* contain a multitude of polyphenolic 'lignan' extractives that have interesting pharmaceutical and antimicrobial properties (Fonseca *et al*. 1979; Céspedes *et al*. 2006). Lignan extractives easily cleave during mass spectrometry to produce abundant molecular fragments (Yamamoto *et al*. 2004). It is possible that differences in the types and relative abundance of such extractives in *A. araucana* and *A. angustifolia* account for differences in the mass spectra observed here. The complexity of the mass spectra produced by DART TOFMS explains why mathematical post-processing of spectra is necessary to separate the two woods, in contrast to the forensic analysis of more homogeneous materials such as explosives, which can be identified using far fewer molecular adducts or fragments (Nilles *et al*. 2010).

Mathematical post processing of DART TOFMS spectra clearly separated the wood of *Wollemia nobilis* and *Agathis australis* from *Araucaria* spp., although most techniques were unable to separate *W. nobilis* and *Agathis australis*. The discovery of *W. nobilis* in 1994 generated a flurry of interest in its phylogenetic status. It was placed in a separate genus within the Araucariaceae by Jones *et al*. (1995) based on the morphology of its leaves, micro-sporangia, pollen, and female strobili. This status is supported by subsequent molecular phylogenetic studies (Escapa & Catalano 2013). However, as pointed out by Escapa and Catalano (2013) these molecular studies differ in terms of their views on the affinity of *Wollemia* to *Araucaria* and *Agathis*. The majority of studies place *Wollemia* as sister to *Agathis*, with *Araucaria* at the base of this clade (Gilmore & Hill 1997; Stefanovic *et al*. 1998; Liu *et al*. 2009; Leslie *et al*. 2012). However, there is also support for the alternatives: *Wollemia* is the sister group to a clade formed by *Agathis* and *Araucaria* (Setoguchi *et al*. 1998) and *Wollemia* and *Araucaria* form a clade sister to *Agathis (*Codrington *et al*. 2009). Furthermore, other studies differ in their views on the affinity of *Wollemia* to *Agathis* or *Araucaria*. For example, *Wollemia*'s leaf anatomy and mechanism of leaf shedding are more similar to those of *Araucaria* than to *Agathis* (Hill 1996; Burrows & Bullock 1999). In contrast, its genome size, morphology of pollen and seed-bearing cones, and chemical composition of volatile oils in leaves more closely resembles those of *Agathis* (Offord *et al*. 1999; Lobreau-Callen & Meagher 2004; Zonneveld 2012). *Wollemia*'s wood anatomy

places it within the Araucariaceae, but "on the basis of its wood anatomy," Heady *et al*. (2002) concluded that "it is not possible to state whether *W. nobilis* is more closely related to *Agathis* or to *Araucaria*." Heady *et al*. (2002) came to this conclusion because *Wollemia* shared with *Agathis* an abundance of resin plugs and light brown heartwood, whereas in common with *Araucaria* it lacked 4-seriate bordered pitting (Jane 1970). The greater abundance of resin in the wood of *Wollemia* and *Agathis* than in *Araucaria* may explain why DART TOFMS suggests a closer affinity between *Wollemia* and *Agathis* than *Araucaria* because volatile ions produced from the surface of the woods would certainly include those generated from abundant resin in rays and adjacent tracheids. Further research, however, would be needed to confirm this hypothesis. Nevertheless, our findings clearly add further weight to the view that *Wollemia* is more closely related to *Agathis* than to *Araucaria*.

Our findings accord with previous studies showing that DART TOFMS can be used to identify and separate hardwoods such as red oak (*Quercus rubra* L.) and white oak (*Q. alba* L.), *Dalbergia* spp. (Wiemann & Espinoza 2017) and samples of wild and cultivated agarwood (*Aquilaria* spp.) (Cody *et al*. 2012; Lancaster & Espinoza 2012a,b; Espinoza *et al*. 2014, 2015; McClure *et al*. 2015). Softwoods are "more uniform in their anatomy than hardwoods and present many more problems of accurate identification" (Gasson 2011). Hence, our finding that DART TOFMS can identify and separate *Araucaria araucana* from *A. angustifolia* and some other members of the Araucariaceae is noteworthy, and suggest it may also be useful in identifying the other softwoods listed by CITES (Gasson *et al*. 2011), many of which are difficult to identify using traditional macroscopic and microscopic anatomical techniques. Softwoods listed by CITES are less frequently encountered by customs than hardwoods (Gasson 2011), but we have received samples of Araucariaceae in the past, and have been able to demonstrate *ex post facto* using DART TOFMS that at least two shipments of *Araucaria* timber that arrived at U.S. ports in the past contained illegally logged *A. araucana*. This recent practical application of our work demonstrates its value, but much more work is needed on many fronts to combat the rampant trade in illegally logged timber: conservation of xylaria to provide authenticated reference timber (Cornish *et al*. 2014; Dadswell *et al*. 2015); more thorough anatomical descriptions of certain CITES-listed species (Gasson 2011); and more widespread deployment and further development of quantitative anatomical and chemometric techniques including DART TOFMS for identification of additional species of Araucariaceae, and other CITES-listed species (Evans *et al*. 2008; Gasson *et al*. 2010). As pointed out by Gasson (2011) there are many challenges ahead. But the analytical devices and most importantly computational capacity now at hand promise significant advances in our ability to meet the challenge of more accurately identifying wood.

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LEGAL NOTE

The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

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Supplementary data for:

Identification of selected CITES-protected Araucariaceae using DART TOFMS

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Content: Supplementary Table 2

Specimens studied and their source.

Species	Accession number	Individual number	Country of origin	Agency	Sample type	Source
Araucaria angustifolia	WD160239	SJR899	Argentina	Michael C. Wiemann USDA Center for Wood Anatomy Research, Forest Products Laboratory, Madison, WI	Collection	SJR
	WD160240	SJR521				
	WD160241	SJR1139				
	WD160242	SJR539				
	WD160247	SJR4127				
	WD160259	SJR23484				
	WD160243	SJR2511	Brazil			
	WD160244	SJR696				
	WD160245	SJR3319				
	WD160246	SJR719				
	WD160248	SJR698				
	WD160249	SJR11043				
	WD160250	SJR35920				
	WD160251	SJR39520A				
	WD160252	SJR15482				
	WD160253	SJR38234				
	WD160254	SJR6078				
	WD160255	SJR38143				
	WD160256	SJR4924				
	WD160257	SJR23796				
	WD160258	SJR4384				
	WD160260	SJR53024				
	WD160261	SJR44703				

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(Table 2 continued)

Species	Accession number	Individual number	Country of origin	Agency	Sample type	Source
Araucaria araucana	WD160101 WD160103 WD160104 WD160106 WD160107 WD160109 WD160110 WD160112 WD160114 WD160117	RAH-01 RAH-03 RAH-04 RAH-06 RAH-07 RAH-11 RAH-12 RAH-14 RAH-15 RAH-19	Rahue, Neuquén, Argentina	Mundo, Ignacio Alberto, Laboratorio de Dendrocronologia e Historia Ambiental, IANIGLA-CONICET, CCT CONICET Mendoza, Mendoza, Argentina	Collection	RAH
	WD160102 WD160105 WD160108 WD160111 WD160113 WD160115 WD160116 WD160118 WD160119 WD160120	RUC2-2 RUC2-5 RUC2-9 RUC2-13 RUC2-15 RUC2-16 RUC2-18 RUC2-19 RUC2-20 RUC2-22	Rucachoroi, Lamin National Park, Argentina		Collection	RUC
	WD160200	planted from seed	Astoria, Oregon USA	Chris Cook, Cook Woods, Klamath Falls	Commercial	CW
Araucaria heterophylla	WD160121 WD160122 WD160123 WD160124 WD160125 WD160126 WD160127 WD160128 WD160129 WD160130		S. Florida, USA	Gary Green IWCS, International Wood Collection Society, Syracuse, IN	Collection	GG
Agathis australis	WD160180 WD160181 WD160182 WD160183 WD160184 WD160185 WD160186 WD160187 WD160194 WD160195		New Zealand	Gary Green IWCS, International Wood Collection Society, Syracuse, IN	Collection	GG

(Table 2 continued)

Species	Accession number	Individual number	Country of origin	Agency	Sample type	Source
(Agathis australis contd)	WD160272 WD160273 WD160274 WD160275 WD160276 WD160277 WD160278 WD160279 WD160280 WD160281		New Zealand	Richard Kuehndorf IWCS, International Wood Collectors Society, Carlton McLendon, Inc., Rare Woods and Veneers, Atlanta, GA	Collection	CMI
Wollemia nobilis	WD160349		Australia	Philip D. Evans, Department of Applied Mathematics, Research School of Physics & Engineering, The Australian National University, Canberra, ACT 0200, Australia	Collection	ANU