Timber Identification in the Taxonomically Challenging Sapotaceae Family

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Research Article

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Abstract

The illegal timber trade is still rampant so robust identification and tracking techniques are necessary to combat this wildlife crime. To follow and enforce timber import laws and adjoining timber species identification, the identity of the botanical species must be well defined. Since the Sapotaceae family is known as a taxonomically challenging family, we focus in this study on the four most valuable Sapotaceae timber species from tropical Africa: *Autranella congolensis* (De Wild.) A.Chev., *Baillonella toxisperma* Pierre, *Tieghemella africana* Pierre and *Tieghemella heckelii* (A.Chev.) Pierre ex Dubard. The wood anatomical characteristic fiber lumen fraction and Direct Analysis in Real Time – Time of Flight Mass Spectrometry (DART-TOFMS) are used to differentiate between the four species and to make inferences on the species delineation and taxonomic identity. Based on visual assessment of the box-plots for the fiber lumen fraction measurements, two groups can be discerned: (1) *A. congolensis* and *B. toxisperma*, and (2) *T. africana* and *T. heckelii*. In addition, all Mann-Whitney U comparisons and the differences in underlying distributions (Kolmogorov-Smirnov) for the fiber lumen fraction measurements were significant between all species. However, when permutating the data within those two groups, significant differences were still found. This could indicate that the differences based on the fiber lumen fraction are more nuanced. The DART-TOFMS analysis shows that *A. congolensis* and *B. toxisperma* have distinct chemotypes, while *T. heckelii* and *T. africana* have remarkably similar chemotypes. Our results provide support for the possibility that *T. africana* and *T. heckelii* are more closely related than previously considered. A taxonomic study would be beneficial to assess the species limits of *T. heckelii* and *T. africana*, as our results suggest they could be conspecific. This would have important implications towards the timber trade and adjoining timber species identification, for the *Tieghemella* species, and their conservation.

Introduction

Illegal logging and timber forensics

It is estimated that 30 to 90% of timber from the tropics is illegally sourced [1–6]. Next to the straightforward ecological damages, there are also substantial economic and social problems associated with timber poaching [4]. These issues have sparked an increased demand in different timber identification and timber traceability techniques, with current frontrunners being wood anatomy, both traditional and with machine vision [7–9], Direct Analysis in Real Time Time-of-Flight Mass Spectrometry (DART-TOFMS) [10–12], genetic analysis [13] and stable isotope ratio analysis [6,14].

Wood anatomy, DART-TOFMS and genetic analysis are currently the most employed methods to determine the species identity of timber. However, timber import laws and adjoining timber species identification can only be followed if the identity of the botanical species is well defined. Until three decades ago, taxonomists mainly used morphological traits to describe and delineate species. However, species can show high levels of intraspecific morphological variation, which complicates accurate species delineation and occasionally results in the erroneous splitting of species. Conversely,
differentiation and speciation are not always accompanied by morphological change, as demonstrated by the abundance of cryptic species [15–17], where two or more distinct species are classified under the same taxonomic unit because they are seemingly indistinguishable from a morphological point of view [15]. For this reason, it is important to include molecular data when new species are described and named. Though, even when DNA-based methods are incorporated, genetic divergence can remain undetected because of homoplasy (shared character that did not arise from a common ancestor) and evolutionary processes such as hybridization (production of offspring by parents from different varieties or species), chloroplast captures (introgression of a chloroplast genome from one species into another), reticulate evolution (or network evolution, where a group of organisms originates through the partial merging of ancestor lineages) or incomplete lineage sorting (common ancestry of gene copies at a single locus extends deeper than previous speciation events) [16,18–20], resulting in wrongly delineated species.

**Sapotaceae**

The Sapotaceae family is known for its highly homoplasious morphological characters and the lack of unambiguous synapomorphies for subfamilies and tribes [21], which are the reasons for the high dynamics of the Sapotaceae taxonomy and the many taxon synonyms. Here, we will focus on the four most important Sapotaceae timber species from tropical Africa: *Autranella congolensis* (De Wild.) A.Chev., *Baillonella toxisperma* Pierre, *Tieghemella africana* Pierre and *Tieghemella heckelii* (A.Chev.) Pierre ex Dubard. All four species represent the largest trees in their respective forested regions, reaching heights of 50 m or more and diameters of sometimes more than 2 m.

*Tieghemella africana* is well known to the international timber trade as Douka [22]. This trade name can occasionally cover timber from *B. toxisperma* and is often considered as the same trade category as wood from *T. heckelii* (Makoré). However, *T. heckelii* is traded under the generic trade name (or pilot name) Makoré, which can include timber from *T. africana* and *B. toxisperma*. *Tieghemella africana* is typically found in the evergreen rainforests from Cameroon to Cabinda (Angola) in the west, and eastward to the Republic of the Congo and Democratic Republic of the Congo (DRC) [23]. The highest species densities are reported in Equatorial Guinea, western Gabon and in the Republic of the Congo, north of Kouilou. In other regions it can be mixed with *T. heckelii* and *B. toxisperma*. Heartwood of *T. africana* is very similar to *T. heckelii*, but tends to be more intensely stained with a more distinct vein pattern. In provenances from the Republic of the Congo, the wood has been noted to darken to a red violet stain. In addition, *T. africana* tends to be slightly harder and heavier than *T. heckelii*. The main distribution area for *T. heckelii* covers eastern Liberia, Côte d’Ivoire and Ghana, but the species also occurs in lower densities in Nigeria [24]. As such, the range of *T. heckelii* overlaps with other morphologically similar Sapotaceae species, creating a challenge for field identification. *Baillonella toxisperma* and *A. conglolensis* occur in low densities in the rainforest of southern Nigeria, Cameroon, Equatorial Guinea, Gabon, Cabinda (Angola), Republic of the Congo and the Democratic Republic of the Congo (DRC) [25,26]. *Baillonella toxisperma* (Moabi), can look very similar to *T. heckelii* but the distinction is clearer than for *T. africana* from Ghana or the Ivory Coast. *Baillonella toxisperma* is also found mixed with
shipments of *T. africana* and *T. heckelii*. *Autranella congolensis* is reported to be falsely sold as *B. toxisperma*, but *Autranella congolensis* wood is harder and darker with a violet stain. Standing trees of *B. toxisperma* and *A. congolensis* are quite similar to each other, with one primary difference being *B. toxisperma* exhibits a distinctively flatter crown [22].

**Taxonomic history**

Although the four Sapotaceae species in this study are currently assigned to three distinct genera, all four species were previously included in the genus *Mimusops*. *Autranella congolensis* seems to be related to the latter, but it differs in having stipules, a longer corolla tube and larger fruits [25], and was thus reinstated as a distinct (monotypic) genus. The genus *Baillonella* was first described by Pierre based on a seed collected in Gabon [27]. Engler then included the genus as a section in the *Mimusops*, but the group was later reinstated as a separate genus because of the thin seed coat and particular nerves that distinguish it from the *Mimusops* [28]. While multiple *Baillonella* species have been described in the 1900s, *B. toxisperma* is currently the only recognised species. The genus *Tieghemella* was first described by Pierre [27], but was later subsequently added to the genera *Dumoria, Mimusops* and *Baillonella*, after which *Tieghemella* was reinstated as a distinct genus. Currently, *T. africana* and *T. heckelii* are the only two species recognised in the genus. However, a taxonomic study is needed to assess the status of the genus and the species limits, since they may be conspecific [23–24].

**Study objectives**

As indicated, these Sapotaceae species all have similar heavy and reddish-brown wood. Because of this similarity, the wood is used for similar purposes and often traded together under the same commercial name. As such it is important (1) to be able to identify these species within the timber trade and (2) to be certain that these are four different species. In this study, we will assess:

1. The robustness of the one wood anatomical characteristic that is claimed to allow for the differentiation of these four Sapotaceae species: the fiber lumen fraction (referred to as the *coefficient de souplesse* by [29]).
2. The possibility to differentiate these four Sapotaceae species using chemical fingerprints via DART-TOFMS.
3. The effect of (1) and (2) towards the species delineation and the taxonomic identity of these four Sapotaceae species.

**Materials And Methods**

**Sampling**

A total of 62 wood specimens were collected from the Tervuren Wood Collection (Royal Museum for Central Africa, Tervuren, Belgium) and three from the World Forest ID project [30] (see Table S1 in Supplementary Materials). Some of these wood specimens were used for wood anatomical analysis and all except one were used to obtain chemical fingerprints via DART-TOFMS. Two samples from the
Tervuren Wood Collection also have a corresponding herbarium voucher at Meise Botanic Garden (BR) in Belgium (see Table S1).

**Wood anatomical analysis**

The anatomical differences between species determined via the IAWA list of microscopic features [31] on InsideWood [32] where compared to the anatomical slices obtained in this study. Anatomical cross-sections (transversal) of 16 wood specimens (Table 1 and Table S1 in Supplementary Materials) were digitized at 20x magnification using Stream Image Analysis Software (StreamMotion, Olympus, Tokyo, Japan) with a scanning stage (Märzhäuser Wetzlar, Wetzlar, Germany) and a UC30 camera (Olympus, Tokyo, Japan) mounted on a light microscope (BX60, Olympus, Tokyo, Japan). For each image, fibers were used to determine the fiber lumen fraction:

\[
\text{Fiber lumen fraction} = \left( \frac{\text{diameter lumen}}{\text{diameter fiber}} \right) \times 100 \, (\%)
\]

Images were aligned in transversal direction and the fiber lumen fraction was determined in two perpendicular directions on the fiber (4 measurements per fiber = 2 fiber lumen fractions, Fig. 1). The average of those two measurements was taken as the fiber lumen fraction of that fiber.

**Figure 1** Example of the fiber lumen fraction measurements. The fiber lumen fraction of the fiber is taken as the average of two measurements in perpendicular directions. Sample: Tw18005, *Tieghemella heckelii*.

Notched boxplots were created using the ggplot2 package [33] in RStudio (Rstudio Team, 2016). Notched boxplots offer a quick visual check whether a statistical difference in mean can be expected. Normality of the data was checked using the Shapiro-Wilk test [34] and significant differences in mean were determined using the non-parametric independent 2-group Mann-Whitney U test [35]. To determine whether the underlying distributions of the data were different, the Kolmogorov-Smirnov test was used. Finally, 50 permutations were run in combination with the non-parametric independent 2-group Mann-Whitney U test to determine whether the comparisons indicate real differences in fiber lumen fraction between the two species groups (*A. congolensis*/*B. toxisperma* and *T. africana/* *T. heckelii*) (see Results section). For the group *A. congolensis*/*B. toxisperma*, four Tw samples belonging to those two species were randomly picked and placed under *A. congolensis*, the same was done for *B. toxisperma*. This was repeated for each of the 50 permutations. Per permutation run, one sample was not used, as there are nine samples between those species (see Table 1). This was to keep the dataset balanced. The same was done for the species groups *T. africana*/*T. heckelii* with four samples randomly picked each permutation run per species.

**Dart-tofms**

The heatmap of the mass spectra shows that the ion pattern from 90–215 $m/z$ was present in all four species (Fig. 3). Higher relative abundance at 409.163 $m/z$ was noted for *A. congolensis*. Higher relative
abundance in ions at 434.316, 440.326 and 452.310 m/z appeared to be indicative of the *Tieghemella* species. *Baillonella toxisperma* showed an ion at approximately 84.081 m/z, which was not found or significantly reduced in the other species, and also showed a higher relative abundance of the ion at 130.087 m/z.

The PCA scatterplot (Fig. 4) showed distinctive grouping for *A. congolensis* and *B. toxisperma*, while *T. africana* and *T. heckelii* group together. There appeared to be three outlier spectra, one from *A. congolensis* and two from *B. toxisperma*. The outlier of *A. congolensis* (Tw4300), and one from *B. toxisperma* (Tw2101), did not group with any other species class, while the other outlier from the *B. toxisperma* class (Tw1675) grouped with *A. congolensis*. These outliers may have been due to misidentifications at the field collection stage or human error. Regardless, they were removed from the PCA model, bringing the total number of ions to n = 792, and from subsequent analysis.

**Figure 4** The scatterplot visualizing the Principal Component Analysis of mass spectra from the four Sapotaceae species. All species exhibited separate clustering trends with the exception of the *Tieghemella* spp.

The DAPC model without the outliers and with *T. africana* and *T. heckelii* spectra in a single class, *Tieghemella* spp., showed distinctive grouping between the three classes (Fig. 5). The calculated LOOCV value for the DAPC model was 96.61%, indicating that two spectra (*B. toxisperma* Tw1666 and *T. heckelii* Tw22612) were misclassified. All test samples (n = 8) were correctly assigned (Table 2).

**Figure 5** Scatterplot of the DAPC model showing the variation between *A. congolensis*, *B. toxisperma* and *Tieghemella* spp. (LOOCV = 96.61%)

| Test Samples | Sample ID   | Class Probability (%) | Assigned Class       |
|--------------|-------------|------------------------|----------------------|
| *A. congolensis* | Tw1765     | 75.19                  | *A. congolensis*     |
| *A. congolensis* | Tw5190     | 91.73                  | *A. congolensis*     |
| *B. toxisperma* | WFID-CBG0030 | 99.97                  | *B. toxisperma*      |
| *B. toxisperma* | WFID-YRNG838 | 100                    | *B. toxisperma*      |
| *B. toxisperma* | WFID-GRGY281 | 100                    | *B. toxisperma*      |
| *T. africana* | Tw22610    | 99.88                  | *Tieghemella* spp.   |
| *T. heckelii* | Tw26511    | 99.95                  | *Tieghemella* spp.   |
| *T. heckelii* | Tw64631    | 98.25                  | *Tieghemella* spp.   |

Analysis of the *Tieghemella* species indicated that the species’ chemotypes are remarkably similar (Fig. 6). Some variation in ion intensity can be seen between the species. However, this variation also
changes from sample to sample (Fig. 3) while the overall ion pattern (Fig. 6) remains constant.

**Figure 6** Comparison spectrum of *T. heckelii* and *T. africana* shows the similarities between the two species’ spectra.

An additional PCA scatterplot (Figure S1 in Supplementary Materials) and DAPC model (Figure not shown) was created using replicates from all *T. heckelii* (*n* = 16) and *T. africana* (*n* = 10) samples. The total number of ions (*n* = 352) was used and, based on the PCA plot, the species did not show separating trends. The ions underwent ANOVA, leaving a total of 29 ions for DAPC analysis. The LOOCV value of the DAPC model was 84.62%; all *T. heckelii* samples were correctly assigned to their class, while only 6 of the 10 *T. africana* samples were correctly assigned.

### Results

#### Wood anatomical analysis

*Autranella congolensis* had the lowest fiber lumen fraction compared to the other species (Table 1), but the values showed some overlap with *B. toxisperma* due to the high standard deviation. The two *Tieghemella* species had a noticeably higher fiber lumen fraction, with *T. heckelii* having the highest value. The notched boxplots of the fiber lumen fraction measurements show that there appeared to be two groups (*A. congolensis/B. toxisperma* and *T. africana/T. heckelii*) and there was no overlap in notches for all four species (Fig. 2). Furthermore, all Mann-Whitney U (MW) comparisons, as well as differences in underlying distributions (KS), were highly significant (*p* < 0.001). However, for *A. congolensis* and *B. toxisperma*, 37 out of 50 permutations were significant based on the independent 2-group Mann-Whitney U test. For *T. africana* and *T. heckelii* this was 34 out of 50 permutations.

| Species               | N    | Average (%) | Std (%) | Samples used from Tw collection            |
|-----------------------|------|-------------|---------|--------------------------------------------|
| *Autranella congolensis* | 210  | 38.67       | 9.04    | 633, 923, 1175, 1578                       |
| *Baillonella toxisperma* | 251  | 44.49       | 6.56    | 10754, 27547, 30909, 44837, 50839          |
| *Tieghemella africana*   | 218  | 64.23       | 9.60    | 10761, 18800, 22610, 26512                 |
| *Tieghemella heckelii*   | 281  | 70.34       | 9.08    | 18005, 21571, 26510, 31670                 |

**Figure 2** Notched boxplots of the fiber lumen fraction for each of the species. The independent 2-group Mann-Whitney U test and Kolmogorov-Smirnov showed significant difference in mean and underlying distribution for each species comparison.

### Discussion
Wood anatomical analysis

When comparing the four species in terms of the IAWA list of microscopic features [31] on InsideWood [32], we only saw minimal differences. The two *Tieghemella* species have vessel-ray pits with distinct borders compared to *A. congolensis* and *B. toxisperma*. The latter two species do have vessel-ray piths of two distinct sizes. The *Tieghemella* species both have gums and other deposits in their heartwood cells. The fibers of *A. congolensis* are very thick-walled, where they can be thin or thick for the other species. *Autranella congolensis* also has prismatic crystals present, which can be in the axial parenchyma cells. Finally, *A. congolensis* and *B. toxisperma* have a higher wood density compared to the *Tieghemella* spp.

When comparing this description with the anatomical slices used in this study, we noticed some important differences. The specimens of *A. congolensis* and *B. toxisperma* also have vessel-ray pits with distinct borders. Moreover, it is not clear whether these species have vessel-ray pits of two distinct sizes. As such, this characteristic could easily be misinterpreted. All four species appear to have deposits of different proportions in their heartwood cells (mainly in ray and parenchyma cells). Our samples confirm the thick-walled fibers for *A. congolensis*, however this also appears to be the case for *B. toxisperma*. In our samples, the *Tieghemella* species have thin-to-thick walled fibers. Only Tw633 (*Autranella congolensis*) appeared to have prismatic crystals clearly present.

For the fiber lumen fraction measurements, all Mann-Whitney U comparisons and the differences in underlying distributions (Kolmogorov-Smirnov) were highly significant (*p* < 0.001) between all species. Based on visual assessment of the box-plots for the fiber lumen fraction measurements, two groups can be discerned: (1) *A. congolensis* and *B. toxisperma* and (2) *T. africana* and *T. heckelii*. This infers that misidentification between the two groups should not be possible if we use the fiber lumen fraction measurements as the diagnostic characteristic. However, when permutating the data per sample, for *A. congolensis* and *B. toxisperma*, 37 out of 50 permutations were significant based on the independent 2-group Mann-Whitney U test. For *T. africana* and *T. heckelii* this was 34 out of 50 permutations. This implies that even when the samples are randomly distributed across species (within one of the two groups), significant differences in fiber lumen fraction are still possible. As such, this characteristic is not consistent enough for unambiguous timber identification of the discussed species, especially when insufficient material is present to study a representative fragment of the specimen.

DART-TOFMS analysis

For the DART-TOFMS analysis, the PCA plot containing all ions from the four species (Fig. 4) showed that *A. congolensis* and *B. toxisperma* formed distinctive clusters, while *T. africana* and *T. heckelii* grouped together in a third cluster. The DAPC model with the *Tieghemella* spp. making up a single class (Fig. 5) resulted in a LOOCV value of 96.61% and all test samples (*n* = 8) were correctly assigned, indicating that the three groups have chemotypes that are distinct enough to allow separation. Analysis of *T. Heckelii* and *T. africana* showed that the two species have remarkably similar chemotypes. This could be (1) due to misidentifications that occurred during field collection, (2) because the two species are closely related, or (3) because the two species are conspecific. The first hypothesis can only be true for samples collected
in Côte d’Ivoire. Although this country does not belong to the distribution range of *T. africana*, there have been some specimens reported to have been found there [23] and as such it could be confused in the field with *T. heckelii*. However, this would indicate a huge gap in distribution of *T. africana* and might be an argument for the case that these two *Tieghemella* species are one species. Our dataset included two *T. heckelii* samples (Tw22612 and Tw26511) collected in Côte d’Ivoire which were grouped in the PCA and correctly classified in the DAPC model with a LOOCV of 84.62% (Figure not shown). However, the LOOCV is likely increased due to the presence of replicate spectra and because of the limited sample size we cannot draw definitive conclusions.

While there were many similar ions between all species analyzed in this study, the ions found in both *T. heckelii* and *T. africana* at 434.316, 440.326 and 452.310 *m/z* were either missing in *A. congolensis* and *B. toxisperma* spectra or relatively low in intensity, allowing for the *Tieghemella* species to be differentiable from *A. congolensis* and *B. toxisperma*. While the PCA provides some support for the hypothesis that *T. africana* and *T. heckelii* are more closely related than previously considered, no definitive conclusion can be drawn without a larger number of samples from the respective species. Future research should solely focus on obtaining both vouchered and field collected samples of the two species in the *Tieghemella* genus. As was previously indicated, a taxonomic study would be beneficial to assess the status of the *Tieghemella* genus and the species limits [23–24], as our results suggest they could be conspecific. This would have important implications towards the timber trade, and adjoining timber species identification.

**Conclusion**

In this study we assessed the wood anatomical characteristic fiber lumen fraction and DART-TOFMS analysis for species differentiation of *Autranella congolensis*, *Baillonella toxisperma*, *Tieghemella africana* and *Tieghemella heckelii*. Based on visual assessment of the box-plots for the fiber lumen fraction measurements, two groups could be discerned: (1) *A. congolensis* and *B. toxisperma* and (2) *T. africana* and *T. heckelii*. In addition, all Mann-Whitney U comparisons and the differences in underlying distributions (Kolmogorov-Smirnov) for the fiber lumen fraction measurements were significant. However, when permutating our data within those two groups, significant differences based on the Mann-Whitney U test were still possible. This indicates that the differences based on the fiber lumen fraction are more nuanced, implying that fiber lumen fraction is not a consistent diagnostic characteristic for the identification of these four species. The chemotypes detected via DART-TOFMS of *A. congolensis* and *B. toxisperma* were distinct from each other and from those of *Tieghemella* spp., demonstrating that they can be identified by their chemotypes. Conversely, *Tieghemella heckelii* and *T. africana* have remarkably similar chemotypes that hinder species identification, though further taxonomic research is needed to assess whether they could be conspecific. Our study shows that chemical profiling can be used to reliably distinguish *A. congolensis*, *B. toxisperma* and *Tieghemella* spp. This has important implications as the ability to separate the members of taxonomically challenging groups, such as the Sapotaceae family, is of utmost importance to decrease the presence of illegally sourced wood within the timber trade.
DECLARATIONS

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests. The findings and conclusions in the article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service or of the U.S. Forest Service.

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Author’s contributions

HB and VD conceptualized the study design. KL and HB prepared the wood anatomical slices and KL and VD performed the wood anatomical analysis. EP and EE performed the DART-TOFMS analysis and interpreted the DART-TOFMS data. VD, EP and SVA were the major contributors in writing the manuscript. SVA was also major contributor in revising the manuscript and was also responsible for the taxonomic and genetic input for this study.

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Figures
Figure 1

Example of the fiber lumen fraction measurements. The fiber lumen fraction of the fiber is taken as the average of two measurements in perpendicular directions. Sample: Tw18005, Tieghemella heckelii.
Figure 2

Notched boxplots of the fiber lumen fraction for each of the species. The independent 2-group Mann-Whitney U test and Kolmogorov-Smirnov showed significant difference in mean and underlying distribution for each species comparison.
Figure 3

Heatmap showing the chemical fingerprint of the samples; each row indicates a single spectrum. The x-axis shows the m/z-value while the y-axis shows sample number; relative abundance of the ion is portrayed through intensity of color, where darker shades indicate a higher relative abundance within the sample. Vouchered specimens are indicated by arrows.

Figure 4
The scatterplot visualizing the Principal Component Analysis of mass spectra from the four Sapotaceae species. All species exhibited separate clustering trends with the exception of the Tieghemella spp.

![scatterplot](image)

**Figure 5**

Scatterplot of the DAPC model showing the variation between A. congoensis, B. toxisperma and Tieghemella spp. (LOOCV = 96.61%)

![comparison spectrum](image)

**Figure 6**

Comparison spectrum of T. heckelii and T. africana shows the similarities between the two species' spectra.

**Supplementary Files**

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- FigureS1.png
- S1SuppMat.docx