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Highlighting a New Morphospecies within the *Dialium* Genus Using Leaves and Wood Traits

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Abstract: During inventories of lesser-known timber species in eastern Gabon, a new *Dialium* morphospecies was discovered. To discriminate it from the two other 2–5 leaflets *Dialium* species, 25 leaf traits were measured on 45 trees (16 *Dialium pachyphyllum*, 14 *Dialium lopense*, 15 *Dialium* sp. nov.). Nine wood chemical traits, as well as infrared spectra, were also examined on harvestable trees (four *Dialium pachyphyllum* and four *Dialium* sp. nov.). This study revealed seven discriminant leaf traits that allowed to create a field identification key. Nine significant differences (five in sapwood and four in heartwood) in terms of wood composition were highlighted. The use of the PLS-DA technique on FT-IR wood spectra allowed to accurately identify the new morphospecies. These results provide strong support for describing a new species in this genus. Implications for sustainable management of its populations are also discussed.

Keywords: new species; leaves; morphology; wood; properties; chemical composition; spectroscopy; chemometrics; unconventional species

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1. Introduction

Despite efforts implemented by Central African countries in sustainable forest management, a progressive depletion of some timber flagship species has been observed [1,2]. This is mainly due to an extremely selective exploitation that focuses on a dozen species [3]. Most of them are light-demanding and logging impacts (canopy openness of less than 10% every cutting cycle of 25 years) are too small to positively influence their regeneration [4,5]. This depletion of species jeopardizes forest biodiversity and the future of productive forests.

The timber sector that covers 53 million ha (29 million with sustainable management plan) contributes significantly to employment and to the Gross National Product (GNP), 4% on average [3]. Apart from conservation areas, legal selective logging appears to be the least degrading use of forest ecosystems [6,7]. Moreover, some certified companies (totaling five million ha for the label Forest Stewardship Council, FSC [3]) go further by bringing significant improvement to life condition of workers, implementing Reduce Impact Logging (RIL) techniques, regulating hunting or enriching the forest [8–10]. By assigning an economic value to a forest, while having a limited impact on it, production forests can strengthen the network of 18 million hectares of protected area [6,7,11] and thus prevent land-use conversion.

Central African states plan to increase areas allocated to timber production and the intensity of logging by 50% by 2030 [3], with a an average logging intensity of 8.7–9.5 m^3/ha. In order to avoid depleting those forests, it is more necessary than ever to reduce the

pressure on traditional species and to redirect the exploitation towards lesser-known timber species [2,10,12] with: (i) a high wood quality, (ii) lower light requirements, and thus, a better regeneration than traditional light-demanding timber species.

Some species of the genus *Dialium* could meet such requirements [13]. They reach high densities in evergreen forests, have sustained regeneration, and very durable wood. However, the systematics of the genus remains unclear [14]. In the forests of central and eastern Gabon, two species of *Dialium* can reach large dimensions and could interest the logging industry: *Dialium pachyphyllum* Harms and *Dialium lopense* Breteler [14]. In the FSC-certified concession of Precious Woods operating in this region, a group of similar individuals appears to have differences in morphological traits from previously known species, especially on the leaves. [15]. Those morphological distinctions suggest the presence of an undescribed *Dialium* morphospecies [16].

The species identification issue is not a recent topic and many tree species continue to be discovered in Central Africa [17–19]. Recently, a species from the same genus, *Dialium heterophyllum* M. J. Falção & Mansano, was discovered in the South Amazonian Basin [20]. An accurate identification of species is crucial to understand their ecology and their timber properties. Therefore, the aim of this study is to evaluate the feasibility of differentiation between closely related species based on vegetative characteristics. More specifically, it aims to: (i) objectify morphological leave traits that discriminate the new morphospecies from the others, (ii) to determine chemical composition variation in the wood of harvestable species, and (iii) to verify whether this chemical composition, using Fourier Transformed Infrared (FT-IR) Spectra and chemometrics, can allow discriminating this new morphospecies.

2. Materials and Methods

2.1. Study Genus

Although there is no consensus among botanists, the *Dialium* genus (*Fabaceae*, *Dialioideae*) could include 44 species, 22 of which are endemic to the Guinean-Congolese region. The differences between species are sometimes tenuous, and descriptions are based on a limited number of individuals. All are hermaphroditic and dispersed by animals with abundant regeneration in the understory of evergreen forests [14]. Some species can reach 40 m in height and a meter in diameter. These species are generally grouped in Gabon under the name of "omvong" and typically have 3–5 leaflets. They have durable woods, resistant to fungi, termites, marine borers, and insects [13,21]. The wood can be used in parquetry, cabinetry, for exterior cladding, bridge construction and other heavy industrial uses [14].

2.2. Study Area

The study was performed in in the logging concession granted to Precious Woods-Compagnie Equatoriale des Bois A.S. (PW-CEB). This FSC-certified logging company is located in Bambidie, Lastoursville (Gabon) (0°41.65′ S–12°59.01′ E). The average temperature and precipitation are 25 °C and 1700 mm, respectively [22]. The forest is evergreen and its canopy is dominated by *Aucoumea klaineana* Pierre, *Scyphocephalium mannii* (Benth.) Warb., and *Julbernardia pellegriniana* Troupin. PW-CEB wishes to diversify its production to guarantee the maintenance of these activities in the long term. From this perspective, the possibility of valorizing *Dialium* wood has been analyzed. During field inventories, 3 morphospecies of "omvong" have been observed: *Dialium pachyphyllum*, *Dialium lopense*, and a new one *Dialium* sp. nov. All are present in the dynamic observation permanent plots set up by the Dynafac network (https://www.dynafac.org (accessed on 1 July 2022)).

2.3. Herbarium Collection and Description

Samples (Figure 1) of *D. pachyphyllum*, *D. lopense*, and *D.* sp. nov. were collected in the field. In addition, reference herbaria from the botanical garden of Meise (Belgium) were studied (Appendix A). They were selected based on the quality of their identification by botanists specialized in the *Fabaceae* family. A total of 45 individuals were analyzed,

including 29 individuals (diameter measured at 1.3 m, height >10 cm) collected in the field and 16 individuals from the Meise collection. For each sample, 25 leaf morphological traits were observed (Table 1). All traits were not systematically visible and/or available (e.g., absence of basal leaflet).

(**a**) (**b**) (**c**)

Figure 1. Field herbarium examples of *Dialium pachyphullum* Harms (**a**), *Dialium* sp. nov. (**b**) and *Dialium lopense* Breteler (**c**).

Table 1. Leaf morphological traits investigated.

Trait	Units/Modalities
Petiole type	"cylindrical"; "bulged"; "canaliculated"
Petiole length	mm
Rachis length	mm
Foliole number	-
Pilosity on the upper face	"pubescent"; "glabrous"
Pilosity on the above face	"pubescent"; "glabrous"
Curved margin	"yes"; "no"
Acumen shape	"oblong"; "elliptical"; "lanceolate"; "ovate"; "obovate"; "oblanceolate"; "falciform"
Asymmetric leaflet base	"yes"; "no"
Tertiary venation	"tight and prominent"; "discrete"
Translucent dots	"yes"; "no"
Terminal leaflet lamina shape	"no acumen"; "sharp"; "tapered"; "retuse"; "obtuse"; "truncated"; "emarginated"
Terminal leaflet lamina length	Mm
Terminal leaflet lamina width	mm
Basal leaflet lamina shape	"no acumen"; "sharp"; "tapered"; "retuse"; "obtuse"; "truncated"; "emarginated"
Basal leaflet lamina length	Mm
Basal leaflet lamina width	Mm
Coriaceous lamina	"yes"; "no"
Twisted lamina	"yes"; "no"
Petiolule length	mm
Ratio petiole/rachis	-
Ratio length/width of terminal leaflet	-
Ratio length/width of basal leaflet	-
Ratio basal leaflet length/terminal leaflet length	-
Ratio terminal leaflet length/petiolule length	-

2.4. Wood Material

For each of the studied species, *D. pachyphyllum* and *D.* sp. nov., four trees were cut following national regulations. Observed stems of *D. lopense* did not reach the legal minimum cutting diameter of 70 cm. Its wood properties were therefore not investigated. For each tree, altitude, floor slope, stem perimeter at the base of the log (measured at 1.3 m height when possible or above buttresses), position in the stand, and tree height were recorded (Table 2). Tree position in the stand was assessed from the ground. A tree with a completely shaded crown was considered as "dominated", a crown situated in the canopy and partially lit as "canopy", and a fully sunny crown above the canopy as "emergent". Wood disk samples, 10 cm thick, were collected at two different heights: at the bottom of the stem, corresponding to base of the log after felling and removing buttresses which are too important for the sawing or rotten heart (h1), and at the top of the stem corresponding to the first living branch (h2). To avoid sample contamination by fuel and oil of the chainsaw, wooden disk faces were planned in the society carpentry. Finally, 100 g of both sapwood and heartwood (taken at least 15 cm away from the pith and 10 cm away from sapwood, to avoid both juvenile wood and potential transition wood) were cut in each disk.

Table 2. Dendrometric and environmental parameters of selected trees. Position: Em = "Emergent" and Can = "Canopy"; altitude (m); slope (°); p is stem perimeter (m); hp is the height at which the perimeter was measured (m); h1 is the sampling height at the base of the stem (m); h2 is the sampling height at the top of the stem (m); ht is the total tree height (m).

Species	Tree	Position	Altitude	Slope	p	hp	h1	h2	ht
D. pachyphyllum	1	Em	369	0	3.30	2.2	1.3	12.0	43.0
	2	Can	358	5	3.10	3.1	1.6	10.2	41.0
	3	Em	360	0	2.90	6.1	2.0	17.1	42.0
	4	Em	365	0	2.86	1.3	1.7	14.0	46.5
D. sp. nov.	1	Can	348	0	3.60	5.1	2.7	18.9	43.0
	2	Em	343	0	2.60	3.3	1.9	19.6	39.0
	3	Em	347	30	2.65	2.4	2.5	21.0	42.0
	4	Can	345	0	2.55	2.8	1.8	16.7	44.5

2.5. Wood Sample Preparation

2.5.1. For Chemical Analyses and Moisture Content

Wood samples were first planed using an electric hand planer Black et Decker© DN 710 and shavings were collected by a dust collector. Shavings were then ground into <1 mm powder using a Fritsch *Pulverisette* universal cutting mill and stored at −18 °C before extraction. Moisture Content (MC) was assessed before each experiment by measuring the mass loss of 1 g of powder drying at 105 °C; this loss of mass was expressed as a percentage of initial mass.

2.5.2. For FTIR Measurements

For each of the 8 trees (4 *D. pachyphyllum* and 4 *D.* sp. nov.) described in Table 1, thin shavings were collected on 5 radially distributed heartwood strips (section 15 × 25 mm). Those strips were cut at the lower sampling height (h1). Shavings were produced using a Veritas© low-angle jack plane and a shooting board. Those thin shavings were then stored in a standard atmosphere of 20 ± 2 °C and 65 ± 5% air relative moisture.

2.6. Wood Primary Metabolites Content and Mineral

For each morphospecies, the variation of primary metabolites was investigated between heartwood and sapwood. Samples were collected at two heights h1 and h2 (Table 2). For each species, wood and sampling height combinations, experimental repetitions are

presented in Table 3. All metabolites are expressed as a percentage of wood dry matter (DM). DM was calculated by subtraction of MC.

Table 3. Experimental iterations on the four selected trees for each species and each modality combination of the studied variables, i.e., wood and sampling height.

	Experimental Repetition (n)				
Tree	**Hemicellulose**	**Cellulose**	**Lignin**	**Nitrogen**	**Ash**
1	2	2	2	3	3
2	2	2	2	3	3
3	2	2	2	-	-
4	2	2	2	-	-

2.6.1. Cellulose, Hemicellulose and Lignin Content

Fibers and lignin content were assessed following the Van Soest method [23–25] using a FOSS© FT 122 FiberTech tm. This method consists in successive matter treatments to remove sequentially soluble hemicellulose, cellulose, and lignin.

2.6.2. Ash Content

Ash content was determined as the mass residue of 1 g wood anhydrous powder after calcining. The calcining was processed according to [26]. It consisted in a first ramp from 21 °C to 575 °C within 2 h, including two temperature stages (102 °C for 12 min and 250 °C for 30 min). Then, a constant heating was processed at the reached temperature during 3 h.

2.6.3. Nitrogen Content

Wood nitrogen content was measured with the Kjeldahl method [27]. A hundred mg of wood powder was weighted on a Nitrogen-free paper and placed into the test tube FOSS Tecator digester for the mineralization. The digestion took place during 2 h, at 360 °C with 7.2 mL of concentrate H_2SO_4 and a Kjeltabs tablette (1.5 g K_2SO_4 + 0.045 g $CuSO_4.5H_2O$ + 0.045 g TiO_2). Tubes were then cooled and titrated with H_2SO_4 0.02 N in a FOSS© Kjeltec 2300.

2.6.4. Silica Content

The silica content was measured on 1 g of wood ash (see the Ash content protocol) by the gravimetric measure after hydrofluoric acid (HF) attack on $HClO_4/HNO_3$ acid-insoluble compounds [28]. Due to economic issues, this test was only applied on 3 samples (from 3 different trees) of sapwood and heartwood, at the lowest sample height (h1), for both *D.* sp. nov. and *D. pachyphyllum*.

2.7. Ethanol-Water Extracts Charaterisation

Extracts were prepared by maceration. After a preliminary test based on the Oreopoulou et al., (2019) review [29], the following extraction parameters were used: five grams of wood powder (W_M) (with a known MC) were incorporated into 100 mL of an ethanol-water (70–30, v/v) solution. Extraction was processed at 50 °C, using a rotary table for 2 h. Extracts were then vacuum filtered using a Büchner funnel and a Whatman© cellulose filter, 11 µm pore size. The ethanol was then evaporated using a rotary evaporator and the filtrate was finally freeze-dried and the dry mass of extracts was recorded (E_{DM}). The extraction yields were finally measured using E_{DM}. All extractions were processed once for each combination of variables modalities (species, heartwood and sapwood, and sampling height) for each tree.

2.7.1. Phenolic Content

The Phenolic Content (PC) was measured using the Folin–Ciocalteu method [30] with some modifications. The tested solution was prepared by dissolving 50 µg of dry extracts into 10 mL of a methanol-water (70–30, v/v) solution. A volume of 100 µL of the tested

solution was added into 500 µL of the Folin–Ciocalteau diluted in water (1:10, *v*/*v*). After 2 min, 2 mL of a 20% Na_2CO_3 solution was added. The mixture was then stirred and kept in a dark place at local temperature for 30 min. Finally, the mixture absorbance was recorded at 750 nm. The PC is expressed as mg gallic acid equivalent per g of dry extract (GAE). The GAE was obtained from a calibration curve of standard gallic acid dilution (from 0.5 g/L to 0.05 g/L). The PC determination was processed in triplicate for each extract.

2.7.2. Condensed Tannins Content

The Condensed Tannins Content (CTC) was measured using the Vanillin assay as mentioned by [31] with some modifications. The tested solution was prepared by dissolving 50 µg of dry extract into 10 mL of a methanol-water solution (70–30, *v*/*v*). In a cuvette, 500 µL of tested solution was added to 1500 µL of a vanillin-methanol solution (4–96, m–v). Then, 750 µL of HCl 37% was added. After stirring the mixture, the reaction took place in a dark place at 20 °C for 20 min. Finally, the absorbance of the solution was recorded at 550 nm. The CTC is expressed as mg catechin Equivalent per g of dry extract (CE). The CE was obtained from a calibration curve of standard catechin dilution (from 0.5 g/L to 0.05 g/L). The CTC determination was processed in triplicate for each extract.

2.8. FT-IR Acquisition

FT-IR spectra acquisition was processed using a Bruker VERTEX 70 paired to a Bruker Platinum Attenuated Total Reflectance (ATR) accessory. ATR principle is based on a reflectance measurement; then, roughly prepared samples such as wood transversal shavings can be used. These have been therefore directly pressed under the crystal diamond of the device for the scans. Spectra were obtained by averaging 32 scans with a resolution of 2 cm^{-1} in a range of 4000 cm^{-1} to 400 cm^{-1}. For each tested heartwood strip, 3 different wood shavings were scanned and considered as 3 repetitions. Spectra were then preprocessed using first a standard normal variate and, then, the first derivative with the Savitzky–Golay algorithm (polynomic order = 1; window = 7) using the *mdatools* package [32]. Regions of 800–1775 and 2810–3000 cm^{-1} were chosen to focus the analyses on relevant spectral information [33,34]. Average preprocessed spectra were calculated for each species and, then, *D.* sp. nov. spectra were subtracted from that of *D. pachyphyllum* to highlight the average differences between species.

2.9. Statistical Analyses

All statistical analyses and graphics were produced using RStudio (v 1.2.5001) [35]. Before the analyses, aberrant values (5 for lignin and 2 for hemicellulose) due to manipulation errors were removed from the datasets.

2.9.1. Conditional Inference Tree

To highlight most discriminant leaf morphological traits that allow morphospecies identification, the Conditional Inference Tree (CIT) was used (ctree function from the *partykit* package [36]). This recursive partitioning method consists in testing the independence of all explicative variables (25 measured leaf traits) and the response variable (species determination) at each partitioning step. The variable that had the strongest association to the response value, and an association test *p*-value lower than the selected alpha (0.05), was selected and the population was segmented. Each subpopulation was submitted to the same procedure until getting homogenous populations (null hypothesis of the independence test accepted for all variables in each subpopulation). To counteract the negative effect of the "multiple comparisons problem" due to inferences, the Bonferroni correction was systematically applied.

2.9.2. PLS-DA

The Partial Least Square Regression Discriminant Analysis (PLS-DA) was used to discriminate *D. pachyphyllum* and *D.* sp. nov. based on their wood shaving FT-IR spectra.

The outlier detection was carried out using Principal Component Analysis (PCA). Spectra were projected on PCs, score distance, and orthogonal distance were measured on the 5 first PCs accounting for 81.5% of the total variance. The region of acceptance proposed by [37] was used on distances and no outliers were found. Then, the dataset was divided into a training set (containing all preprocessed spectra from 3 trees of each species) and a validation set (containing the remaining spectra from the last trees of each species). Each set was composed by an explicative X matrix (FT-IR spectra) and a response Y matrix (species affiliation). To process PLS on a categorical Y, the matrix was first coded as dummy blocks. Then, using *plsda* and *perf* functions from the *mixomics* package [38], a PLS-DA model was fitted. The PLS-DA procedure aims to create a linear combination of variable from X, called Latent Variables (LVs), that maximize the covariance with Y. Scores of samples are calculated for those new variables and projected on the new LVs n-dimensional space, where n corresponds to the number of LVs. For each Y modality (*D.* sp. nov. and *D. pachyphyllum*) parameter (such as centroids) of the n-dimensional scores, a distribution is calculated. Using those parameters and a distance metric, it is possible to measure the distribution proximity of a new sample projected in the LVs space. Those distances to distributions are finally used to classify the samples. In this study, the Mahalanobis distance metric, which takes into account the shape of the distribution [39], was chosen. The optimal number of LVs to select (that explain most of the Y variance while avoiding overfitting) was assessed using a k-fold (fold = 5) cross-validation repeated a hundred times. This cross-validation processed tree operations for a hundred times: (i) five samples were randomly selected in the training set, (ii) the PLS-DA model was fitted on the remaining dataset for 20 LVs, (iii) the five samples were predicted by the model and the Overall Classification Error rate (OCE) is computed for each LVs of the model. After all repetitions, mean and standard deviation of OCE were measured for each LV. The lowest LV number that minimized the OCE was selected. During this procedure, the Variable Importance in Projection score (VIP score) confidence interval ($\alpha = 0.05$) was calculated from its distribution for each variable on each selected LV. This interval was used to verify whether variable VIP scores were significantly higher than 1, the commonly used threshold to assess the importance of a variable in prediction [40]. Within LVs, VIP scores' 95th percentile were also measured to identify variables that were situated among the 5% most important in the considered LV. After fitting the model on the training dataset, the validation set was predicted by it and a confusion matrix has been built. A confusion matrix allows to measure model accuracy and classes sensitivity/specificity.

2.9.3. Variance Analyses

Analysis of variance (ANOVA) was used to investigate leave trait differences between morphospecies but also the influence of wood (sapwood and heartwood) and sampling height (h1 and h2) on wood chemical properties. To confirm ANOVA preconditions, normality and homoscedasticity, the Ryan–Joiner and the Levene tests were used, respectively. If means were unequal, they were compared by using the post-HOC *t*-test (t). If the homoscedasticity precondition was not met, the non-parametric test of Kruskall–Wallis (χ^2) was used and medians were compared by using the Wilcoxson–Mann–Whitney (W) test. The selected α was 0.05 for all analyses excepted when variable modalities to compare were superior to two. In this case, the Bonferroni correction was applied to limit the family-wise error rate. Those analyses were conducted using the *rstatix* package [41].

3. Results

3.1. Leaf Morphological Traits

3.1.1. Conditional Inference Tree

The algorithm only selected qualitative variables for the CIT construction (Figure 2). The first partition, based on venation type (variable independence test: $p < 0.001$) allowed discriminating all 15 *D. pachyphyllum* individuals that had a discrete tertiary venation from the 25 other trees. The second partition, based on terminal leaflet's acumen shape

(variable independence test: $p = 0.024$) produced a pure node with only *D. lopense* and a node with 68% of *D.* sp. and 32% of *D. lopense*. All individuals that presented leaves with a tight, prominent tertiary nervation and an obtuse/no acumen can therefore be predicted as *D. lopense*. However, trees which presented leaves with tight, prominent tertiary venation and a sharp, tapered or retuse acumen had 68% probability to belong to the *D.* sp. and 32% to *D. lopense*.

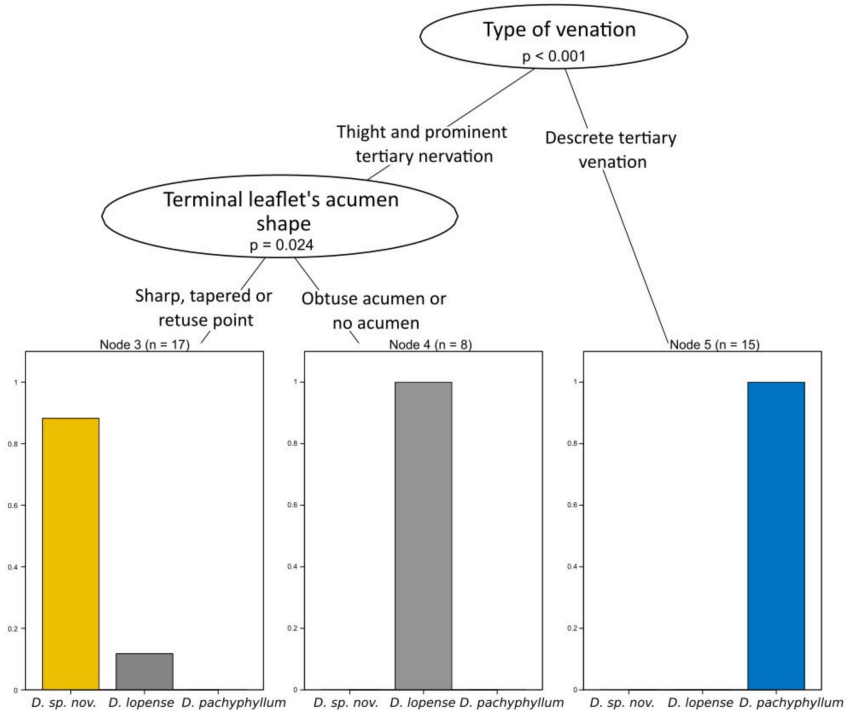

Figure 2. Conditional inference tree using the 25 leaf morphological variables to discriminate the 40 individuals for which all leaf traits could be measured.

3.1.2. Variance Analyses

Variance analyses on quantitative variables highlighted many significant differences between *D. lopense* and *D.* sp. nov. (Figure 3). The mean width/length ratio of the basal leaflet was the most significant (t = 3.31, $p = 0.0043$), with 0.54 ± 0.08 and 0.45 ± 0.06 for *D. lopense* and *D.* sp. nov., respectively. The mean terminal leaflet width also differed (t = 2.96, $p = 0.0073$) between the two morphospecies, 51.93 ± 13.48 mm and 39.4 ± 8.63 mm respectively. The mean width/length ratio on terminal leaflet is higher (t = 2.64, $p = 0.015$) for *D. lopense* (0.44 ± 0.084) than for *D.* sp. nov. (0.37 ± 0.05). The median petiole/rachis ratio is lower (W = 38, $p = 0.01$) for *D. lopense* (0.25 ± 0.24) than for *D.* sp. nov. (0.49 ± 0.44). The mean length of terminal petiolule/length of terminal leaflet ratio is lower (t= −2.68, $p = 0.012$) for *D. lopense* (0.06 ± 0.02) than for *D.* sp. nov. (0.07 ± 0.02).

D. pachyphyllum only differed significantly (W = 59.5, $p = 0.018$) from *D.* sp. nov. based on a lower petiole/rachis ratio median (0.22 ±0.18 for *D. pachyphyllum*). It however varied from *D. lopense* in two traits. *D. pachyphyllum* had a higher (t = −2.24, $p = 0.034$) mean length of the terminal petiolule/length of terminal leaflet ratio (0.072 ± 0.022 and 0.057 ± 0.016, respectively, for *D. pachyphyllum* and *D. lopense*) and a higher (W = 35, $p = 0.013$) basal leaflet length/terminal leaflet length ratio median (0.67 ± 0.11 and 0.58 ± 0.08, respectively, for *D. pachyphyllum* and *D. lopense*).

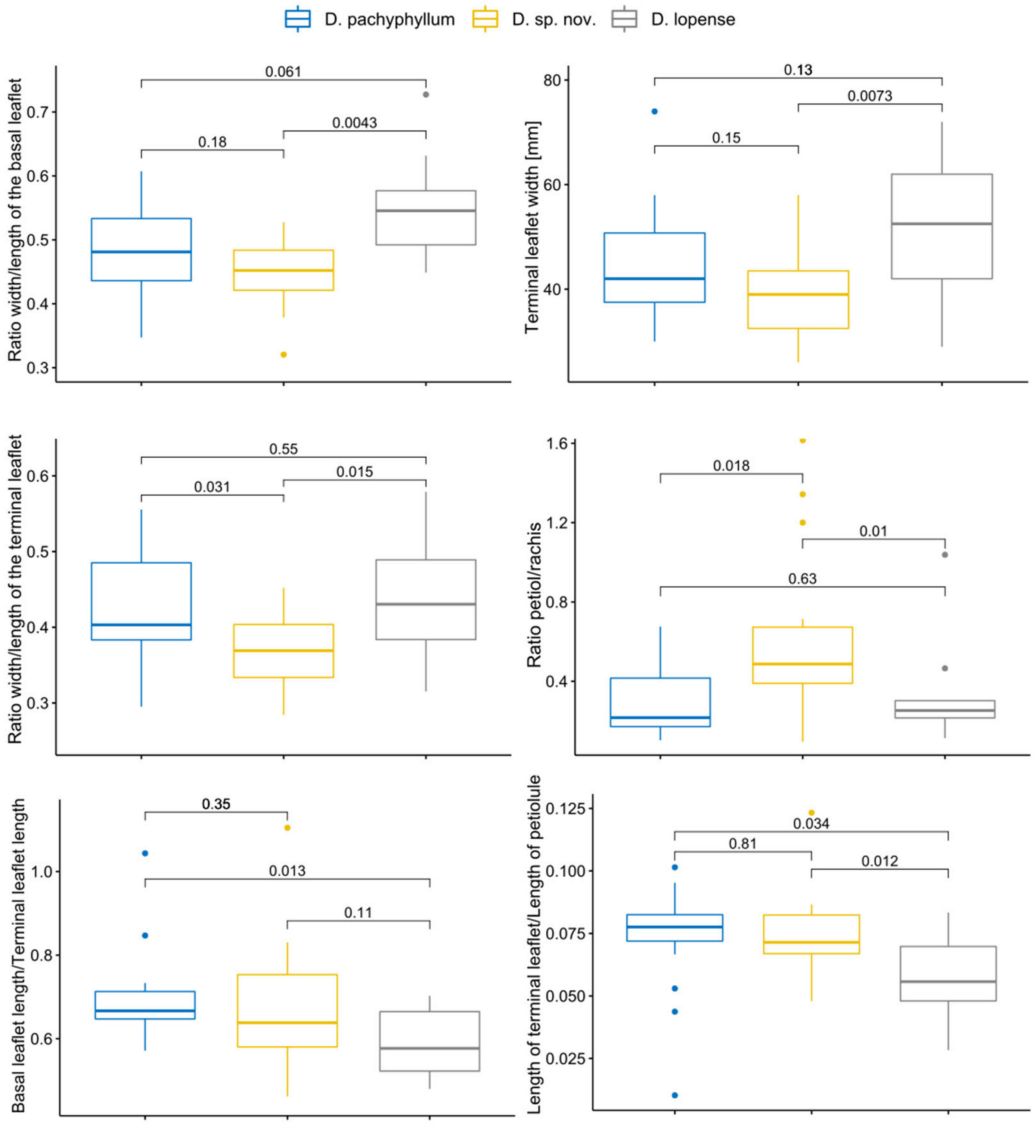

Figure 3. Significant leaf morphological trait variations between *Dialium pachyphyllum*, *Dialium* sp. nov., and *Dialium lopense*. *p*-values for species comparison are related to *t*-tests (ratio *w*/*l* of the basal leaflet, terminal leaflet width, ratio length of terminal leaflet/length of petiolule) and Wilcoxon tests (ratio petiol/rachis, ratio basal leaflet length/terminal leaflet length).

3.2. Chemical Composition

3.2.1. Primary Metabolites and Minerals

No significant effect of the sampling height was observed on primary metabolites. The mean cellulose content significantly differed (F = 3.56, $p = 0.0014$) between sapwood of *D. pachyphyllum* (55.4 ± 2.98%DM) and *D.* sp. nov. (52.0 ± 2.52%DM). The mean lignin content was significantly different between heartwood and sapwood (F = 72.3, $p < 0.001$) with a higher content in heartwood (27.9 ± 2.01%DM) than sapwood (23.5 ± 1.93%DM). As for lignin, the mean hemicellulose content was only significantly different between heartwood and sapwood (F = 54.4, $p < 0.001$). However, heartwood had a lower hemicellulose content (12.4 ± 1.14%DM) than sapwood (14.9 ± 1.4%DM).

The median ash content was significantly different ($\chi^2 = 25.9$, $p < 0.001$) between species (2.46 ± 0.78%DM and 0.90 ± 0.282%DM for *D.* sp. nov. and *D. pachyphyllum*, respectively). The median nitrogen content was significantly ($\chi^2 = 25.9$, $p < 0.001$) different within sapwood (0.28 ± 0.04%DM) and heartwood (0.35 ± 0.05%DM). The median of this metabolite also significantly differed ($\chi^2 = 6.65$, $p = 0.009$) between species: 0.31 ± 0.04%DM

for *D. pachyphyllum* and 0.35 ± 0.07 for *D.* sp. nov. However, this difference between species was due to samples from heartwood for which species differentiation (0.32 ± 0.03%DM for *D. pachyphyllum* and 0.39 ± 0.05%DM for *D.* sp. nov.) was very significant (W = −4.53, $p < 0.001$) unlike sapwood (Figure 4).

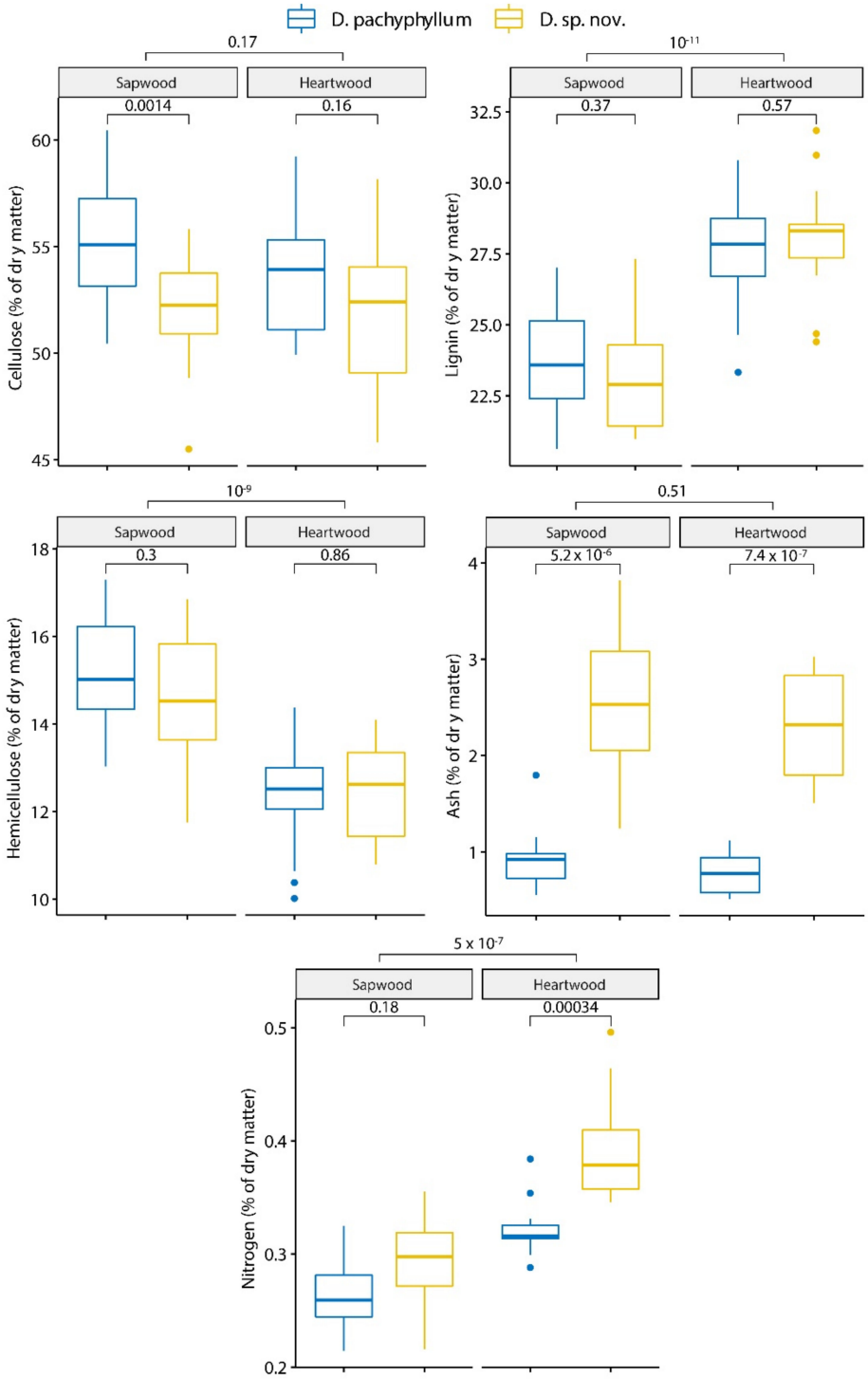

Figure 4. Primary metabolite's variation in heartwood and sapwood among the different studied species; *p*-values concerning heartwood and sapwood are related to ANOVA test (Cellulose, Lignin and Hemicellulose) and Kruskal–Wallis test (Ash and Nitrogen); *p*-values that compare species are related to the *t*-test (Cellulose, Lignin and Hemicellulose) and Wilcoxon's test (Ash and Nitrogen).

The mean silicate content was higher in *D.* sp. nov. (2.42 ± 0.57%DM and 2.64 ± 0.59%DM for sapwood and heartwood, respectively) than *D. pachyphyllum* (1.23 ± 0.39%DM and 0.92 ± 0.22%DM for sapwood and heartwood, respectively).

3.2.2. Ethanol Extracts

Median extraction yields differed significantly (W = 698, $p < 0.001$) with the sampling height. Samples from the top of the stem had higher extraction yields (2.6 ± 1.0%DM) than samples at the bottom (2.2 ± 0.6%DM). *D.* sp. nov. sapwood (2.7 ± 0.7%DM) had a higher extraction yield (W = 158, $p = 0.007$) than *D. pachyphyllum* sapwood (2.13 ± 0.36%DM).

The mean PC was significantly affected by the three studied variables: for sampling height (F = 5.0, $p = 0.028$), for heartwood and sapwood (F = 77.4, $p < 0.001$), and for the species (F = 37.5, $p < 0.001$). The mean PC was lower at the lower sampling height (330 ± 84 GAE) than top of the stem (359 ± 11 GAE). The mean PC was higher for heartwood (402 ± 82 GAE) than sapwood (280 ± 72 GAE). Finally, it was lower for *D. pachyphyllum* (304 ± 93 GAE) than *D.* sp. nov. (384 ± 82 GAE). Figure 5 emphasizes the significant difference of mean PC between the two species for both sapwood (253 ± 72 GAE for *D. pachyphyllum*; 320 ± 57 GAE for *D.* sp. nov.) and heartwood (355 ± 83 GAE for *D. pachyphyllum*; 448 ± 4.6 GAE for *D.* sp. nov.).

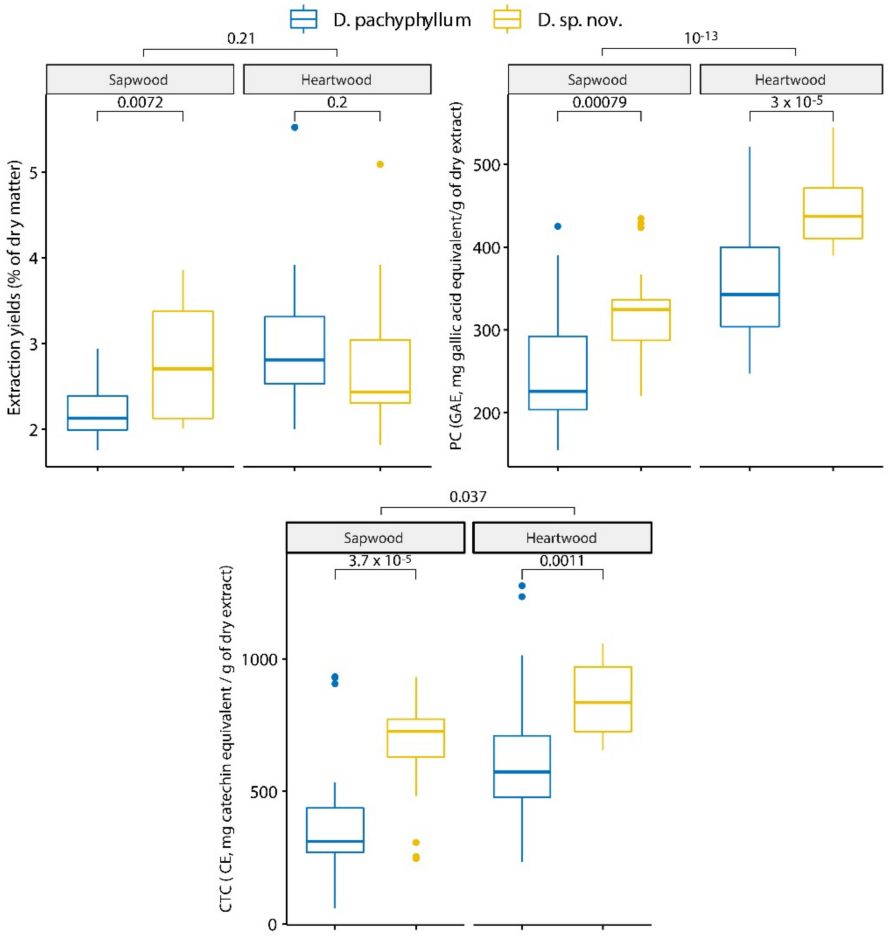

Figure 5. Extract yields and extract composition variation in heartwood and sapwood among the different studied species. *p*-values concerning heartwood and sapwood are related to ANOVA test (Phenolic content and Condensed tannin content) and Kruskal–Wallis test (Extraction yields); *p*-values that compare species are related to the *t*-test (Phenolic content and Condensed tannin content) and Wilcoxon's test (Extraction yields).

The three variables also induced a significant variation of mean CTC: for sampling height (F = 4.5, $p = 0.037$), for heartwood and sapwood (F = 22.3, $p < 0.001$), and for species

(F = 35.3, $p < 0.001$). The same PC variation pattern was observed: the mean CTC was lower at the lower sampling height (585 ± 265 CE and 677 ± 275 CE for lower and higher sampling height, respectively), higher for the heartwood (733 ± 248 CE and 528 ± 259 CE for heartwood and sapwood, respectively), and lower for *D. pachyphyllum* (502 ± 287 CE and 760 ± 185 CE for *D. pachyphyllum* and *D.* sp. nov., respectively). Figure 5 highlights the difference of mean CTC between the two species for both sapwood (385 ± 241 CE for *D. pachyphyllum*; 672 ± 190 CE for *D.* sp. nov.) and heartwood (619 ± 285 CE for *D. pachyphyllum*; 848 ± 132 CE for *D.* sp. nov.).

3.2.3. Wood FT-IR Distinction

Average preprocessed spectra for each species are presented in Figure 6. The spectral differences (Δ) highlight 15 wavenumbers that present high peaks. The PLS-DA model was fitted using 7 LVs which reached an average OCE of 2% during the cross-validation procedure (Figure 7). After predicting the validation set, composed with an independent tree of each species, the accuracy (29/30) of 96.6% is close to cross-validation (Table 4). The sensitivity and specificity of *D. pachyphyllum* are, respectively, 93.8% and 100%. For *D.* sp. nov., specificity and sensitivity are 100% and 87.5%, respectively. Those results highlight a high accuracy of the model and a low risk of predicting *D.* sp. nov. as *D. pachyphyllum*.

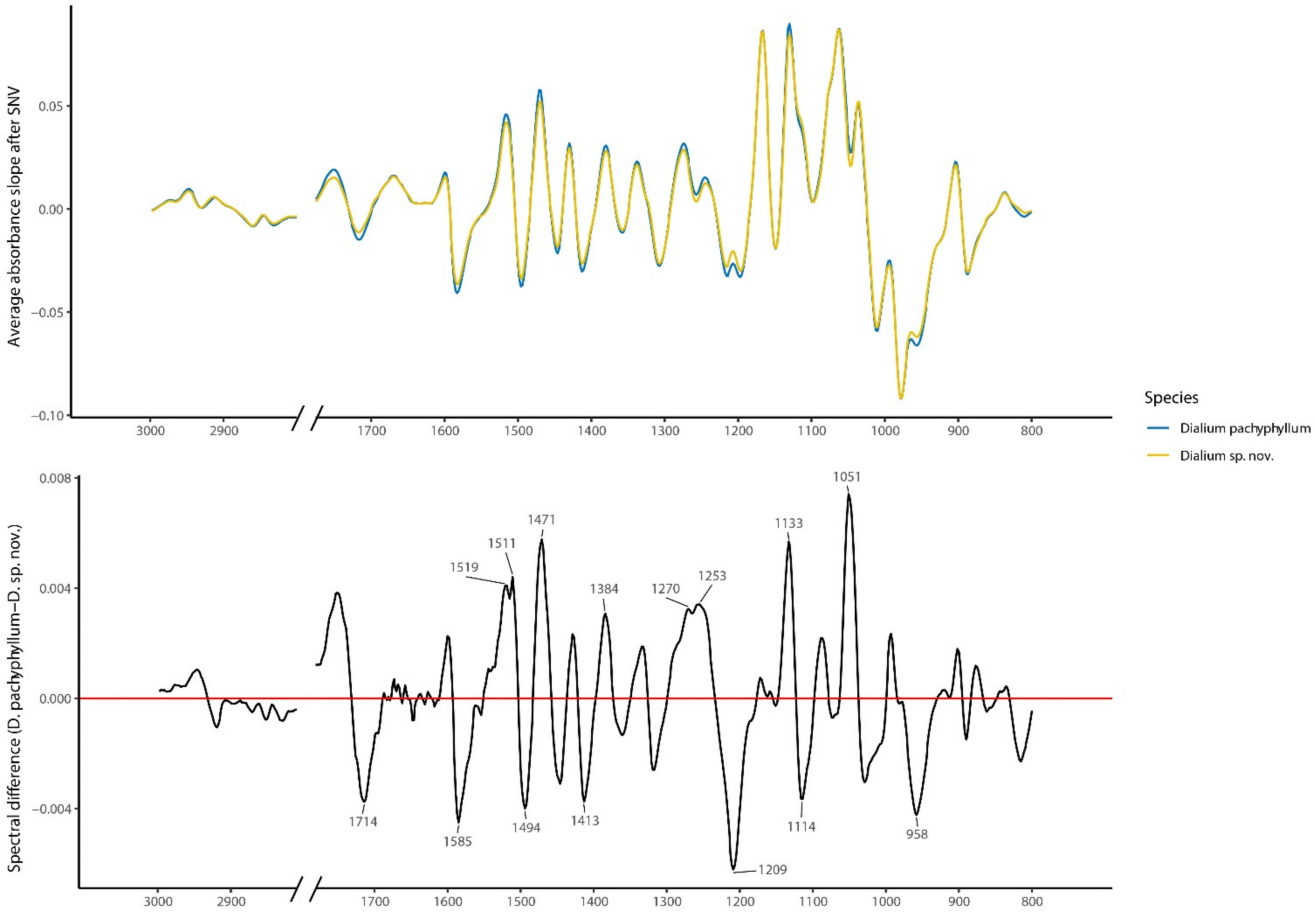

Figure 6. Upper plot presents average preprocessed (SNV + first derivatitive) spectrum of each species. The lower graph represents the difference (*D. pachyphyllum-D.* sp. nov.) between the average spectra. The 15 wavenumbers correspond to 15 high peaks of difference between the species.

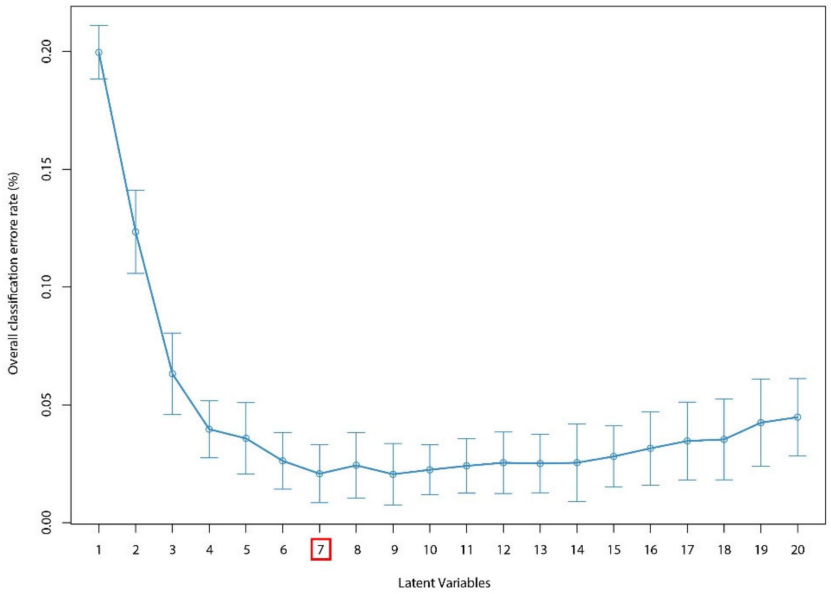

Figure 7. Overall classification error rate from the cross-validation procedure. The number of LVs selected is red-circled.

Table 4. Confusion matrix obtained by predicting the independent validation dataset.

		D. pachyphyllum	***D.* sp. nov.**
Predicted	***D. pachyphyllum***	15	0
	***D.* sp. nov.**	1	14

Table 5 compares the wavenumbers identified by the spectral difference (Δ) and their importance in the prediction model. The 1051 cm^{-1} shows the largest peak difference and reaches the top 5% of VIP scores for each LV. This variable therefore highly participated to species distinction. According to the literature, this band can be attributed to the C-H stretching bond from tannins (*Terminalia chebula* (Gaertner) Retz., *Caesalpinia spinosa* (Molina) Kuntze, and *Schinopsis lorentzii* Engl. tannin extracts in their study [42]). The 1114 cm^{-1} peak, although not the highest Δ, reaches top 5% VIP scores of 6 LVs and strongly contributes to the predictive model. As for the previous band, this wavenumber can be attributed to tannins (C-H bending observed in *S. lorentzii* and *Castanea sativa* Mill. extracts [42]). In opposition, the 1253 cm^{-1} and 1270 cm^{-1} peaks did not show VIP scores significantly higher than 1, except for LV1 and 2, respectively. Those wavenumbers, assigned to lignin bonds, are not important in the discriminant model. Wavenumbers from 1714 cm^{-1} to 1511 cm^{-1} were assigned to bonds in lignin or phenols. Except the 1511 cm^{-1} band for LV5 and LV6, this range presents a significant importance in species differentiation. Peaks at 1413, 1471, and 1494 cm^{-1} had VIP scores higher than 1 and reached the top 5% for 1413 cm^{-1} on LV1 and 1471 cm^{-1} on LV1 and LV2. Those bands are associated to lignin bonds. The 1209 band, assigned to a cellulose bond, has a high Δ and a significant importance in all LVs. The two last bands investigated, 1133 and 958 cm^{-1}, are less important in the model (VIP scores around 1 or not higher) while presenting a medium Δ.

Table 5. Wavenumbers (W), expressed in cm^{-1}, that present 15 large peak differences ($\times 10^{-3}$) between average species spectrum (Δ); Mean VIP scores from of PLS-DA model, shaded scores are upper to the 95th percentile of vip score within the LV, scores with [n] are not significantly higher than 1 according to the confidence interval from the cross-validation process; Bond: chemical bond assignement to the wavenumber; Mol corresponds to the molecular class in which the chemical bonds are found (L = Lignin, H = Hemicellulose, C = Cellulose, P = Polyphenol, T = Tannin); Ref. corresponds to references that mention the bond assignment.

W	Δ	VIP Scores							Chemical Bond Assignment	Mol	Ref.
		LV1	LV2	LV3	LV4	LV5	LV6	LV7			
1714	38	1.47	1.19	1.14	1.12	1.10	1.08	1.09	**1712 cm^{-1}:** C=O groups	L	[43]
1585	45	1.62	1.28	1.25	1.22	1.21	1.20	1.18	**1585 cm^{-1}:** Aromatic ring stretching	L	[44]
1519	41	1.56	1.29	1.22	1.20	1.19	1.16	1.15	**1517 cm^{-1}:** Aromatic skeletal vibration	L	[43]
1511	44	1.37	1.13	1.07	1.05	1.04 [n]	1.02 [n]	1.05	**1508–1513 cm^{-1}:** Aromatic skeletal vibration	L, T	[42,43]
1494	40	1.60	1.31	1.24	1.23	1.22	1.19	1.19	**1495 cm^{-1}:** aromatic vibration	P, T	[45]
1471	58	1.77	1.40	1.33	1.32	1.30	1.28	1.26	**1460–1470 cm^{-1}:** C-H vibration	L	[46,47]
1413	83	1.93	1.53	1.46	1.43	1.41	1.38	1.37	**1413 cm^{-1}:** C=C aromatic	L	[48]
1384	31	1.49	1.20	1.14	1.13	1.14	1.11	1.10	**1384 cm^{-1}:** -OH bending of phenolic bond	L	[49]
1270	32	1.09 [n]	1.14	1.09	1.07	1.05 [n]	1.03 [n]	1.01 [n]	**1265 cm^{-1}:** C-O vibration	L	[50,51]
1253	33	1.07	0.89 [n]	0.85 [n]	0.84 [n]	0.83 [n]	0.82 [n]	0.81 [n]	**1250 cm^{-1}:** C-O-C asymmetric stretch	L	[34]
1209	62	1.50	1.21	1.15	1.13	1.11	1.10	1.09	**1203–1210 cm^{-1}:** O-H bending	C	[52]
1133	57	1.18	0.94 [n]	1.07 [n]	1.11	1.10	1.09	1.08	**1134 cm^{-1}:** C-O-C glycosidic vibration of xylan	H	[50]
1114	37	1.54	1.64	1.63	1.62	1.60	1.56	1.54	**1112–1113 cm^{-1}:** C-H bending in plane	T	[42]
1051	98	1.73	1.64	1.56	1.53	1.51	1.50	1.48	**1050 cm^{-1}:** C-H stretching in plane	T	[42]
958	42	1.33	1.12 [n]	1.12	1.13	1.11	1.09	1.08 [n]	**957–961 cm^{-1}:** C-H aromatic out of plane deformation	-	[45]

4. Discussion

4.1. Species Distinction in Forest Inventories

Species discrimination criteria based on leaf morphology is frequently used in the *Fabaceae* family [53,54] and, particularly in the *Dialium* genus [55]. Furthermore, during field surveys, reproductive characteristics are generally not visible and only trunks and leaves can be observed. Leaves in *Dialium* can contribute to distinguishing species from each other [56]. For example, distinction of *Dialium heterophyllum* from other species in the Amazonian Basin is done by its reduced rachis and unifoliate to trifoliate leaves [20]. Using the 7 significant leaf traits to classify this species seems therefore relevant. Table 6 presents the identification key for the 3–5 leaflets *Dialium* species that occur in the study area.

4.2. Wood Chemical Properties and Their Variation

4.2.1. Primary Metabolites and Minerals

As Mbagou (2017) already observed, sampling height has no influence on structural primary metabolites [57]. However, many differences can be observed considering the radial position of the sample. Indeed, considering both species, the lignin content was 4.4%DM higher in heartwood than sapwood. This pattern was already observed by many authors [58–60]. Hemicellulose varies oppositely and, considering both species, is on average 2.5%DM lower in heartwood than sapwood. The difference is less pronounced but remains encountered in the literature [60].

Table 6. Identification key for 3–5 leaflets *Dialium* species present in PW-CEB forests; on the left side of the table, the sections with the observable traits are presented; on the right side is either the species classification corresponding to the observed trait, or the reference of the section to continue the identification key; the range of values presented for the quantitative variables, in the third section, corresponds to the interval in which 50% of the observations made during this study occurs for each species.

1.	- Tight and prominent tertiary venation - Discrete tertiary venation		2. *D. pachyphyllum*
2.	- Sharp, tapered or retuse terminal leaflet acumen - No terminal leaflet acumen or obtuse acumen		3. *D. lopense*
3.	- Ratio width/length of the basal leaflet: [0.42–0.48] - Terminal leaflet width: [33–44] mm - Ratio width/length of the terminal leaflet: [0.33–0.40] - Ratio petiole/rachis: [0.39–0.67] - Length of terminal leaflet/length of petiolule: [0.06–0.08]		*D.* sp. nov.
	- Ratio width/length of the basal leaflet: [0.49–0.57] - Terminal leaflet width: [42–62] mm - Ratio width/length of the terminal leaflet: [0.38–0.49] - Ratio petiole/rachis: [0.22–0.30] - Length of terminal leaflet/length of petiolule: [0.05–0.07]		*D. lopense*

The highest cellulose content (55.4%DM) was obtained from the sapwood of *D. pachyphyllum* which appears to reach higher values than generally observed in African tropical woods. According to Gérard et al., (2019), the average is 42.2%DM, and the maximum observed is 58.1%DM [28]. For instance, *Aucoumea klaineana* present a cellulose content of 46.1%DM [61], *Lophira alata* Banks ex C.F. Gaertn. of 40.0%DM [28]. This difference could be explained by the cellulose determination method. Heartwood lignin content (27.9%DM in average) did not significantly differ between species and was a bit lower than in other tropical woods for both species. Gérard et al., (2019) measured an average lignin content of 29.2%DM for 549 tropical species tested [28]. Nuopponen et al., (2006) reported that the lignin content of tropical hardwoods can exceed that of softwoods and reach between 29%DM and 41%DM [62]. Hemicellulose did not differ between species. Heartwood hemicellulose content seems comparable to the average pentosane content (major constituent of hemicellulose) for tropical hardwood of 15.9%DM [28]. Ash content was significantly higher in *D.* sp. nov. which had a high value (2.46%DM) compared to other tropical species that, on average, have 1.1%DM [28]. Concerning nitrogen content, *D.* sp. nov. heartwood has the highest value which is almost twice the average of 0.24%DM observed for 59 Panamanian tree species [63]. Results about silica content follow the same pattern. *D.* sp. nov. has twice more silica content in sapwood (2.42%DM) and nearly three times more in heartwood (2.64%DM) than *D. pachyphyllum*. Both species had a very high silica content according to the 0.1%DM average silica content of 599 hardwoods [28].

4.2.2. Ethanol-Water Extracts

Extract yields were higher at the top of the stem for both species. Gérardin et al., (2020) also obtained higher yields for water-ethanol extracts at the top of the trees [64]. Concerning species distinction, extracts yields were higher in *D.* sp. nov. sapwood. However, using the same extraction method, several authors obtained higher yields than observed for both species. Saha Tchinda (2015), using hot water as solvent, obtained 8.4%DM from *Baillonella toxisperma* Pierre heartwood and 3.8%DM for *Distemonanthus benthamianus* Baill. heartwood. PC and CTC are significantly higher in the heartwood than sapwood. Those results are probably species-dependent since Engozogho et al., (2020) reported higher polyphenol and tannin levels in sapwood (2 ± 0.8%DM) than heartwood (0.7 ± 0.1%DM) of *Aucoumea*

klaineana, whereas Mbagou et al., (2017) bring out significant higher PC in heartwood than sapwood for *Tessmania Africana* Harms, and Bikoro et al., (2018) had significantly more PC in sapwood but significantly more CTC in heartwood of *Khaya ivorensis* A. Chev. [65]. Considering species variation, *D.* sp. nov. has higher PC and CTC than *D. pachyphyllum*. According to Nisca et al., (2021), those metabolite are interesting to discriminate species [66]. *D.* sp. nov. heartwood PC is comparable to *Pterocarpus soyauxii* Hooker acetone extract (430 GAE) and both species have higher PC than *Baillonella toxisperma* for the acetone extract [67].

4.3. FT-IR Wood Distinction

The high accuracy of the PLS-DA model (96.6%) on two independent trees of the training set added to the significant differences in analytical metabolite measurements, and proves the difference in wood composition between the two species. As already highlighted by Wang et al., (2016), spectroscopy is an adapted tool to discriminate closely related species as *Dalbergia* spp. [34]. However, the interpretation of the spectra without the use of statistical techniques can be hazardous. Indeed, as it has been shown, the peak differences observed on the average spectra are not always associated to discriminant bands. This can come from a high intra-species variation being greater than inter-species variation. Knowing this, it is likely that the peaks at 1270 and 1253 cm^{-1}, despite a high Δ, present a large inter-species variation that do not allow discrimination. The peak at 1114 cm^{-1} assigned to tannin bonds, has the same delta but its importance in the predictive model might be due to low intra-species variations. The higher Δ peak at 1051 cm^{-1} is the most interesting because it represents the greatest average difference between the species and participates very strongly in the PLS-DA model. Added to the significant difference in CTC between *Dialium* species, those results are in lines with Nisca et al., (2021) to highlight the efficiency of this class of molecule in the distinction of closely related species.

4.4. Impacts on Tree Ecology, Forest Management, and Wood Production

Leaf traits are highly correlated to tree ecology [68]. For instance, venation density significantly influences water management [69] and, therefore, tree light strategy. According to Martin A. et al., (2014), wood nitrogen content is negatively correlated to the relative growth rate and higher values could correspond to late successional species [63]. As those two traits significantly discriminate the studied *Dialium* species, their ecology might differ. Consequently, *D.* sp. nov. should be discriminated in annual monitoring plots on the study site and its population dynamic should be separately modelled from the other species. Once population dynamic parameters will be known, management practices should be adapted. Moreover, before any valuation of the wood, the conservation status of the species, in relation to its distribution area, should be specified.

Wood properties are directly related to chemical composition [70]. The cellulose proportion variation between *D.* sp. nov. and *D. pachyphyllum* could therefore lead to differences in mechanical properties [71]. The silica content increases resistance to marine borers [72] while PC, CTC, and suberin increase durability against fungi [73–75]. However, silica content is highly abrasive and increases sawing difficulties [14]. To know if the species should be promoted to a different timber sector, it will be interesting to check whether the difference in chemical properties induces a significant difference in wood properties and processing.

Dialium pachyphyllum has a wide distribution in Central Africa while *Dialium lopense* is restricted to Gabon [14]. As this new species has not yet been described, its area of occurrence as well as its exploitation potential are not known. If those are restricted, it may be interesting to control the production of this species. This control can be done through customs checks before export. In view of the ease of preparation of shavings chips and the accuracy of the model, these techniques could be used routinely to identify wood packages. However, this requires a large increase in the number of individuals to train the model to

capture inter-specific variation. In addition, the model should favor a sensibility of 100% for *D.* sp. nov. to avoid classifying the samples into another species.

5. Conclusions

This study brought out many results that support the existence of a new *Dialium* species. Using 7 significant leaf traits to discriminate *D.* sp. nov. from other 3–5 *Dialium* leaflets, an identification key was proposed for forest inventories. A total of nine significant differences in chemicals properties were highlighted (five in sapwood and four in heartwood). The PLS-DA model trained on heartwood shavings FT-IR spectra accurately discriminate *D.* sp. nov. from *D. pachyphyllum*. Those outcomes may have consequences on the newly discovered morphospecies ecology, population dynamic, wood properties, and timber production potential. It is therefore advisable to investigate those aspects along with the study of its reproductive system before considering its large-scale logging.

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Data Availability Statement: Datasets will be deposited on DRYAD after paper acceptance.

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Conflicts of Interest: The work is all original research carried out by the authors. All authors agree with the content of the manuscript and its submission to the journal. No part of the research has been submitted nor published in any form in this journal or elsewhere. The manuscript is not being considered for publication elsewhere while it is being considered for publication in this journal. All sources of funding are acknowledged in the manuscript, and authors have no financial benefits that could result from publication.

Appendix A

Table A1. Herbarium information. Herbarium reference that mentions the "BR00000" prefix comes from the botanical garden of Meise (Belgium), "field" corresponds to the herbarium collected in PW-CEB forest for this study.

Harvester	Herbaria Reference	Date	Identification	Country
de Wilde	BR0000016564503	19-12-96	*Dialium lopense*	Gabon
Breteler	BR0000016564534	23-01-99	*Dialium lopense*	Gabon
Wieringa	BR0000016564589	17-11-94	*Dialium lopense*	Gabon
Wieringa	BR0000016564572	02-04-04	*Dialium lopense*	Gabon
Breteler	BR0000016564527	05-02-99	*Dialium lopense*	Gabon
Breteler	BR0000016564541	17-02-99	*Dialium lopense*	Gabon
Doucet J-L	BR0000016564558	01-05-96	*Dialium lopense*	Gabon
Wilk	BR0000016564565	17-05-87	*Dialium lopense*	Gabon
McPherson	BR0000016564596	22-11-93	*Dialium lopense*	Gabon
McPherson	BR0000016564602	18-05-92	*Dialium lopense*	Gabon
McPherson	BR0000016564626	15-05-92	*Dialium lopense*	Gabon
Bibang & Doucet J-L.	field	30-03-21	*Dialium lopense*	Gabon
Doucet R.	field	17-06-21	*Dialium lopense*	Gabon
Doucet R.	field	May 2019	*Dialium lopense*	Gabon
McPherson	BR0000013636012	04-05-92	*Dialium pachyphyllum*	DRC
Tshibamba	BR0000013639617	01-07-11	*Dialium pachyphyllum*	DRC
van de Burgt	BR0000013639631	12-09-11	*Dialium pachyphyllum*	Gabon
Wieringa	BR0000013635824	16-11-94	*Dialium pachyphyllum*	Gabon
Wieringa	BR0000013635916	31-10-03	*Dialium pachyphyllum*	Gabon
Doucet R.	field	May 2019	*Dialium pachyphyllum*	Gabon
Doucet R.	field	May 2019	*Dialium pachyphyllum*	Gabon
Doucet R.	field	16-06-21	*Dialium pachyphyllum*	Gabon
Doucet R.	field	16-06-21	*Dialium pachyphyllum*	Gabon
Doucet R.	field	16-06-21	*Dialium pachyphyllum*	Gabon
Doucet R.	field	16-06-21	*Dialium pachyphyllum*	Gabon
Doucet R.	field	17-06-21	*Dialium pachyphyllum*	Gabon
Doucet R.	field	17-06-21	*Dialium pachyphyllum*	Gabon
Doucet R.	field	17-06-21	*Dialium pachyphyllum*	Gabon
Doucet R.	field	19-06-21	*Dialium pachyphyllum*	Gabon
Doucet R.	field	19-06-21	*Dialium pachyphyllum*	Gabon
Bibang & Doucet J-L.	field	30-03-21	*Dialium* sp. nov.	Gabon
Bibang & Doucet J-L.	field	30-03-21	*Dialium* sp. nov.	Gabon
Bibang & Doucet J-L.	field	31-03-21	*Dialium* sp. nov.	Gabon
Bibang & Doucet J-L.	field	31-03-21	*Dialium* sp. nov.	Gabon
Bibang & Doucet J-L.	field	31-03-21	*Dialium* sp. nov.	Gabon

Table A1. *Cont.*

Harvester	Herbaria Reference	Date	Identification	Country
Bibang & Doucet J-L.	field	31-03-21	*Dialium* sp. nov.	Gabon
Bibang & Doucet J-L.	field	31-03-21	*Dialium* sp. nov.	Gabon
Doucet R.	field	May 2019	*Dialium* sp. nov.	Gabon
Doucet R.	field	May 2019	*Dialium* sp. nov.	Gabon
Doucet R.	field	May 2019	*Dialium* sp. nov.	Gabon
Doucet R.	field	May 2019	*Dialium* sp. nov.	Gabon
Doucet R.	field	17-06-21	*Dialium* sp. nov.	Gabon
Doucet R.	field	19-06-21	*Dialium* sp. nov.	Gabon
Doucet R.	field	19-06-21	*Dialium* sp. nov.	Gabon
Doucet R.	field	19-06-21	*Dialium* sp. nov.	Gabon

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