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Occurrence and anatomical features of growth rings in tropical rainforest trees in Peninsular Malaysia: a preliminary study

Amir Affan Abdul Azim^{1,2*} and Naoki Okada^{1,3}

¹ Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto, 606-8502, Japan

² Faculty of Forestry, Universiti Putra Malaysia, Selangor, 43400, Malaysia

³ Graduate School of Global Environmental Studies, Kyoto University, Yoshida Honmachi, Kyoto 606-8501, Japan

* Corresponding author: amiraffan.aa@gmail.com

ABSTRACT The occurrence and anatomical features of growth rings were investigated in tropical tree species in Peninsular Malaysia. The species studied included five species from two plantations, in a tropical monsoon and tropical rainforest climate, and 26 species from a natural forest in a tropical rainforest climate. Most of the trees were diffuse-porous species, with the exception of *Peronema canescens*, which was ring-porous. The growth rings were observed macroscopically and microscopically and were classified as having fiber zones, radially flattened fibers, marginal parenchyma, thick-walled fibers, or variations in vessel size and density. Whereas *P. canescens* from two plantations and *Intsia palembanica* from the natural forest showed well-defined growth rings, possibly formed annually following environmental stresses, 17 species showed indefinable growth rings and 10 species had no growth rings. Many of the samples presented intermittent growth rings, ambiguous growth rings, discontinuous growth rings, or resin canals and/or traumatic canals that made them difficult to classify. Bands of axial parenchyma were observed macroscopically in *Sindora wallichii* and *Azadirachta excelsa*, but microscopic analysis revealed that these bands were formed by concentric traumatic canals. Parenchyma bands with resin ducts or traumatic canals were likely not to be formed periodically. We concluded that some tree species that grow in a tropical rainforest climate do form growth rings and may be used in tree-ring analysis studies.

Key words: Tropical tree; Growth ring; Tropical rainforest; Peninsular Malaysia

INTRODUCTION

Worldwide interest in tropical dendrochronology has been increasing. Rozendaal and Zuidema (2011) and Pumijumnong (2013) reviewed the potential of performing tree-ring chronology in the tropics. The development of tree-ring chronologies of teak (*Tectona grandis*) in India (e.g., Bhattacharyya et al. 1992; Borgaonkar et al. 2007), Java (e.g., D'Arrigo et al. 1994), Thailand (e.g., Pumijumnong et al. 1995), and Myanmar (e.g., Pumijumnong et al. 2001) has stimulated interest in annual ring formation in other tropical trees. Development of tropical tree-ring chronologies would make a valuable contribution to climatology, forest ecology, and forest management in the tropics. However, tree-ring analyses can only be performed when trees form anatomically distinct annual growth rings. There were some basic studies reporting growth rings in tropical trees and their potential for tropical dendrochronology (e.g., Roig et al. 2005; Maingi 2006; Worbes and Fichtler 2011) and few applications for tropical seasonal forests (e.g., Brien et al. 2009) and mangrove forests (e.g., Menezes et al. 2003; Chowdhury et al. 2008). Limited studies have

been conducted in tropical rainforest (e.g., Fichtler et al. 2003; Soliz-Gamboa et al. 2011, Lopez et al. 2012), because most tropical rainforest trees were thought not to produce anatomically distinct annual growth rings.

Thus, investigating the characteristics of growth rings in tropical trees and identifying the species that have potential for dendrochronological studies in the tropics represent a major new endeavor in tropical dendrochronology. Stahle (1999) has proposed identifying the temperate or subtropical species that have known dendrochronological value and a wide natural distribution and extending the survey into the tropics. However, the distribution of these species is limited to certain growing conditions and they are not widely found in tropical rainforests.

In Malaysia, some tropical tree species are grown in plantations, such as *T. grandis*, *Acacia* spp., *Cedrela* spp., *Khaya* spp., and *Swietenia* spp., because of their fast growth and the promising quality of their timber. Most of these form anatomically distinct annual growth rings in their native habitat (Worbes 1989, 1995, 2002). Less pronounced seasonal changes in the tropical rainforest environment, however, might prevent these tree species from forming

anatomically distinct growth rings. Although it is important to study these exotic species to understand their growth behavior in the tropics, it is more important to study the indigenous tree species in order to understand tree growth, forest development, and recovery after disturbances in tropical rainforests.

In this study, we examined growth rings in common tropical rainforest tree species in Peninsular Malaysia to evaluate their potential for use in tropical dendrochronology. We described and categorized the anatomical features of growth ring markers in trees from two plantations and a natural forest.

MATERIALS AND METHODS

Study site

The study trees were selected from two plantations and one natural forest (Fig. 1). Both forest plantations are managed by the Forest Research Institute Malaysia, and are located at Mata Ayer Forest Reserve, Perlis (MAFR, 6° 40' N, 100° 15' E) and Bukit Hari, Forest Research Institute Malaysia, Selangor (BHFRIM, 3° 14' N, 101° 38' E). The climate of MAFR is classified as tropical monsoon (A_m

according to Köppen-Geiger climate classification; Peel et al. 2007), with a 2–3-month dry season from December to February. The climate of BHFRIM is classified as tropical rainforest (A_f according to Köppen-Geiger climate classification), in which the monthly precipitation is usually much greater than 100 mm. The natural forest is a secondary disturbed lowland dipterocarp forest, Ayer Hitam Forest Reserve, Selangor (AHFR, 3° 1' N, 101° 39' E). The climate of AHFR is also classified as tropical rainforest, but with a short dry period for 1–2 months in June and July.

Study species and methods

Table 1 summarizes the selection of tree species, as well as the information regarding their growth rings from the literature available (FRIM 1993; PROSEA 1994a, b, c; Ogata et al. 2008). We investigated the occurrence and anatomical features of growth rings in five tree species, belonging to three families, planted in MAFR; five tree species, belonging to four families, planted in BHFRIM; and 26 tree species, belonging to 18 families that grow naturally in AHFR. All species selected in MAFR was planted in year 2000 and trees in BHFRIM were planted in year 1998 (*Azadirachta excelsa*, *Dyera costulata* and

Fig. 1. Location of research sites and climate diagram for each area. Monthly precipitation (bar) and temperature (line) are the mean from 2000 to 2011. Climate data for the Ayer Hitam Forest Reserve (AHFR) was provided by Universiti Putra Malaysia, and data for the Mata Ayer Forest Reserve (MAFR) and Bukit Hari, Forest Research Institute Malaysia (BHFRIM) were obtained from the Malaysian Meteorological Department.

Table 1. Selection of tree species and the characteristics of growth rings in the literature and the present study

Study site Family Species	Diameter (cm)	Characteristics of growth ring			
		Literature	Distinctiveness	Type	Difficulties
Mata Ayer Forest Reserve					
Dipterocarpaceae					
<i>Hopea odorata</i>	15.2–19.9	Indistinct (FRIM 1993; PROSEA 1994a)	C	–	RTC
<i>Shorea roxburghii</i>	12.0–15.3	Indistinct (FRIM 1993; PROSEA 1994a)	C	–	RTC
Meliaceae					
<i>Azadirachta excelsa</i>	13.5–22.4	Marked by differences in pore size, fiber wall thickness, and initial parenchyma (PROSEA 1994b)	B	MP	IT, AR, RTC
Verbenaceae					
<i>Gmelina arborea</i>	16.2–27.7	Marked by differences in pore size and fiber wall thickness (PROSEA 1994a)	B	VSD	AR
<i>Peronema canescens</i>	13.7–21.2	Marked by differences in vessel frequency and size (ring-porous porosity) (PROSEA 1994a; Ogata et al. 2008)	A	VSD	–
Bukit Hari, FRIM, Selangor					
Apocynaceae					
<i>Dyera costulata</i>	15.3–28.1	1. Marked by periodic layers without wood parenchyma (FRIM 1993) 2. Marked by differences in fiber wall thickness (PROSEA 1994b)	B	RF	AR
Dipterocarpaceae					
<i>Hopea odorata</i>	16.2–22.8	Indistinct (FRIM 1993; PROSEA 1994a)	C	–	RTC
<i>Shorea roxburghii</i>	13.6–22.0	Indistinct (FRIM 1993; PROSEA 1994a)	C	–	RTC
Meliaceae					
<i>Azadirachta excelsa</i>	15.5–21.7	Marked by differences in pore size, fiber wall thickness, and initial parenchyma (PROSEA 1994b)	B	VSD	AR, RTC
Verbenaceae					
<i>Peronema canescens</i>	13.3–18.5	Marked by differences in vessel frequency and size (ring-porous porosity) (PROSEA 1994a; Ogata et al. 2008)	A	VSD	–

Distinctiveness: A: Well defined, B: Poorly defined (with difficulties), C: Absent.

Type: FZ: Fiber zones, RF: Radially flattened fibers, MP: Marginal parenchyma, TF: Thick-walled fibers, VSD: Variations in vessel size and/or density.

Difficulties: IT: Intermittent growth ring, AR: Ambiguous growth ring, DC: Discontinuous growth ring, RTC: Occurrence of resin and/or traumatic canal in parenchyma band.

Table 1. (continued)

Study site Family Species	Diameter (cm)	Literature	Characteristics of growth rings		
			Distinctiveness	Type	Difficulties
Ayer Hitam Forest Reserve					
Apocynaceae					
<i>Dyera costulata</i>	16.0–36.2	1. Marked by periodic layers without wood parenchyma (FRIM 1993) 2. Marked by differences in fiber wall thickness (PROSEA 1994b)	B	RF	AR
Clusiaceae					
<i>Garcinia urophylla</i>	12.8–16.1	If present, marked by different regions of fewer parenchyma bands (PROSEA 1994c)	B	FZ, TF, RF	AR
Dipterocarpaceae					
<i>Hopea odorata</i>	22.0–27.9	Indistinct (FRIM 1993; PROSEA 1994a)	C	–	RTC
<i>Shorea acuminata</i>	18.2–33.9	Indistinct (FRIM 1993; PROSEA 1994a)	C	–	RTC
<i>Shorea parvifolia</i>	15.3–21.0	Indistinct (FRIM 1993; PROSEA 1994a)	B	TF	AR, RTC
Euphorbiaceae					
<i>Macaranga gigantea</i>	14.1–26.4	Indistinct (PROSEA 1994c)	B	RF	AR
Lauraceae					
<i>Litsea costalis</i>	20.7–31.5	1. Concentric markings produced by terminal bands or wood parenchyma (FRIM 1993) 2. Differences in fiber wall thickness and radial fiber diameter (PROSEA 1994b)	B	RF	IT
Leguminosae					
<i>Intsia palembanica</i>	15.6–25.6	1. Produced by terminal layers of wood parenchyma (FRIM 1993) 2. Marked by marginal parenchyma bands and/or differences in vessel frequency (PROSEA 1994a)	A	MP	–
<i>Sindora wallichii</i>	15.7–34.7	1. Produced by terminal layers of wood parenchyma (FRIM 1993) 2. Marked by terminal layers of wood parenchyma (PROSEA 1994a)	B	MP	IT, RTC
Melastomataceae					
<i>Pternandra echinata</i>	12.6–15.1	Indistinct (PROSEA 1994c)	B	FZ	AR
Meliaceae					
<i>Aglaia oligophylla</i>	15.1–23.0	Indistinct (PROSEA 1994b)	C	–	–

Distinctiveness: A: Well defined, B: Poorly defined (with difficulties), C: Absent.

Type: FZ: Fiber zones, RF: Radially flattened fibers, MP: Marginal parenchyma, TF: Thick-walled fibers, VSD: Variations in vessel size and/or density.

Difficulties: IT: Intermittent growth ring, AR: Ambiguous growth ring, DC: Discontinuous growth ring, RTC: Occurrence of resin and/or traumatic canal in parenchyma band.

Table 1. (continued)

Study site Family <i>Species</i>	Diameter (cm)	Characteristics of growth rings		
		Literature	Distinctiveness	Present study Type Difficulties
Ayer Hitam Forest Reserve				
Meliaceae				
<i>Azadirachta excelsa</i>	16.1–27.2	Marked by differences in pore size, fiber wall thickness, and initial parenchyma (PROSEA 1994b)	B	VSD, MP AR, DC, RTC
Moraceae				
<i>Artocarpus scortechini</i>	17.5–22.4	1. Indistinct (FRIM 1993) 2. Usually indistinct, but when present generally marked by long wings of parenchyma and smaller pores (PROSEA 1994b)	C	– –
<i>Streblus elongatus</i>	12.6–26.6	Indistinct, occasionally visible, indicated by darker colored bands with relatively few vessels and parenchyma (PROSEA 1994c)	C	– –
Myrtaceae				
<i>Syzygium polyanthum</i>	17.0–30.1	Indistinct or inconspicuous, but occasionally concentric with 3-5 mm-thick bands with few or no vessels (PROSEA 1994b)	B	FZ AR
Oleaceae				
<i>Ochanostachys amentacea</i>	16.3–37.9	Indistinct (PROSEA 1994b)	B	FZ AR
Passifloraceae				
<i>Paropsia vareciformis</i>	13.8–27.1	No literature	B	FZ AR
Rhizophoraceae				
<i>Gynotroches axillaris</i>	12.7–18.6	Indistinct (PROSEA 1994c)	C	– –
<i>Pellacalyx axillaris</i>	14.2–20.5	Indistinct (PROSEA 1994c)	C	– –
Rubiaceae				
<i>Aidia densiflora</i>	10.0–19.6	No literature	B	RF, FZ IT, AR
<i>Diplospora malaccense</i>	10.6–15.1	No literature	B	FZ IT, AR
<i>Timonius wallichianus</i>	12.3–18.1	Sometimes visible, indicated by layers of marginal parenchyma (PROSEA 1994c)	B	MP IT, AR, DC

Distinctiveness: A: Well defined, B: Poorly defined (with difficulties), C: Absent.

Type: FZ: Fiber zones, RF: Radially flattened fibers, MP: Marginal parenchyma, TF: Thick-walled fibers, VSD: Variations in vessel size and/or density.

Difficulties: IT: Intermittent growth ring, AR: Ambiguous growth ring, DC: Discontinuous growth ring, RTC: Occurrence of resin and/or traumatic canal in parenchyma band.

Table 1. (continued)

Study site Family Species	Diameter (cm)	Characteristics of growth rings		Present study	
		Literature	Distinctiveness	Type	Difficulties
Ayer Hitam Forest Reserve					
Sapindaceae					
<i>Pometia pinnata</i>	14.9–20.5	1. Vague concentric marking produced by terminal layers of wood parenchyma may be visible on end surface (FRIM 1993) 2. Marked by concentric bands of marginal parenchyma (PROSEA 1994a)	C	–	–
Sapotaceae					
<i>Palaquium maingayi</i>	17.3–24.1	1. Generally absent but occasionally concentric layers of dense fibers may occur that simulate growth rings (FRIM 1993) 2. Indistinct, if present, marked by differences in spacing on tangential parenchyma bands and/or in fiber wall thickness on either side of the ring boundary (PROSEA 1994a)	C	–	–
Thymelaeaceae					
<i>Gonystylus confusus</i>	12.1–21.9	Indistinct (FRIM 1993; PROSEA 1994a)	B	FZ	AR
Ulmaceae					
<i>Giromniera nervosa</i>	12.3–20.1	Indicated by banded parenchyma (PROSEA 1994c)	B	MP	IT, DC

Distinctiveness: A: Well defined, B: Poorly defined (with difficulties), C: Absent.

Type: FZ: Fiber zones, RF: Radially flattened fibers, MP: Marginal parenchyma, TF: Thick-walled fibers, VSD: Variations in vessel size and/or density.

Difficulties: IT: Intermittent growth ring, AR: Ambiguous growth ring, DC: Discontinuous growth ring, RTC: Occurrence of resin and/or traumatic canal in parenchyma band.

Hopea odorata), 1999 (*Shorea roxburghii*) and 2000 (*Peronema canescens*). The tree species in AHFR were selected because they are common species that grow in primary and secondary forest. The investigation of anatomical features was conducted using wood cores, as destructive methods are not permitted. Depending on the number and size of trees available in the study sites, up to 20 individuals were sampled for each species with an increment borer (diameter = 5.15 mm). Wood cores were extracted from the north, west, east, and south sides of each tree stem at breast height at the end of December 2011. Each core was examined macroscopically under an Olympus SZX12 stereomicroscope. Transverse sections 20–30 µm in thickness were cut using a sliding microtome and double-stained with safranin and fast green. All transverse sections were observed microscopically under an Olympus BX50 compound microscope.

Description of anatomical feature of growth rings

We classified growth rings as (A) well defined, (B) poorly defined, and (C) absent, on the basis of microscopic observation. Following Worbes (1985), the IAWA Committee (1989), and Carlquist (2001), we then considered the anatomical features of growth ring boundaries for each species and classified them into one or combination of the following five types:

1. Fiber zones (FZ): The tissue is mainly composed of wood fiber, which appears as a tangentially darker band macroscopically. Microscopically, it is a zone with fewer or no vessels and/or axial parenchyma cells.
2. Radially flattened fibers (RF): The sample contains radially narrow fibers.
3. Marginal parenchyma (MP): There is a tangentially arranged axial parenchyma band at the initial or terminal stage of xylem formation.
4. Thick-walled fibers (TF): The cell wall changes from thin-walled cells at the beginning of the growth period to thick-walled cells at the end of the growth period.
5. Variation in the vessel sizes and/or density (VSD): The vessel diameter and/or density changes from large diameter and low density to small diameter and high density in the xylem layer.

Some samples presented difficulties and their growth ring could not be easily classified. The difficulties encountered were categorized as:

1. Intermittent growth rings (IT): non-periodical and sporadic occurrence of growth rings.
2. Ambiguous growth rings (AR): indefinite or indiscernible

ring boundary.

3. Discontinuous growth rings (DC): discontinuous along a circumference.
4. Occurrence of resin canals and/or traumatic canals (RTC): resin canals and/or traumatic canals were included in parenchyma band.

RESULTS

Anatomical features of growth rings

Table 1 summarizes the degree of growth ring distinctiveness, types of growth rings, and types of difficulties encountered in each species studied. Unlike FRIM (1993) and PROSEA (1994a, b, c), our study revealed that even though some of the species investigated formed growth rings, these were either weak or faint. All five types of growth rings were observed and some species showed a combination of several types.

Growth rings were well defined in *Peronema canescens* (Verbenaceae; MAFR and BHFRIM), featuring ring-porous porosity (Figs. 2a and 2b); and *Intsia palembanica* (Leguminosae; AHFR), featuring marginal parenchyma (Figs. 6a and 6b). In these species, the rings showed essentially the same pattern and their occurrence might be caused by environmental factors. Growth rings were absent in 10 species. Poorly defined growth rings were encountered in 17 species.

Fiber zones (FZ) were most frequently observed as growth ring boundaries in eight species. These species were *Garcinia urophylla*, *Pternandra echinata*, *Syzygium polyanthum*, *Ochanostachys amentacea*, *Paropsia vareciformis* (Fig. 3), *Aidia densiflora*, *Diplospora malaccense*, and *Gonystylus confusus*. All these species grow naturally in the tropical rainforest climate of AHFR.

Growth rings featuring radially flattened fibers (RF) were identified in five species, on the basis of microscopic observation. These species were *Dyera costulata* (BHFRIM and AHFR), *G. urophylla* (AHFR), *Macaranga gigantea* (AHFR), *Litsea costalis* (AHFR, Fig. 4), and *A. densiflora* (AHFR).

Like RF growth rings, those that featured thick-walled fibers (TF) and clear ring boundaries were observed microscopically. Fiber wall thickness changed from thin-walled at the beginning of one growth period to thick-walled at the end of the period. Tree species featuring TF as growth rings were *G. urophylla* (Fig. 5) and *Shorea parvifolia*, both of which grow naturally in AHFR.

Five species showed marginal parenchyma (MP). This

Fig. 2. Transverse sections of ring-porous *Peronema canescens*. (a) Sample collected from Mata Ayer Forest Reserve; (b) Sample collected from Bukit Hari, Forest Research Institute Malaysia. Arrows indicate the direction of the bark side.

Fig. 3. Transverse sections of *Paropsia vareciformis* showing growth ring boundary marked by fiber zone. (a) Arrowheads indicate darker and clear ground tissue of growth rings. (b) Arrowheads indicate fiber zones with few or no axial parenchyma and/or vessels. Arrows indicate the direction of the bark side.

feature was distinct macroscopically, depending on the spacing between boundaries (Fig. 6a). Microscopically, MP appears as parenchyma bands associated with abrupt changes in radial diameter or wall thickness of fibers (Fig. 6b). Tree species classified as MP were *Azadirachta excelsa* (MAFR, AHFR), *I. palembanica* (AHFR), *Sindora wallichii* (AHFR), *Timonius wallichianus* (AHFR), and *Gironniera nervosa* (AHFR).

Clear variation in the vessel sizes and/or density (VSD) was found only in *P. canescens* (MAFR and BHFRIM), although weak VSD types included *A. excelsa*

(BHFRIM and AHFR) and *Gmelina arborea* (MAFR). We classified both *A. excelsa* and *G. arborea* as weak VSD because the changes in vessel sizes were not as clear as in *P. canescens* and there were only faint differences in vessel density. In this study, we primarily observed the anatomical features and did not measure the size of vessels, but we were able to determine the position of growth ring boundaries as the vessel feature changed from high density and small diameter to low density and large diameter in one xylem layer (e.g., Fig. 7). *A. densiflora* and *G. urophylla* growing in AHFR showed a combination of types of fibers

Fig. 4. Transverse sections of *Litsea costalis* showing growth ring boundary marked by radially flattened fibers. (a) Arrowheads indicate darker ground tissue of growth rings. (b) Arrowheads indicate the changes in radial diameter of fibers from larger diameter in the beginning of growth to smaller diameter at the end of the growth ring. Inset image shows the enlargement (from dashed circle) of the radially flattened fibers (arrowhead). Arrows indicate the direction of the bark side.

Fig. 5. Transverse sections of *Garcinia urophylla* showing a growth ring boundary marked by thick-walled fibers. (a) No clear darker ground tissue was observed. (b) Arrowheads indicate the differences in fiber wall thickness of the growth ring. Inset image shows the enlargement (from dashed box) of thick-walled fibers (TF). Arrow indicates the direction of the bark side.

in their growth rings, and *A. excelsa* (AHFR) showed a combination of growth rings between the MP and VSD types within the xylem layer.

Irregularities in growth rings

Table 1 includes 17 species that showed poorly defined growth rings. IT was occasionally found in the xylem layer and was distinguished morphologically from normal growth boundaries by its sporadic character or circuit of non-uniform growth zones (e.g., Figs. 8a, 8b and 8c). AR

Fig. 6. Transverse sections of *Intsia palembanica* showing a growth ring boundary marked by marginal parenchyma. (a) Arrowheads indicate the band of axial parenchyma. (b) Arrowhead indicates the marginal parenchyma band associated with the abrupt changes in fiber wall thickness and radial sizes. Inset image shows the enlargement (from dashed box) of axial parenchyma cells (AP) associated with abrupt changes in fiber wall thickness and radial sizes (F). Arrows indicate the direction of the bark side.

Fig. 7. Transverse section of *Azadirachta excelsa* showing a growth ring boundary marked by weak changes in vessel diameter from large to small diameter (arrowheads) and vessel density from high (HVD) to low (LVD) density. Arrow indicates the direction of the bark side.

included growth rings that were indefinite, macroscopically as well as microscopically (e.g., *D. costulata*; Fig. 9). In the DC type, ring boundaries are discontinuous at one or more points along the circumference. This feature was well observed macroscopically (e.g., Fig. 10). Another irregularity encountered was RTC in the parenchyma band. Minute observation revealed that there were differences between resin and traumatic canals with respect to the thickness of epithelial cell walls and the number of axial parenchyma cells surrounding these canals (e.g., Figs. 11b and 11d). Traumatic canals had thicker epithelial cell walls and were surrounded by more axial parenchyma cells than the resin canals.

A total of seven species showed IT irregularities: four species belonging to the MP group (*A. excelsa* in MAFR, *S. wallichii*, *T. wallichianus*, and *G. nervosa*), two belonging to the FZ group (*A. densiflora* and *D. malaccense*), and one belonging to the RF group (*L. costalis*). In this study, the AR irregularity type was the main problem in judging the growth ring boundary and was observed in 14 species. The majority of those species belonged to the FZ and RF groups; some also belonged to the VSD group (*A. excelsa* in BHFRIM and *G. arborea*). Most of the tree species with growth rings in the MP group showed DC irregularities (*A. excelsa* in AHFR, *T. wallichianus*, and *G. nervosa*). In this study, all the species belonging to Dipterocarpaceae formed

Fig. 8. Transverse sections showing intermittent occurrence of growth ring boundaries in the xylem layer of *Litsea costalis* (a) and *Azadirachta excelsa* (b and c). (a) Arrowheads indicate the intermittent occurrence of radially flattened fibers in the xylem layer. The exact growth ring boundary is still unknown and the tree may have formed many growth rings in a single year. (b) Arrowheads indicate the intermittent occurrence of axial parenchyma bands. (c) Arrowheads indicate the intermittent occurrence of marginal parenchyma in the xylem layer. Inset image 1 shows the enlargement (from dashed box 1) of axial parenchyma cells (AP) not associated with abrupt changes in fiber (F) wall thickness and radial diameter. Inset image 2 shows the enlargement (from dashed box 2) of axial parenchyma cells (AP) associated with abrupt changes in fibers from thin-walled (TnF) to thick-walled (TkF) fibers. Marginal parenchyma from dashed box 2 may be used as a growth ring boundary. Arrows indicate the direction of the bark side.

concentric bands of resin canals (e.g., Figs. 11a and 11b), and *A. excelsa* and *S. wallichii* formed concentric bands with traumatic canals (e.g., Figs. 11c and 11d).

DISCUSSION

Occurrence of growth rings in tropical rainforest trees

All of the tree species we studied had diffuse-porous porosity, except for ring-porous *P. canescens*. Most classifications of growth rings of temperate hardwood

Fig. 9. Transverse sections of *Dyera costulata* showing an ambiguous growth ring boundary. (a) No clear darker ground tissue can be observed. (b) Thin arrows indicate radially flattened fibers. Inset image shows the enlargement (from dashed box) of radially flattened fibers (arrowheads) in the region of the ambiguous growth ring. Arrow indicates the direction of the bark side.

Fig. 10. Transverse section of *Gironniera nervosa* showing discontinuous growth ring boundaries (circles). The parenchyma bands are either disconnected or disappear on the circuit. Arrow indicates the direction of the bark side.

species in the literature are based on vessel diameter changes, but it was not possible to judge the growth rings in most tropical rainforest trees. Even though most tropical rainforest trees do not form growth rings, mostly because there is less seasonality in tropical climates, this research proves that some of them do form growth rings, and that they are characterized by the anatomical features of FZ, RF, TF, MP, and variation in VSD.

Changes of fibers in the xylem layer were the most common feature to be characterized as a growth ring boundary in this study. In a zone in which the volumetric percentage of fibers is high, xylem is considered to function more in mechanical support than in water transportation and/or storage of carbohydrates. In species with the FZ type of growth ring boundary, the absence of vessels and/or axial

parenchyma in the growth ring boundary was obvious. In the tropical rainforest climate, where precipitation and temperature are high throughout the year, we may assume that trees, in general, grow continuously and tree physiological activities continue to function. We consider the appearance of fewer or no axial parenchyma cells and vessels in a xylem layer is attributable to the conditions under few carbohydrates to be stored and less soil water to be taken up during the time of their formation. Unexpected short drought may be one of such conditions.

Several species showed the growth ring boundary types of RF and TF. Faint dark tangential bands were observed macroscopically in some of the species. Using a microscope, we can observe the changes in the radial size of fibers and the thickness of the fiber wall close to the

Fig. 11. Transverse sections showing resin and traumatic canals in a parenchyma band. (a) Photomacrograph of *Hopea odorata*. Arrowhead indicates the parenchyma band. (b) Photomicrograph of *H. odorata*. Arrowheads indicate the concentric band of resin canal. Inset image shows an enlargement (from dashed box) of a resin canal (RC) surrounded by thin-walled epithelial cells (EC) and a few axial parenchyma cells (AP). (c) Photomacrograph of *Sindora wallichii*. Arrowheads indicate a series of parenchyma bands. (d) Photomicrograph of *S. wallichii*. Arrowheads indicate a concentric band of traumatic canals. Inset image show an enlargement (from dashed box) of a traumatic canal (TC) surrounded by thick-walled epithelial cells (EC) and massive axial parenchyma cells (AP). Arrows indicate the direction of the bark side.

growth ring boundary.

The formation of RF and TF may be explained through the lens of tree phenology. After observing darker rings on a disk of young para rubber (*Hevea brasiliensis*) grown in Malaysia, Ogata et al. (2001) found that the variation of fiber cell wall thickness and radial diameter of fibers in the darker rings were formed during the leafless period. Jalil et al. (1998) reported that such features are formed by the “wintering effect” during annual defoliation at the beginning of each year. Ogata et al. (2001) suggested that

the RF and TF are formed at the time of defoliation rather than during the final phase of shoot elongation. It is important to investigate the disappearance of axial parenchyma and/or vessels in the fiber zone and the changes of fiber radial size and cell wall thickness in order to better understand this group of growth ring boundaries.

There were five species with MP as the growth ring boundary. This feature was easy to observe macroscopically but still must be confirmed microscopically to avoid being confused with parenchyma bands, following the IAWA

Committee (1989). The IAWA Committee (1989) recommended using the marginal parenchyma bands only when they were associated with abrupt changes in fiber wall thickness and/or radial sizes as growth rings. MP appears to function primarily to store starch. Dünisch and Puls (2003) observed in Meliaceae species that starch is predominantly stored in axial parenchyma and that soluble carbohydrates are mobilized at the beginning of the growing period, to support the formation of new leaves and secondary xylem before the new foliage produces carbohydrates through photosynthesis. Ogata et al. (2001) reported that banded parenchyma in mature *H. brasiliensis* grown in Malaysia are formed during the leafless period. In summary, the formation of MP could be best explained as starch storage for flushes of growth. In this study, therefore, we consider MP to indicate the start of new growth, associated with an increase in fiber wall thickness and/or the decrease in fiber radial diameter. Fiber changes indicate the end of current growth in trees.

We found clear VSD in *P. canescens* (MAFR and BHFRIM) and weak VSD in *A. excelsa* (BHFRIM and AHFR) and *G. arborea* (MAFR). The variation of vessel size and/or density is most likely caused by changes in water availability to trees (e.g., Baas 1976; Baas et al. 1983; Alves and Angyalossy-Alfonsi 2000; Ohashi et al. 2009). Ohashi et al. (2013) reported that vessel features varied independent of water availability in all species studied grown in rainforest climate and most species grown in savanna and monsoon climate. Ohashi et al. (2013) also stressed that even water availability influences variation in vessels for some trees in the tropics, it is also depend on the species characteristics such as vessel porosity, leaf phenology and drought adaptations, and internal factors such as phytohormones. However, the actual factor causing variation in vessel size and/or density in tropical trees are still in dispute.

Through microscopic observation of wood sections and information from the MAFR officers on general phenological observation of tree species and site conditions, we consider that VSD in *P. canescens* and *G. arborea* grown in MAFR were influenced by the genetic factor controlling vessel porosity, phenomena of leaf shedding and adaptation to 3- to 4-month drought. VSD of *P. canescens* in BHFRIM was mostly considered to be influenced by the genetic factor since the site was along a river and always humid. *A. excelsa* grown in BHFRIM and AHFR were considered to be influenced by the genetic factor controlling semi-ring porous feature and short term leaf shedding. Further investigation on factors causing vessel changes in tropical rainforest trees should be conducted to deepen our

understandings.

Intra-specific variation in growth ring characteristics

The growth ring characteristics of several species in our study differ from those reported in the literature (FRIM 1993; PROSEA 1994a, b, c). For example, FRIM (1993) described *D. costulata* as being marked by periodic layers without axial parenchyma, whereas PROSEA (1994b) described *D. costulata* as being marked by differences of fiber wall thickness. In this study, we observed changes in fiber radial sizes as growth ring boundaries in *D. costulata* growing in BHFRIM and AHFR, and these samples were categorized as RF.

We present the following reasons for the differences: (1) most of the findings in the literature were intended for timber use, and so accurate descriptions of each species were unnecessary; (2) the trees examined were grown under different climatic conditions; and (3) the small number of sample replicates failed to cover the anatomical variations. Information provided by FRIM (1993), for instance, was used mostly for wood identification. They grouped different genera from the same family together in a single commercial timber group. Therefore, the information about growth rings in a particular trade group might actually refer to any of several genera within the group. Information provided by PROSEA (1994a, b, c) mostly covered the tree species grown in southeast Asia, and thus the growth ring information from this source might come from trees grown in a tropical seasonal area.

Different climatic and site conditions also caused different growth boundaries to develop in a single species. *A. excelsa* growing in MAFR had MP as the growth ring boundary, *A. excelsa* growing in BHFRIM showed weak VSD, and *A. excelsa* growing in AHFR had a combination of MP and weak VSD. *A. excelsa* in MAFR was growing in a tropical monsoon climate and sheds leaves over a 2–3-month dry period, resulting in the formation of MP as its growth ring boundary. *A. excelsa* in BHFRIM grows on a hill plot. Water availability during some low precipitation periods was distorted, perhaps triggering weak VSD in growth rings. In AHFR, the *A. excelsa* tree was growing in an open field. AHFR experienced at least 2 months of low precipitation, during which the tree continuously sheds leaves. Nobuchi et al. (1995) observed bands of parenchyma with small-diameter vessels as a growth ring in *H. odorata* in Thailand, and found that the growth rings were formed in the dry season. In our study, *H. odorata* grown in all study sites did not display distinct growth rings. Thus,

we suggest that the species reacts to environmental stressors, producing different growth ring boundaries.

Irregularities in poorly defined growth rings

In this study, irregularities were classified as IT, AR, DC, or RTC. The most common problems in dendrochronological studies in temperate and tropical seasonal areas are false rings and wedging rings (e.g., Worbes 1985, 2002; Bhattacharyya et al. 1992; Brien and Zuidema 2006; Rozendaal and Zuidema 2011; Worbes and Fichtler 2011). These features, also known as discontinuous, partial, or incomplete rings, were classified as DC in this study.

The irregularities IT, AR, and DC may be attributed to unusual cambial activity at certain positions of the stem. The explanation for wedging rings given by Schweingruber (1988) and Worbes and Fichtler (2011) is that they develop when a tree changes its direction of growth relative to the light source due to competition with surrounding trees. This direction change probably leads to differences in the local supply of carbohydrates, water, mineral elements, and phytohormones (Dünisch et al. 1999). Other explanations for wedging rings are unusual climatic extremes or traumatic events. In the case of IT in species that have MP as their growth rings, we differentiated the actual growth ring boundaries from the other parenchyma bands by strictly using the MP associated with abrupt changes in fiber wall thickness and/or radial size as the growth ring boundaries, following the IAWA Committee (1989).

We identified RTC in parenchyma bands for some species. Macroscopic observation alone suggested that the growth ring would be featured by MP. Minute microscopic observation revealed that in some cases, these tree species also produced parenchyma bands associated with resin canals and/or traumatic canals. Even though these canals became one of the main features that assisted in wood identification, their occurrence is considered to be contingent. We recommend avoiding the use of concentric parenchyma bands with resin canals as growth ring boundaries; as such canals are stimulated by traumatic events, such as physical or mechanical injuries.

Future extension of anatomical approach to studying growth rings

Our results from this study suggest that some tropical rainforest trees produce growth rings that are useful for dendrochronological studies. To further develop this

anatomical approach, it is necessary to use an appropriate sampling method, use the correct terminology for the anatomical description, and consider the effect of usual (periodical) and unusual (abrupt) changes.

Destructive sampling is often difficult, and disk samples may not be available for analysis, making it difficult to conduct circumferential observation of growth rings. Multiple samples of wood cores from a single tree are necessary in order to confirm the occurrence of growth rings. As wood anatomical terminology has been developed mainly in temperate regions, some technical terms are inappropriate for use in tropical dendrochronology. For example, a growth ring in a temperate tree has a ring width; whereas fiber zones in tropical trees, sometimes referred to as growth rings, have actually no width. It is necessary to standardize the terminology.

Finally, the anatomical structure of a growth ring includes the physiological effect of usual (periodical) changes during the dry season and unusual (abrupt or non-periodical) changes by ecological phenomena such as El Niño. Worbes and Fichtler (2011) stressed that weak or abrupt changes in endogenous and exogenous factors resulted in structural changes in the xylem layer and alterations to growth ring characteristics. Brien and Zuidema (2006) mentioned that the abrupt changes are of special interest in dendrochronological studies, as they can be used as to date certain incidents and analyze the impact of these events, such as extreme climatic events or suppression and release effects. Future anatomical studies should take the above points into consideration.

CONCLUSION

This study confirmed that some tropical rainforest tree species do form growth rings. Anatomical features that were considered as growth ring boundaries were fiber zones, radially flattened fibers, thick-walled fibers, marginal parenchyma, and variation in vessel size and/or density. *Peronema canescens* showed well-defined growth rings, featuring ring-porous porosity characteristics. *Intsia palembanica* also showed well-defined growth rings, featuring marginal parenchyma. A total of 17 species showed indefinable growth rings when some irregularities were included, such as intermittent growth rings, ambiguous growth rings, discontinuous growth rings, and resin and/or traumatic canals in the parenchyma band. Growth rings were absent from nine of the studied species. Our results confirmed the importance of selecting species that produce well-defined growth ring boundaries in tropical

dendrochronology. In a future task, it is recommended to extend the survey of potential tree species and to confirm whether those growth rings are formed annually or not, for precise tropical dendrochronological studies.

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