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GROWTH-RING DISTINCTNESS AND BOUNDARY ANATOMY VARIABILITY IN TROPICAL TREES

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ABSTRACT

The phenomenon of distinct, absent or indistinct growth rings is a highly variable feature used for wood identification and a wide range of tree-ring studies. Causes for its variability are not yet fully understood. There is also a lack of consensus within the scientific community about how distinct and indistinct tree rings should be defined and classified. We use a selection of 103 Central African rainforest trees to analyse the anatomy of growth-ring boundaries of 103 Central African rainforest species and assessed the influence of the climate, tree organ and leaf shedding behaviour on growth-ring distinctness and anatomy. We observed a high variability of tree-ring boundaries anatomy and distinctness within and among individuals and species. Although, for some semi-deciduous species, higher incidence of distinct growth rings appears to be related with a more pronounced seasonal climate, no general trends are observed for the assembly of studied species. Growth rings are variable within individuals depending on the considered organ: trunks tend to show more distinct rings than branches. Growth-ring distinctness is difficult to implement as a trait to measure tree performance when only based on abrupt changes in fibre size and cell wall thickness. From the potential growth-ring markers identified in the IAWA list of hardwood features, those applying to vessel and parenchyma density and distended rays appear to be more useful in tropical trees than abruptly flattened latewood fibres or abrupt changes in vessel diameter.

Keywords: Growth rings, Democratic Republic of the Congo, increment core, branch, wood anatomy, phenology.

Note: Supplementary raw data can be accessed in the online edition of this journal via <http://booksandjournals.brillonline.com/content/journals/>

INTRODUCTION

The presence or absence of (in)distinct growth rings is a wood anatomical feature familiar to everybody involved in wood research. Distinct growth rings contribute to the decorative value of wood and also drew the attention of the first scientific observers.

The first observations of growth rings with the unaided eye were done by Theophrastes (McBride 1983) and Da Vinci (Studhalter *et al.* 1963). Van Leeuwenhoek was the first to study growth rings in temperate trees under a microscope (Schierbeek 1959). He also made microscopic observations of tropical *Diospyros* wood (Baas 1982; Baas & Vetter 1989) and noted the absence of distinct growth rings. Presence or absence of distinct growth rings is the start of many dichotomous identification keys for commercial timbers (IAWA Committee 1989), whereas in fossil wood it is also a criterion to characterise the climate of the geological era where the fossils originate from (Francis & Hill 1996).

Growth-ring formation is supposed to be triggered by a succession of favourable and unfavourable growing conditions (Schweingruber 1996; Whitmore 1998). The cambial response of trees varies mainly with the environment, the species and the age of a tree (*e.g.*, Fonti *et al.* 2010; De Ridder *et al.* 2013; De Micco *et al.* 2016). Besides, a variety of wood anatomical structures defining growth rings are reported. This complexity leads to different 'levels' of growth-ring distinctness (Schweingruber 1996). For example, Fahn (1962) recognised five types of periodicity of cambial activity in trees and shrubs from Israel, essentially based on growth-ring distinctness. The first type shows growth rings, the middle three types "more or less" distinct growth rings and the fifth does not show growth rings at all. Carlquist (2001) described 15 growth-ring types and Worbes (2010) reduced them to four basic types. Next to these classifications, many other studies in temperate and tropical regions use their own classification, mostly directed towards the applicability in dendrochronological studies (Tarhule & Hughes 2002; Schweingruber *et al.* 2006; Sonsin *et al.* 2012; Groenendijk *et al.* 2014). Thus, the necessity of a clear and at the same time broad definition of growth-ring distinctness is obvious in order to construct databases on ring characteristics for different species and individuals in different environments. Especially under tropical circumstances there is a high level of growth-ring distinctness variability and uncertainty (Wils *et al.* 2009).

The most used and well-known definition proposed by IAWA (IAWA Committee 1989) defines distinct growth-ring boundaries by an abrupt structural change at the boundary, usually including change in fibre wall thickness and/or radial fibre diameter. In addition six other growth ring-markers, often co-occurring with changes in fibre morphology are listed. The abrupt changes should allow for an exact association of each cell to one single growth ring. IAWA Feature 1 is reserved for species showing distinct growth rings with an abrupt structural change at the growth-ring boundaries, while Feature 2 is for indistinct growth rings, with a more or less gradual change in cell characteristics on a radial axis, and absent rings. However, the classification between Features 1 and 2 remains often arbitrary and largely depends on the judgement of the individual wood anatomist, often biased by his/her origins. Cherubini *et al.* (2013) used one olive branchwood sample and circulated it for growth-ring analysis to different laboratories. The number of counted growth rings varied considerably among the experts showing the subjective character of growth-ring distinctness.

An alternative approach to wood anatomical descriptions consists of chemical composition studies along growth rings with markers like calcium crystals or oxygen or carbon isotopes (Gourlay 1995; Poussart *et al.* 2004). Growth seasonality is then

studied by the periodic variation in concentration of isotopes or mineral inclusions stored in wood. Finally, wood specific gravity profiles from pith to bark may also indicate a periodicity in cambial activity (Worbes 1995). High-resolution X-ray tomography scans can detect small zones with anatomical fluctuations that influence wood specific gravity (*e.g.*, flattened fibres) and could thus provide quantitative data for the determination of growth-ring boundaries complementary to classical wood anatomy (Van den Bulcke *et al.* 2009; Bastin *et al.* 2015).

Next to wood anatomy and dendrochronology, protocols on trait measurements and trait analyses *per se* are becoming increasingly important to study complex ecosystems (*e.g.*, Cornelissen *et al.* 2003; Pérez-Harguindeguy *et al.* 2013; Beeckman 2016; Bräuning *et al.* 2016). Violle *et al.* (2007) define traits as “any morphological, physiological or phenological feature measurable at the individual level, without reference to the environment or any other level of organisation”. Lachenbruch and McCulloh (2014) introduce a temporal notion in the definition of traits. For them, traits are “features of the plant unit that influence hydraulic or mechanical function and (...) will maintain the same value over the period in which the performance is evaluated”. Growth-ring distinctness is supposed to enable the measurement of a tree’s sensitivity to environmental variations. These variations can be seasonal or extreme events (*e.g.*, ENSO). These stressful events may induce the creation of intra-annual density fluctuations (IADFs) or false rings which might have the same anatomical features as growth rings (De Micco *et al.* 2016). Both the study of growth-ring width and IADFs anatomy may improve understanding of climate-growth relationships and climate reconstruction in tropical regions (Fonti *et al.* 2010; Venegas-Gonzales *et al.* 2015).

In this study, we use the IAWA features and definition of growth rings to describe growth-ring boundaries of a set of species from Central Africa with particular attention to the anatomical diversity. Based on these case studies, the following questions are addressed: does growth-ring distinctness and its variability depend on: a) the climate (species at two distant sampling sites with different precipitation regimes), b) tree organ (trunks *vs* branches), c) deciduous or evergreen phenology? Finally, the use of growth-ring distinctness as a trait is discussed based on the results of this study.

MATERIAL AND METHODS

Use of existing collections and database

Wood collection of the RMCA — The XylaREDD collection of tropical Congolese wood specimens stored in the Royal Museum of Central Africa (RMCA) was browsed and 103 species (Annex 1) were selected based on the number of available high-quality microtome sections. Those microscopic samples were observed under the microscope (Olympus BX60) and the anatomy of detected growth rings was described. Some samples did not show any visible ring boundaries, in which case rings were marked absent. Growth rings were tested for the presence or absence of several features such as abrupt changes in fibre or vessel dimensions or parenchyma frequency variation (Table 1). After the analyses of the different growth-ring markers growth rings were defined as present if fibre wall thickness or radial fibre diameter abruptly changed according to

Table 1. List of anatomical features used to describe growth-ring boundaries.

Abbreviation	Anatomical feature
AC_FW	Abrupt change in fibre wall thickness
AC_FD	Abrupt change in radial fibre diameter
GC_FD	Gradual change in radial fibre diameter
GC_VD	Gradual change in vessel diameter
VDV	Vessel density variation
MP	Marginal parenchyma present
PDV	Parenchyma bands density variation
DR	Distended rays

the IAWA definition (IAWA Committee 1989). All analysed features are illustrated in Figure 1. The influence of site, tree organ and phenology was addressed with statistical analyses described in the “Data analysis” paragraph.

For 13 out of 103 species, sampled at the Luki Biosphere Reserve in 2015, both trunk and branch samples were available, and these were compared for any differences in growth ring distinctness. Observations were done either on long micro sections according to Gaertner and Nievergelt (2010), on thin sections, or on finely sanded transverse surfaces of increment cores. The species and number of samples per trunk or branch are presented in Table 2.

Phenology

Phenological data for each species were extracted from the INERA (Institut National pour l’Etude et la Recherche Agronomiques) observations that took place from 1948 to 1957 in the Biosphere Reserve of Luki. Two categories were created: evergreen and semi-deciduous. Species were classified as semi-deciduous if several individuals were

Table 2: Species for which both branches and trunkwood samples are available. All samples originate from the Luki Biosphere Reserve.

Species	Number of branch samples	Number of trunk samples
<i>Blighia welwitschii</i>	5	4
<i>Carapa procera</i>	6	3
<i>Celtis mildbraedii</i>	5	4
<i>Chrysophyllum africanum</i>	5	3
<i>Cola griseiflora</i>	6	5
<i>Erythrophleum suaveolens</i>	5	3
<i>Garcinia punctata</i>	5	1
<i>Petersianthus macrocarpus</i>	5	6
<i>Polyalthia suaveolens</i>	6	5
<i>Pycnanthus angolensis</i>	5	4
<i>Staudtia kamerunensis</i>	5	4
<i>Trichilia gilgiana</i>	6	3
<i>Trilepisium madagascariense</i>	5	3

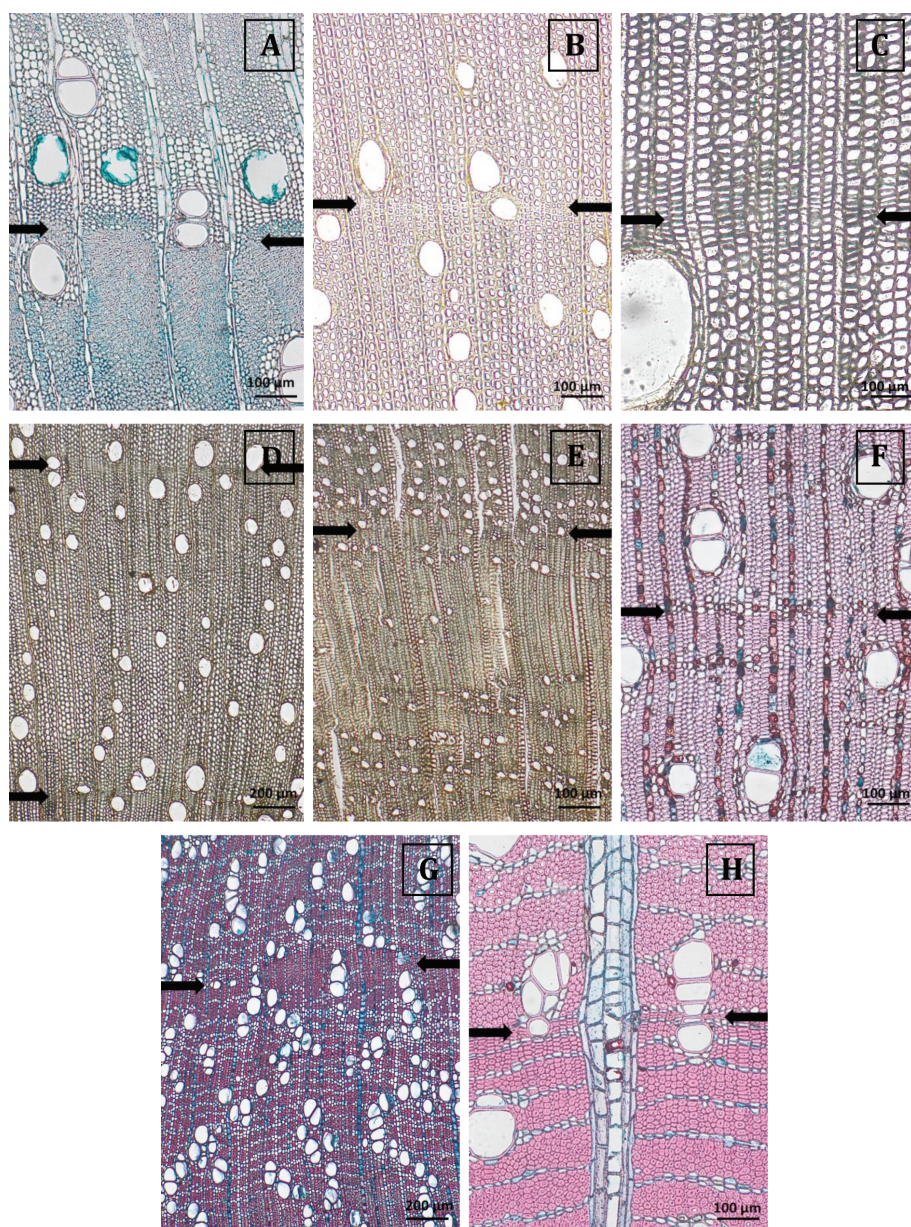


Figure 1. Illustration plates for the studied anatomical features. – A: Abrupt change in fibre wall thickness (*Copaifera mildbraedii*). – B: Abrupt change in radial fibre diameter (*Agelaea pentagyna*). – C: Gradual change in radial fibre diameter (*Ricinodendron heudelotii*). – D: Gradual change in vessel diameter (*Agelaea pentagyna*). – E: Vessel density variation (*Dichapetalum madagascariense*). – F: Marginal parenchyma present (*Trichilia rubescens*). – G: Parenchyma bands density variation (*Synsepalum subcordatum*). – H: Distended rays (*Polyalthia suaveolens*). Arrows represent growth ring boundaries.

observed leafless during the observation period (Couralet *et al.* 2010). Other databases were available (CJB – African Plants Database, Prota4U), however, not all the species were described for their phenological characteristics and some species were described as both “evergreen” and “deciduous”. The INERA database had the advantage of being site specific. Also, information about the methodology used to construct the database was available and identical for all the species.

Study sites

Analyses were conducted on samples collected in the Democratic Republic of the Congo (DRC). Site selection was based on the availability of sufficient numbers of samples from those sites and contrasting rainfall regimes in order to compare growth dynamics between different zones. The sites are located in the Biosphere Reserves of Yangambi (0.46°N 24.28°E) and Luki (5.39°N 13.4°E). Annual precipitation is highest in Yangambi (around 1700 mm) and decreases to around 1200 mm in Luki.

The Biosphere Reserve of Yangambi is located on the northern bank of the Congo River, just north of the equator in the Oriental Province. The region has an Af type tropical rainforest climate (following Köppen-Geiger; Peel *et al.* 2007). Soils on the Yangambi plateau are ferralsols primarily formed from eolian sediments, composed mostly of quartz sand, kaolinite clay and hydrated iron oxides (Gilson 1956; Van

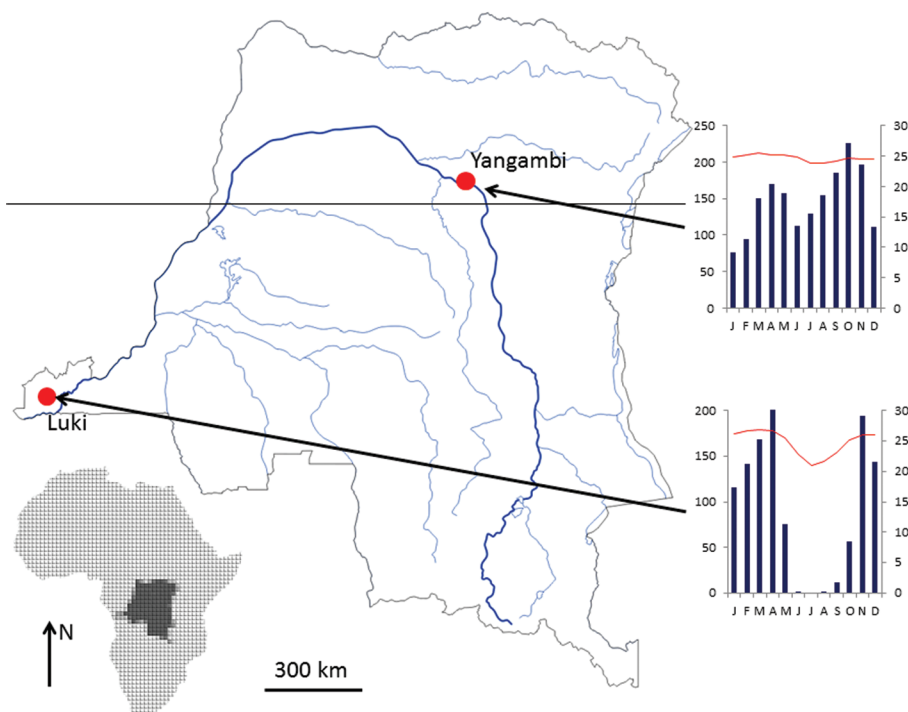


Figure 2. Geographical location of the two study sites with climate diagrams: monthly precipitation left, average day temperature (red line) right. The horizontal line represents the equator.

Ranst *et al.* 2010). The Yangambi region receives an annual precipitation of 1762 mm (1961–2012) with, on average, two to three months with lower monthly precipitation around 100 mm, during December–February (Fig. 2). A smaller season with less precipitation occurs in June to July. Temperatures are fairly constant throughout the year with a minimum of 24.2 °C in July and a maximum of 25.5 °C in March. Vegetation in the reserve is characterized by both old-growth and disturbed forests (Gilson 1956). Forest disturbance is mainly caused by slash-and-burn agricultural activities followed by abandonment allowing forest regeneration (Kearsley 2013).

The Biosphere Reserve of Luki is situated in the Lower Congo Province at an altitude of < 300 m above sea level, in a semi-evergreen rainforest occurring in a mosaic landscape with patches of savanna and successional forest types. The dry season lasts approximately four to five months, from May to September–October. The proximity of the ocean, however, buffers the intensity of the dry period with relative humidity more or less constant throughout the year. Average temperatures are around 26 °C and annual precipitation varies around 1100 mm (De Ridder *et al.* 2013). The soils of the Luki Reserve are classified as orthic ferralsols (FAO 2007). The rainforest is a mixture of deciduous and evergreen tree species in the upper canopy and mostly evergreen species in the understory (Donis 1948; Lebrun & Gilbert 1954; Couralet *et al.* 2010). Also, the semi-evergreen mature rainforest encloses other vegetation types which can be relatively widespread (*e.g.*, Marantaceae open forests, woodland savanna types dominated by *Xylopia aethiopica* and regenerating forest dominated by *Terminalia superba*, especially abundant in and around the Luki reserve (Donis 1948; Hubau *et al.* 2015)).

Data analysis

Each observed growth ring was described by the presence/absence of eight different anatomical features (Table 1) and a score of respectively 1 or 0 assigned per feature. In case of several rings with different anatomical features per individual, an average was calculated per individual per feature. A PCA was then conducted on the sample set to determine how anatomical features interact. A dissimilarity matrix was also constructed with Euclidian distances between PCA's site scores and a clustering (Ward's method) performed (Fig. 4). Five groups were subsequently defined and trends in growth-ring anatomy of the samples within these different groups were analysed. The formation of five clusters was retained because it allowed the best separation of individuals in anatomically homogeneous groups. It also allowed working with a limited number of groups which is easier for the discussion of general trends. The proportion of samples from different sites, organs and phenological character was calculated for each group. All these analyses were performed with R software with *vegan* and *gclust* packages.

RESULTS

Growth-ring boundaries anatomy variability

324 individuals sorted in 103 species from the XylaREDD collection were analysed with, on average, 3.2 individuals per species; 28 % (91 individuals) presented distinct

growth rings. Another 28 % (91 individuals) presented indistinct growth rings while 44 % (142) had no growth rings at all. Growth-ring distinctness was decided on the basis of IAWA’s definition of distinct growth rings. Results for XylaREDD samples are presented in Annex 1 (supplementary material).

Climate

The proportion of individuals originating from Yangambi in our sample set is 60 % (194 individuals) and samples from Luki make up 40 % (130 individuals). These 60–40 % proportions are also observed in groups exclusively composed of individuals with distinct, indistinct and absent rings. In Yangambi, 26 % (50) of individuals have distinct growth rings, 30 % (58) indistinct and 44 % (86) show no rings at all, while in Luki those proportions are very similar.

Tree organs

The sample set was composed of 51 % (162) of individuals sampled from branches with a diameter of a few centimetres, 42 % (131) from the trunk and 7 % (23) of samples of ambiguous origin, *i.e.*, either from thick branches or of thin main stems (8 individuals had no metadata about the sampled organ). In branch samples, 27 % (43) of individuals had distinct growth rings, 25 % (41) showed indistinct rings and 48 % (78) showed no visible rings. In trunk samples, those proportions shifted towards 28 % (36) with distinct rings, 31 % (41) with indistinct and 41 % (54) without rings.

The comparison of trunk- and branchwood of the same tree is given in Table 3. In all cases, trunks had the same number or more samples with distinct rings than branches.

Phenology

Only 158 samples were used for this study due to the lack of information about leaf-shedding behaviour of other species. Among evergreen species 31 % (28) of the samples have distinct rings, 23 % (21) indistinct and 46 % (41) show no growth rings. The proportion of distinct rings in semi-deciduous species is quite similar: 28 % (19).

Table 3: Number of trunk- or branchwood samples with distinct growth rings.

Species	Number of branch samples	Number of branch samples with distinct rings	Number of trunk samples	Number of trunk samples with distinct rings
<i>Blighia welwitschii</i>	5	5	4	4
<i>Carapa procera</i>	6	1	3	2
<i>Celtis mildbraedii</i>	5	5	4	4
<i>Chrysophyllum africanum</i>	5	1	3	1
<i>Cola griseiflora</i>	6	0	5	0
<i>Erythrophleum suaveolens</i>	5	2	3	2
<i>Garcinia punctata</i>	5	0	1	0
<i>Petersianthus macrocarpus</i>	5	3	6	0
<i>Polyalthia suaveolens</i>	6	3	5	5
<i>Pycnanthus angolensis</i>	5	2	4	4
<i>Staudtia kamerunensis</i>	5	4	4	4
<i>Trichilia gilgiana</i>	6	3	3	3
<i>Trilepisium madagascariense</i>	5	0	3	0

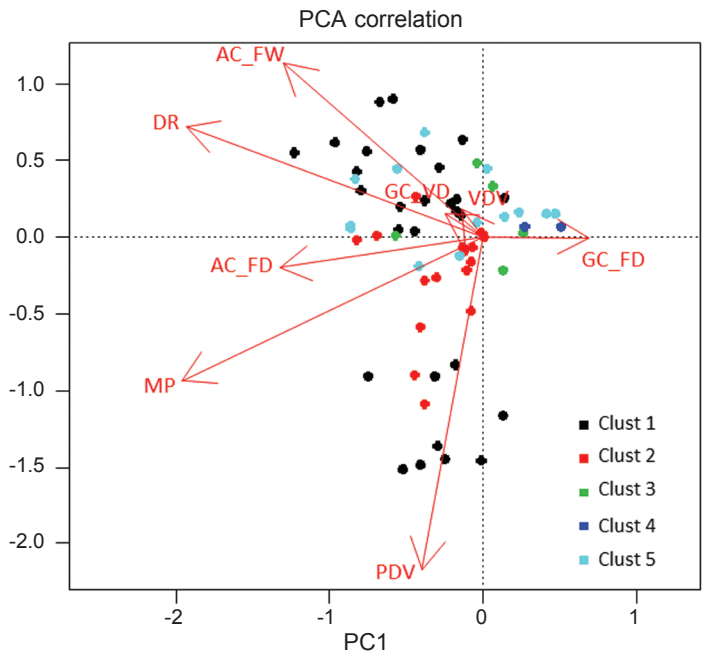


Figure 3. PCA of anatomical features defining growth rings. Correlations between 8 observed features mapped onto first two principal components. For abbreviations used see Table 1. Points represent individuals that belong to a particular cluster (see the paragraph “Clustering”).

However, indistinct rings make up to 39 % (27) of the samples and only 33 % (23) show no rings.

PCA of anatomical descriptors

The PCA maps the anatomical features and individuals along the first two axes (Fig. 3). Three features contribute together to the first axis: changes in fibre dimensions correlated to marginal parenchyma and distended rays. Parenchyma density variation seems to be independent from the previous anatomical features and contributes mostly to the second axis. Finally, the changes related to vessel anatomy are not related to either of the first two axes.

Individuals from the first two clusters are widely spread across the correlation map. These clusters are composed of individuals with variable growth-ring anatomies. The other three clusters are more homogeneous. It should be noted that the proportion of the variance explained by the first two axes is relatively low (Table 4).

Table 4. Scores from the PCA. The first two axes explain 38% of the total variance.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Eigenvalue	18.377	11.738	11.261	10.157	0.9699	0.853	0.66415	0.35951
Proportion explained	0.2297	0.1467	0.1408	0.127	0.1212	0.1066	0.08302	0.04494
Cumulative proportion	0.2297	0.3764	0.5172	0.6442	0.7654	0.872	0.95506	100.000

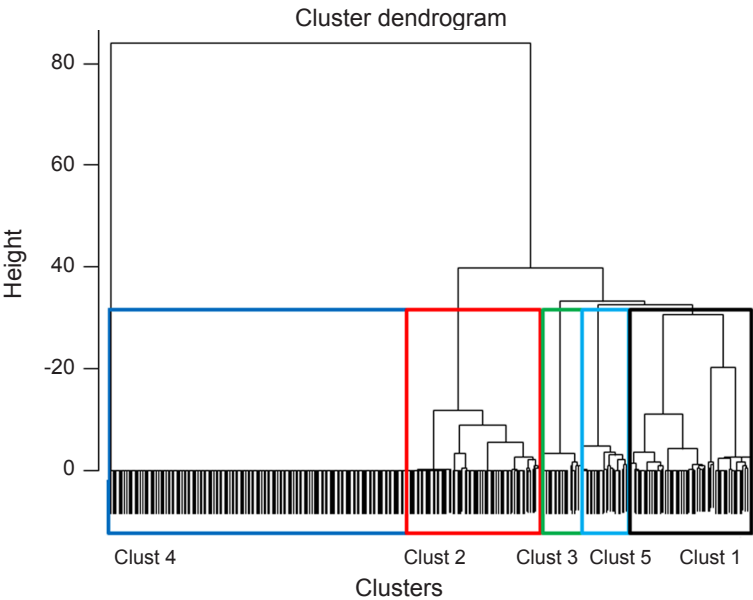


Figure 4: Cluster dendrogram with dissimilarity levels.

Clustering

Five groups of 62 (19%), 68 (21%), 20 (6%), 150 (47%) and 23 (7%) individuals were created (Fig. 4). Clusters 1, 2, 3, and 5 are more closely related among each other than to cluster 4.

The clusters were created based on the anatomical features defining their growth rings and an average was calculated for the occurrence of each anatomical descriptor for each group. The results are presented in Table 5. The first cluster is characterised by an important anatomical variability with individuals presenting abrupt changes in fibres, marginal parenchyma and distended rays. The majority of individuals have indistinct rings. The second cluster has mainly individuals with distinct growth rings defined by an abrupt change in fibre diameter, sometimes associated with marginal parenchyma and, to a lesser extent, distended rays. The third cluster is composed almost exclusively of individuals with indistinct rings characterised by gradual change in fibre diameter, sometimes associated with marginal parenchyma or distended rays. The fourth cluster

Table 5. Proportion of samples with studied anatomical features in each cluster. For abbreviations see Table 1.

Cluster	AC_FW	AC_FD	GC_FD	GC_VD	VDV	MP	PDV	DR	distinct	indistinct	absent
1	0.381	0.219	0.016	0.028	0.032	0.630	0.298	0.516	0.359	0.641	0
2	0	0.873	0	0	0.020	0.548	0.026	0.25	0.814	0.221	0
3	0.025	0.05	0.925	0	0	0.15	0	0.125	0.05	0.95	0
4	0	0	0.007	0	0	0	0	0	0.007	0.027	0.947
5	0.152	0.264	0.076	0	0.994	0.348	0	0.087	0.319	0.681	0

Table 6. Proportions of samples from different sites and different organs.

Cluster	Luki	Yangambi	Trunk	Branch
1	40 %	60 %	62 %	38 %
2	49 %	51 %	52 %	48 %
3	40 %	60 %	44 %	56 %
4	39 %	61 %	41 %	59 %
5	22 %	78 %	5 %	95 %
Whole set	40 %	60 %	45 %	55 %

Table 7. Systematic affinity. Numbers of individuals of each family distributed in the five clusters.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Anacardiaceae		4		3	
Annonaceae	9	9		1	1
Apocynaceae	3	6	2	8	
Asparagaceae				2	
Burseraceae				3	
Cannabaceae			1	1	
Celastraceae		1		7	
Chrysobalanaceae				4	1
Clusiaceae				4	
Connaraceae	3	6	5	7	
Dichopetalaceae	3	1		16	9
Ebenaceae				3	
Euphorbiaceae		1	1	2	
Fabaceae	17	14	4	22	1
Icacinales				3	
Irvingiaceae			1	8	
Lamiaceae		1		1	1
Linaceae		1		2	
Malvaceae	1			3	
Meliaceae	10	4	1	5	
Moraceae	1	1		3	
Myristicaceae			3		
Olacaceae			1	10	
Pandaceae				2	
Polygalaceae				1	
Rhamnaceae				3	
Rubiaceae	4	3	1	16	7
Rutaceae		4		4	3
Salicaceae	2	2			
Sapindaceae	1	6		2	
Sapotaceae	7	2		4	1
Violaceae	1	2			

is made of individuals with no visible rings. Finally, the fifth cluster has the same proportions of distinct and indistinct rings as the first. Its principal anatomical feature is vessel density variation. Some of its individuals also show abrupt changes in fibres associated with marginal parenchyma.

The influence of climate, organ and phenology within clusters is presented in the next paragraphs. The results of Luki/Yangambi and trunk/branch repartition within different clusters are presented in Table 6.

The botanical families composing different clusters are presented in Table 7. A total of 32 families were represented in the sample set. Each family is present, on average, in 2.5 clusters. In our sample set, almost every family is represented in the cluster with absent growth rings. Families represented in only one or two clusters are mostly present in cluster 4 characterised by samples with no visible rings. Most of the families are characterised by a high variability of anatomical features marking the growth ring boundaries.

Influence of the climate and tree organs

The proportions of samples from Luki or Yangambi are similar to the proportion in the whole sample set in every cluster. The fifth cluster slightly differs from the other clusters and the whole sample set. The inter-cluster variability and the deviation from the average are higher in the Trunk-Branch proportions (Table 6).

Table 8. Proportion of samples with different phenological character in different clusters and in different sites.

Cluster	Number of samples	Evergreen	Semi-deciduous
1	15 (10 %)	40 %	60 %
2	59 (37 %)	51 %	49 %
3	10 (6 %)	50 %	50 %
4	67 (42 %)	64 %	36 %
5	7 (5 %)	86 %	14 %
Whole set	158	57 %	43 %
Yangambi	71 (45 %)	63 %	37 %
Luki	87 (55 %)	52 %	48 %

Table 9. Anatomy of growth rings in different clusters. The subset is composed of species with available information about their phenology. For abbreviations see Table 1.

Cluster	AC_FW	AC_FD	GC_FD	GC_VD	VDV	MP	PDV	DR	distinct	indistinct	absent
1	0.9	0.355	0	0	0	0.667	0	0.733	0.7	0.3	0
2	0.059	0.606	0.008	0.025	0.232	0.641	0	0.458	0.602	0.432	0
3	0	0	1	0	0	0.1	0	0.1	0	1	0
4	0	0	0	0	0	0	0	0	0.015	0.015	0.955
5	0	0.143	0	0	0	0.714	0.857	0	0.143	0.857	0

Influence of the phenology

The sample set is composed of 158 individuals of which 71 are from Yangambi and 87 from Luki. These two sites contribute equally to the group of individuals of evergreen species. The semi-deciduous species group is composed of 62 % of individuals from Luki and 38 % from Yangambi (Table 8). A new PCA and a new clustering were conducted on the 158 individuals subset and five subgroups were created from the subset of species with information on their leaf-shedding behaviour and their anatomy described (Table 9). The highest proportions of evergreen species are in clusters 4 and 5 where the highest proportions of indistinct or absent growth rings can be found. Cluster 1, with the highest proportion of distinct rings as well, has the highest proportion of semi-deciduous species. Cluster 2 is anatomically similar to cluster 1 but their species composition is different. There are more evergreen species in the second cluster than in the first.

Table 10 gives a more precise view about the contribution of evergreen and semi-deciduous species to the distinctness of growth rings. The only difference between Luki and Yangambi is that individuals with distinct rings are mostly evergreen species in Yangambi and semi-deciduous in Luki.

Table 10. Number of individuals with distinct, indistinct or absent rings in evergreen and semi-deciduous species groups in Luki and Yangambi. SD: semi-deciduous; E: evergreen.

		Distinct	Indistinct	Absent
Luki	SD	58 % (14)	57 % (17)	36 % (12)
	E	42 % (10)	43 % (13)	64 % (21)
Yangambi	SD	22 % (5)	56 % (10)	35 % (11)
	E	78 % (18)	44 % (8)	65 % (20)

DISCUSSION

We observed variation in growth-ring anatomy between species, within species and even within individuals. These observations are in accordance with Fichtler and Worbes (2012). So far, growth-ring distinctness studies mainly focused on growth-ring boundaries distinctness on the species level without acknowledging possible intra-species variation (Worbes 1999; Groenendijk *et al.* 2014). Our study highlights the high level of variation of growth-ring anatomy and distinctness in the tropics and raises questions about the sources of this variation. Here, we address some of these questions.

By analysing the results of clustering on Figure 4 we can observe that clusters 1, 3 and 5 have very small dissimilarity levels. Moreover, counting them as only one cluster would enable us to work with three different clusters: one cluster with a majority of distinct growth rings, one with a majority of indistinct and one with absent growth rings. However, most of the variability lies within these three groups and considering them as one unique cluster would prevent several analyses provided in the upcoming paragraphs.

Influence of the climate on growth-ring distinctness

In our sample set, the proportion of trees with distinct, indistinct or invisible rings is similar in Luki and Yangambi. Moreover, the relative proportions of individuals from Luki and Yangambi remain similar in four groups out of five. Only cluster 5, with only 23 individuals, has different proportions (22% from Luki and 78% from Yangambi). This cluster is dominated by samples with indistinct rings defined by vessel density variations (areas with very few or even without vessels throughout the samples). This could be linked to water availability (Zanne *et al.* 2010). However, most of the individuals with these features are originating from Yangambi which shows a more uniform precipitation distribution than Luki. It is to be noted that the size of the sample subset might play a role and that the percentage share might not be representative of a larger population with these anatomical characteristics.

Cluster 2, larger than the previous (68 individuals), also deviates from the overall proportions Luki/Yangambi. Individuals from Luki make up to 49% of cluster 2 (against 40% in the total sample set). This cluster is characterised by individuals with distinct growth rings showing abrupt changes in radial fibre diameter. The increase in the proportion of samples from Luki in this cluster is in accordance with the hypothesis that a stronger seasonality leads trees to the formation of more distinct growth rings. According to Borchert (1999), plants react to drought but responses vary widely among trees and their annual development is not well synchronised by climatic seasonality. Also, the length of the dry season may not be sufficient as a proxy for water availability. Soils with a better water retention or sites with higher relative air humidity levels can create conditions that satisfy the trees' water needs and buffer a longer dry season (Borchert 1999).

Luki and Yangambi differ in terms of annual rainfall and the length of the dry season but other factors such as a constantly high relative humidity in Luki can make up for the decrease in rainfall during several months. Finally, according to Fichtler and Worbes (2012), a greater variability is observed between individuals at one site rather than between different sites. Although the number of studied individuals is limited, our observations appear to be in line with Fichtler and Worbes' findings (Tables 5 & 6). This may indicate that cambial activity is more sensitive to climatic variability from year to year than to the average climate conditions per site.

It is also important to keep in mind that our study is a general overview of different individuals in two contrasting sites. It gives us a first insight of the possible trends but there is no precise information about the response of a single species to different climatic conditions. In several species, individuals came from only one site and, in general, not enough individuals per species per site were available for a thorough analysis of within-species response to the climate. An adapted sampling design with enough replications per species per site is necessary to effectively tackle the question of the behaviour of growth-ring distinctness under the influence of climate within the tropics.

Influence of the organs on growth-ring distinctness

A bigger variability is observed among clusters in terms of branch/trunk proportions in the samples. Trunk sample proportion shifts from 62% in the first cluster to

5 % in the last one. Data suggest that a slightly higher proportion of distinct rings is to be found in trunkwood (cluster 2 defined by distinct rings has a higher proportion of trunk samples). Features like abrupt change in fibre diameter or wall thickness, marginal parenchyma or distended rays seem to occur more often in trunkwood than in branches. Storage tissues associated with growth-ring boundaries are thus more evident in trunks than in branches. As cambial activity normally starts nearby the site of photosynthesis and moves further down the trunk (Tepper & Hollis 1967), storage tissues in the trunk may be useful to resume stem cambial activity faster by using reserves stored close to the cambium layer. However, a very limited number of species present both branch- and trunkwood in our samples set. In some species trunks present a lot of storage tissues while branches do not (*Hylodendron gabunense*, *Trichilia prieuriana*, *Zanthoxylum gillettii*), in some species the contrary can be observed (*Chytranthus macrobotrys*, *Maranthes glabra*), some species have abundant storage tissues in both trunks and branches (*Synsepalum subcordatum*) and some species do not show storage tissues at growth-ring boundaries regardless of the organ (*Klainedoxa gabonensis*, *Oncoba welwitschii*, *Strombosia grandifolia*).

We can also note that the fifth cluster is largely dominated by branchwood. Branchwood tends to show more variation in the vessel density than trunkwood according to our results. Tree water status is an important variable controlling phenology and cambium activity (Borchert 1999). Vessel conductivity is function of the size and number of vessels per unit area (Zanne *et al.* 2010). To avoid important moisture losses by transpiration during dry periods, branches with zones of a lower vessel density might be one of the solutions adopted by tropical trees.

We can also take into account the third and fourth clusters. They are defined by samples with indistinct or absent growth rings and share almost exactly the same proportions of trunk/branchwood as in the whole sample set. We can conclude that a tree with distinct growth rings in the trunk might still have indistinct rings in the branches. On the other hand, if rings are indistinct or simply absent in the trunkwood, it is improbable to find distinct rings in the branches. This statement is supported by our results in Table 3.

However, the scientific potential of growth rings in the branchwood must not be underestimated. Nicolini *et al.* (2012) have studied growth rings in branchwood of a tropical species (*Parkia velutina*) and could date with a reasonable error the age of the tree. Further research is needed to fully assess the potential of branchwood in growth-ring studies and possibly use branch rings to reconstruct tree growth.

Influence of phenology

The original phenological observations were carried out between 1948 and 1957 and brevi-deciduousness was not taken into account (Couralet *et al.* 2010) which meant that trees being observed leafless for more than 10 days during the observation period were marked as being deciduous. To avoid such sweeping generalisations we limited our analyses to “evergreen” and “semi-deciduous” species. This category is coarser but is better adapted from an ecological point of view to our sample set and available information.

Our observations about the influence of leaf phenology on growth-ring distinctness in the 158 individuals reveal interesting trends. The share of individuals of semi-deciduous species is the highest in clusters with the most distinct rings while evergreen species are more represented in the clusters with a majority of indistinct or absent rings. This is in accordance with the previous statements that growth-ring distinctness is linked to tree phenology (Worbes 1999; Borchert 1999; Brien & Zuidema 2005). However, Table 10 shows us that within the group of individuals with distinct rings in Luki, the share of individuals of semi-deciduous species (58 %) is much higher than in Yangambi (22 %). For individuals with indistinct or absent rings, proportions of semi-deciduous species remain the same in Luki and Yangambi. This is supported by Worbes' statements that not all deciduous species form distinct rings (Worbes 1999). According to Borchert (1999), phenology and cambial growth are correlated with each other in tropical dry climates and get progressively uncoupled with the increase in precipitation. This difference in semi-deciduous species proportions between individuals with distinct or indistinct/absent rings might thus be in accordance with the longer dry season recorded in Luki and the low storage capacity in its soils (FAO 1979). This may also explain the relatively high cumulated proportion (72 %) of samples with indistinct and absent rings in the semi-deciduous species in the whole sample set.

However, we must keep in mind that our sample set does not allow for testing climate influence on leaf shedding behaviour at the individual level (which was not the goal of this study). Based on the observations conducted in Luki, we assigned the evergreen or semi-deciduous character to the species as a whole, which may be incorrect for the two contrasting sites.

Remaining problems and future research for growth-ring distinctness

Although our sample set contains many individuals representing a considerable number of species, it is important to keep in mind that some limitations exist. The Xylarium samples are most often variable in size and metadata are not very precise; some of them are cubes of c. $2 \times 2 \times 2$ cm with no indication of their position within the trunk. Most of the samples used for this study were only a few centimetres long. Although the absence of distinct or indistinct growth rings in those samples is valuable information about anatomical and growth trends, it does not provide the information about possible growth rings in other parts of the trunk. Moreover, even if growth rings are visible, we possibly describe only a part of their variability and know little about growth-ring anatomy variation over the years. This is especially true if only few samples are available. To avoid the lack of representativeness, we recommend studying growth-ring distinctness and variability on pith-to-bark cores or complete disks.

It is also important to consider the link between “distinct” or “indistinct” tag and growth-rings anatomy. During this study, we focused on distinctness understood as abrupt changes in fibre diameter and/or wall thickness. This raised several problems during the process. First of all, the IAWA definition of distinct growth ring boundaries uses the adverb “usually” when referring to changes in fibre wall thickness and/or radial diameter. We believe that in the tropical context, this definition emphasises too much the changes in fibres anatomy compared to other descriptors. The term

“usually” might lead to an underestimation of the proportion of species with distinct rings in the tropics. Moreover, the meaning of “indistinct” is vague and an arbitrary one (as is the separation of “indistinct” and “absent” as judged from the illustrations in the Hardwood List). In several cases in our material, boundaries were defined by parenchyma patterns (parenchyma bands variation in size or frequency, parenchyma cell shape), vessel organisation (same size but vessels aligned in a tangential row) or distended rays associated with more-or-less gradual variation in fibre anatomy. We classified these structures as indistinct growth rings when they were not accompanied by abrupt changes in fibres anatomy. We think that a more flexible approach in classifying tropical growth-ring distinctness while providing a rigorous methodology in growth ring research is possible. The ability to tell whether a single cell belongs to the Nth or the N+1th ring should be emphasised. A quantitative assessment of growth-ring distinctness could also be elaborated (*e.g.*, a measurement of the distance between consecutive parenchyma bands, high-resolution densitometry or parenchyma cell measurements). Schweingruber *et al.* (2006) propose a more complete description of growth-ring distinctness with attention paid to the possibility to cross-date rings and conduct dendrochronological studies.

Growth-ring distinctness needs further research to be used as a trait in ecological studies. A consensus on different levels of distinctness needs to be reached among scientists and classification must be as objective as possible. A measure of the degree of distinctness (*e.g.*, the amplitude of variation of radial fibre diameter) can be implemented to measure the sensitivity of trees to variable environmental conditions. This can complement the previously researched features like ring width (Worbes 1999) or the variation in vessel pattern (Fichtler & Worbes 2012). To comply with Lachenbruch and McCulloh’s (2014) definition, a time frame for the analyses also needs to be defined. Since the feature is supposed to maintain the same value over the study period, a period representative of different life stages (juvenile, mature, old) of trees needs to be defined per species.

It is important for further dendrochronological and dendroecological studies to take into account the possible geographical variation in growth-ring distinctness in different databases. To assess the annual nature of growth zones boundaries and consider them as growth rings, additional studies as cambial marking, cross-dating, radiocarbon (Rozendaal & Zuidema 2011), oxygen isotopes (Poussart *et al.* 2004) or calcium (Poussart *et al.* 2006) measurements might be necessary in case of doubt. Finally, we need to understand growth-ring diversity and escape the artificial binary vision of distinct *versus* indistinct to absent growth rings.

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