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ANATOMICAL STRUCTURES AND DIFFERENCES OF RATTAN GENERA FROM SOUTHEAST ASIA

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WEINER, G. & LIESE, W. 1988. Anatomical structures and differences of rattan genera from Southeast Asia. The anatomical structure of the rattan stem is presented. A detailed investigation of 53 species of the genera *Calamus, Daemonorops* and *Korthalsia* from Malaysia, Indonesia as well as the Philippines reveal characteristic differences. All genera investigated show the same general differentiation of the stem into peripheral and central corpus. Main structural features are the epidermis, parenchyma cells in the peripheral zone, and in the central corpus as well as the composition of the vascular bundles. Special structural features are represented by the "yellow cap", "ducts", and SiO_2-particles. Of diagnostic significance are the composition and arrangement of the vascular bundles, the peripheral parenchyma, and the morphological pattern of the parenchyma tissue.

Key words: Palmae - arecaceae - calamoideae - rattan - stem anatomy - vascular bundles - metaxylem - protoxylem - phloem - parenchymatous tissue.

Introduction

The large subfamily of the Calamoideae comprises 13 genera with about 568 species (Uhl & Dransfield 1987). Most of the genera (10) and their species occur in the Southeast Asian region with a limited number (4 genera; 3 endemic) in western Africa. From the many species only about 20 belonging to the genera *Calamus, Daemonorops* and *Korthalsia* are regularly used in Southeast Asia for the rattan trade, but almost all species are used by rural people to some extent.

The identification of rattan is based so far on morphological characters of the Palmae such as flower, fruit, leaf sheaths and leaves which, however, are removed during the processing of the rattan stem. An identification of only the stem is often difficult.

A comprehensive anatomical characterization of the rattan stems has not been undertaken so far. In 1845 H.V. Mohl investigated *Calamus* and described the large metaxylem vessel of its vascular bundle as peculiar in comparison with other genera of the Palmae. Solereder and Meyer (1928) supplemented this observation with further details for *Calamus* and *Daemonorops.* The studies of Cheadle and Whitford (1948), Parthasarathy and Klotz (1976) and Klotz (1978) on the metaxylem and phloem of Palmae hardly considered the subfamily Calamoideae. Tomlinson (1961) described briefly nine rattan genera in "Anatomy of the Monocotyledons". The first detailed description of the rattan stem anatomy of various

genera and species with the aim of a structural differentiation was undertaken by Siripatanadilok (1974) for five genera from Java, and by Teoh (1978) for eight genera from Malaysia. Whereas Siripatanadilok considered a differentiation of the genera in Java as feasible, such a possibility for Peninsular Malaysia was discounted by Teoh. Renuka *et al.* (1987) investigated the morphology and anatomy of nine *Calamus* species from India and concluded that anatomical characteristics were only applicable for the differentiation of genera but not for species.

In this paper, we made a detailed analysis of the structural components of 27 species from eight genera collected in the Peninsular Malaysia and Kalimantan, Indonesia. It revealed distinct anatomical features, which permitted an anatomical characterization of the genera (Liese & Weiner 1988, Weiner 1988). The general anatomy of the rattan stem is dealt with first, followed by an elaboration of characteristic differences between the main three genera used in the trade from Southeast Asia.

Materials and methods

Altogether 53 species of the genera *Calamus, Daemonorops* and *Korthalsia* were investigated. Thirteen species originating from East Kalimantan, Indonesia, were provided by the Universitas Mulawarman, Samarinda, and 19 species from Peninsular Malaysia were collected in cooperation with the Forest Research Institute Malaysia, Kepong, in the States Selangor and Negri Sembilan. Furthermore, 18 species of the genus *Korthalsia* from Malaysia and Indonesia were provided by J. Dransfield, Royal Botanic Gardens, Kew, United Kingdom; and another 18 species belonging to these three genera were made available by the Forest Products Research and Development Institute, Laguna, Philippines. The botanical identity of all samples investigated was checked at the herbarium of the respective institutes, based on vegetative features. Information about the herbarium vouchers is available from the authors.

The dried sample specimens were taken from an internode of a mature stem. Since earlier investigations (Weiner 1988) did not reveal distinct anatomical differences along the length of a stem, no specific designation of the sample's place was made. The material was boiled for *ca.* 10 *min* in distilled water, embedded in a mixture of 20 *ml* Polyetheleneglycol (PEG) [MW 2000] and 100 *ml* distilled water and then placed in an oven for 4-5 days at 60° *C* until all water had evaporated. For light microscopy, cross and longitudinal sections of 18-24 μm thickness were cut from the PEG penetrated blocks and the PEG rinsed thoroughly with distilled water. The sections were then double-stained with Acridin/Crysoidin red and Astra blue. Cross sections were embedded in glycerin, the more fragile longitudinal sections in Canada balsam. To recognize crystals and $Si0_2$, cross sections were bleached in a household bleaching agent (DAN KLORAX) containing chlorin, rinsed in water and embedded in clove oil (method after Ter Welle 1976, modified by Richter 1981).

Results and discussion

General anatomical structure of the rattan stem

In cross section, a rattan stem can be divided into two zones, the periphery without vascular bundles, and the central corpus with vascular bundles. The periphery consists of an epidermis followed immediately by rows of parenchyma cells. The epidermis is characterized by one row of radially oriented, unlignified, isodiametric cells with only few stomata. The cells are equal in size; their wall thickness varies between species. With the light microscope, the outer tangential cell wall appears rather homogeneous but exhibits a differential shadowing after double staining. Electron microscopical observations revealed that the cell wall of the epidermis is heavily impregnated with silica ($Si0_2$). The peripheral zone between the epidermis and the central corpus extends inwards to the first vascular bundles. It is composed of thick-walled, heavily lignified and partly sclerified parenchyma cells. They are rectangular to oval in outline and are oriented in tangential rows contrary to the parenchyma cells of the central corpus. The cell lumen partly contains red-brown substances. Within the parenchyma, small fibre islands as well as fibre islands with phloem rudiments, so-called "incomplete vascular bundles," are embedded.

The central corpus conforms with the typical structure of monocotyledons featuring dispersed vascular bundles embedded in a ground parenchyma tissue. The collateral vascular bundles consist of a centrally located xylem and external phloem, both of which are surrounded by a fibre and a parenchyma sheath. The xylem consists of metaxylem vessel(s) and protoxylem vessels. The metaxylem vessel is by far the largest element of the vascular bundle and can already be seen with the naked eye. Its form is round to oval. The lignified metaxylem vessel has simple perforation plates on transverse end walls. According to Parathasarathy and Klotz (1976) no correlation exists between the diameter of the stem and the diameter of the vessel or the size of the palm and the length of tracheary elements, respectively. The protoxylem vessels located near the metaxylem vessel consist of several unlignified, thin-walled cells, with ring-like wall thickenings. In general, the protoxylem contains one to five vessels, much smaller in diameter than the metaxylem vessel(s).

The phloem, situated between the metaxylem vessel and the fibre sheath, is formed by unlignified, thin-walled sieve tubes and companion cells. The sieve tubes have simple sieve plates as already described by Parathasarathy and Klotz (1976). The sieve tubes may be grouped in one or two fields. In a vascular bundle with only one phloem field, the field is situated opposite the protoxylem vessels, and the sieve tubes are located in a cluster. In vascular bundles with two phloem fields, the sieve tubes are arranged in two groups laterally with respect to the metaxylem vessel. The orientation of the sieve fields can be either at an acute angle or diametrically tangential (see Figures 2&7). These sieve tubes are lying mostly in one row in a "pearl-like" pattern (see Figure 2) or are arranged in a double row (see Figure 7). In genera with two phloem fields the number of sieve tubes with

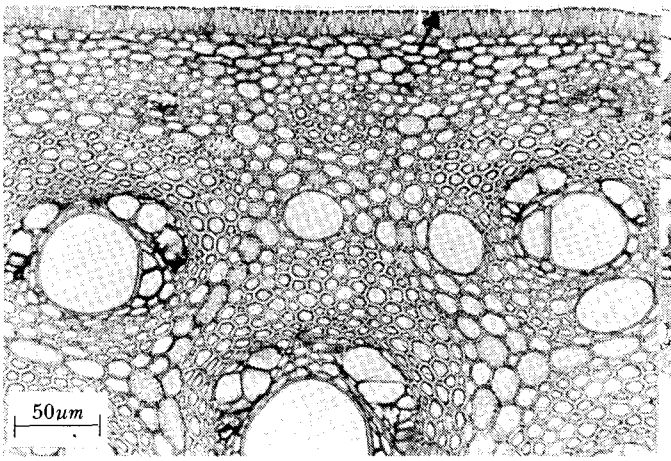

Figure 1. *Calamus exilis* periphery: epidermis (arrow) and peripheral zone

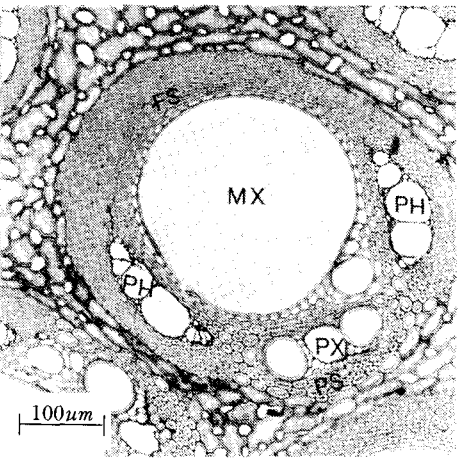

Figure 2. *Calamus caesius* vascular bundle with two phloem fields and one metaxylem vessel. FS: fibre sheath, PH: phloem fields, MX: metaxylem vessel, PX: protoxylem vessels, PS: parenchyma sheath

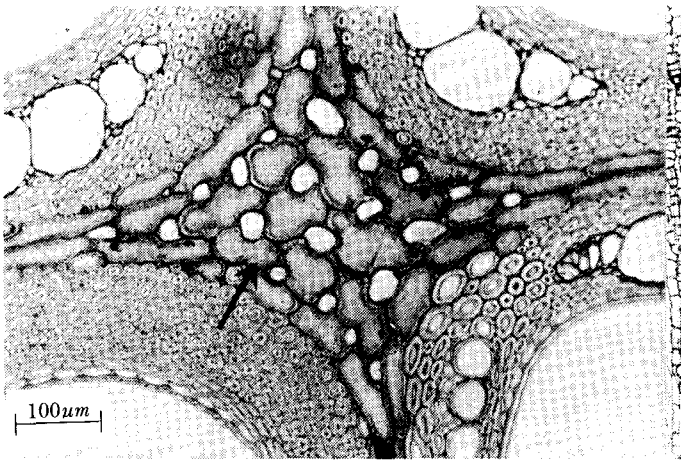

Figure 3. *Calamus caesius* ground tissue type A ("puzzle-like") [arrow], in cross section

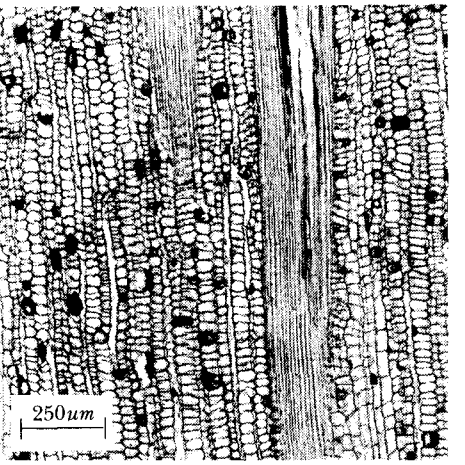

Figure 4. *Calamus conirostris* ground tissue (like "coins in a pile") in longitudinal section

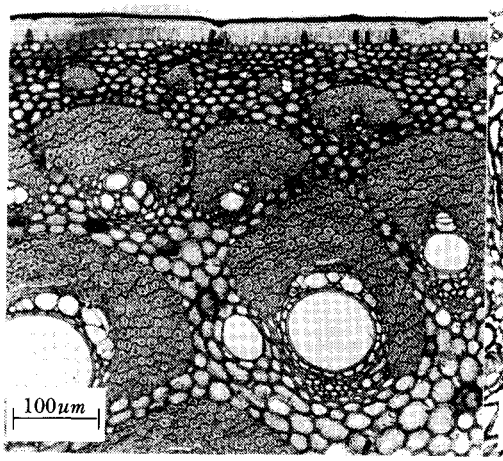

Figure 5. *Daemonorops angustifolia* epidermis and peripheral zone with fibre islands

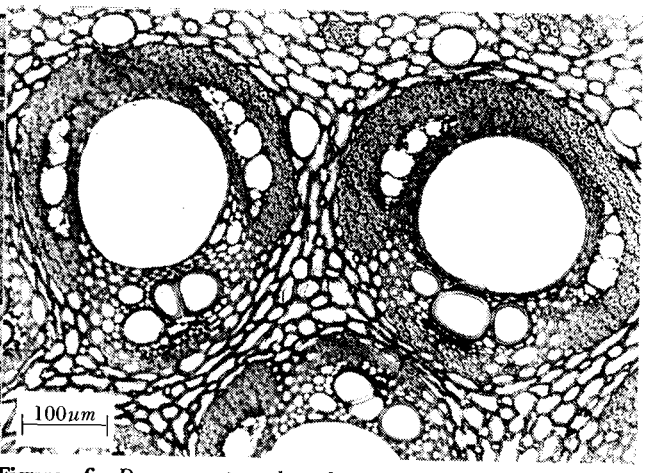

Figure 6. *Daemonorops melanochaetes* vascular bundles with two phloem fields and one metaxylem vessel

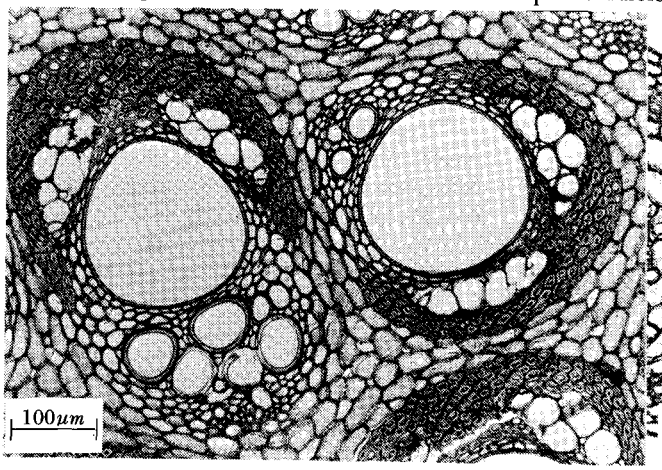

Figure 7. *Daemonorops angustifolia* vascular bundles with two phloem fields, sieve tubes arranged in a row and in a cluster, and one metaxylem vessel

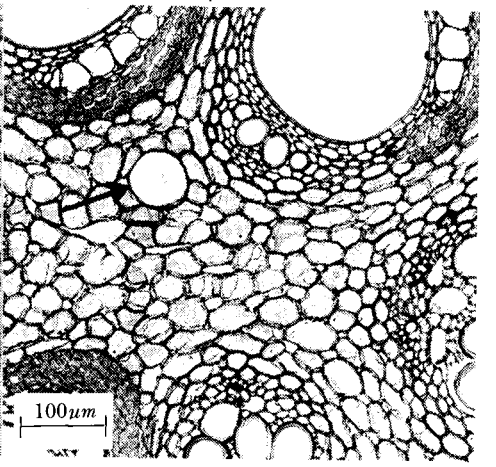

Figure 8. *Daemonorops angustifolia* ground tissue type B ("pebble-like") in cross section with a "duct" (arrow)

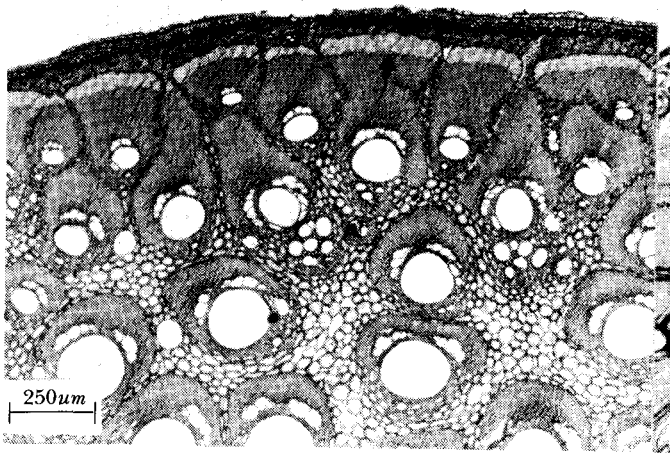

Figure 9. *Korthalsia echinometra* epidermis, peripheral zone and outer vascular bundles with "yellow cap" (arrow)

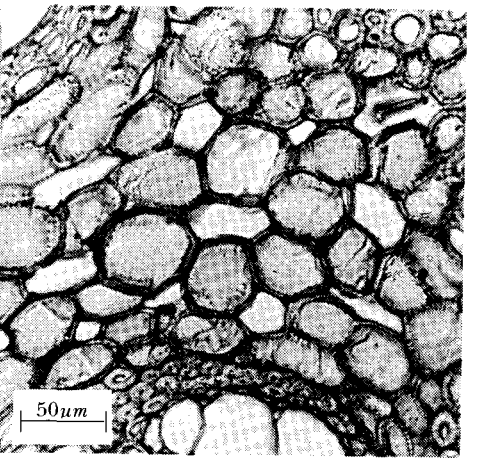

Figure 10. *Korthalsia echinometra* ground tissue type B ("pebble-like")

companion cells may vary from four to seven per field.

Species of the genera *Calamus, Daemonorops* and *Korthalsia* investigated in this study, always possess two phloem fields. The same anatomical characteristics apply to the genera *Calospatha, Ceratolobus, Pogonotium* and *Retispatha.* One phloem field is characteristic for only three (*Myrialepis, Plectocomia, Plectocomiopsis*) out of eight genera investigated by Weiner (1988).

The xylem, metaxylem and protoxylem are enclosed by a parenchymatous tissue of two morphologically different types: flat and rectangular cells surround the metaxylem vessel in an uniserate layer. This tissue may be interpreted as "paratracheal parenchyma." It appears quite different from the parenchyma of the vascular bundle, which surrounds sheath-like the metaxylem vessel(s) and protoxylem vessels towards the ground parenchyma. This parenchyma is characterized by small spheroidal cells with a distinct lumen and lignified walls, and is therefore different in form and size from the ground parenchyma. The fibre sheath surrounding the phloem and the metaxylem vessel(s) consists of thick-walled, heavily lignified fibres. Their walls show, already in the light microscope, a polylamellate structure which becomes distinct in the electron microscope (Parameswaran & Liese 1985). In longitudinal section, the fibres show unlignified septa. The fibre sheath varies in size and form over the cross section of the stem: in the outer third of the central corpus they are more voluminous, and the fibres are thicker-walled than in the central part. The extention of the fibre sheath correlates with the position of the phloem fields.

The ground tissue surrounding the vascular bundles consists of isodiametric parenchyma cells with lignified walls. These cells form a tissue with interspersed intercellular spaces. On account of their distinct form and arrangement, several types can be distinguished in cross section (Weiner & Liese 1987):

Type A: Large cells, irregular in form with numerous large intercellular spaces (they appear "jigsaw puzzle-like") (see Figure 3); and

Type B: Smaller cells with thick walls and an oval form with small inter- cellular spaces ("pebble-like") (see Figures 8 & 10).

In the longitudinal section, both types of parenchyma tissue are arranged in tidy rows of exact vertical orientation (like "coins in a pile") (see Figure 4). The genera which contain only one phloem field *(Myrialepis, Plectocomia, Pletocomiopsis)* evince a third type of ground parenchyma :

Type C: Large, thin-walled parenchyma cells, arranged in an orderly opposite pattern ("net-like").

In longitudinal section, the cells are elongated in series either vertically or horizontally. Besides the diffusely distributed vascular bundles, all genera show small fibre islands, consisting of few thick walled fibres.

The stem of the rattans exhibits some special structural features. The eight

genera investigated by Weiner (1988) contain in the ground parenchyma a few much larger cells with a diameter of 50 - 160 μm. These cells possess thin unlignified walls and are either arranged solitarily or in small clusters (see Figure 8, arrow). In a longitudinal section they appear as "ducts", but remnants of regular cross walls exist. Bundles of raphides 200 μm in length are present in these "ducts".

Particles of silicon dioxide ($Si0_2$) are located in small rectangular parenchyma cells between the fibre sheath or the fibre islands, respectively, and the neighbouring ground tissue. The $Si0_2$-particles display a "star-like" form and appear in all taxa with considerable variation in size and number.

Noteworthy is a differentiation of the fibre sheaths of the outer vascular ring in the genera *Korthalsia, Myrialepis, Plectocomia* and *Plectocomiopis*. In these genera, the outer part of the fibre sheath appears honey yellow after double staining, in contrast to the usual red colour of the fibre walls. A lumen is hardly discernible; the cell walls are polylamellar. Longitudinal sections confirm that these yellow-staining cells are fibre sclereids. As a result, the outer ring of vascular bundles in these genera seems continuous through these fibre sclereids (see Figure 9). This striking appearance has been named "yellow cap" by Siripatanadilok (1974).

The ground parenchyma of all genera contains reddish cell contents, which were described as tannins by Tomlinson (1961), Siripatanadilok (1974) and Teoh (1978). Reactions with $Fe^{II}Cl$ and $Fe^{III}Cl$ for the material of this study, however, proved to be negative.

Tyloses have been observed in the metaxylem vessels of several species. A possible relation to ageing or wounding has not been observed so far.

Differentiation and identification of the rattan genera Calamus, Daemonorops and Korthalsia

The results obtained from the investigation of the 53 species of three genera have shown that a differentiation of these genera on the basis of structural features appears possible. This confirms earlier observations based on 27 species from eight genera (Liese & Weiner 1988, Weiner 1988). Characteristics of diagnostic significance are:

a) vascular bundles
- number of phloem fields
- number of metaxylem vessel(s)
- presence or absence of the "yellow cap",

b) ground parenchyma
- type A
- type B
- type C

} arrangement in cross and longitudinal section, and

c) peripheral zone
- arrangement of parenchyma cells.

In the following, a description of the three genera is presented based on these characters.

Calamus: The species investigated are listed in Table 1. The **epidermis** consists of one row of isodiametric cells with unlignified walls. They are slender, equal in size, and 30-60 μm in radial direction. The ratio between tangential to radial diameter is approximately 1:4. The outer tangential wall of the epidermis is two to three times thicker than the radial cell walls. The **peripheral zone** consists of up to ten rows of lignified parenchyma cells, lying in a ring around the central corpus. The outer three or four cells are rectangular and appear more strongly lignified (Figure 1).

Table 1. Rattan material investigated

Calamus	*Daemonorops*	*Korthalsia*
caesius Blume (I,P.M.,P)	*angustifolia* (Griff.) Mart. (I,P.M.)	*angustifolia* Blume (I)
conirostris Becc. (P.M.)	*crinita* Blume (I)	*celebica* Becc. (I)
diepenhorsti Miq. (P)	*geniculata* (Griff.) Mart. (P.M.)	*cheb* Becc. (M)
discolor Mart. (P)	*grandis*(Griff.) Mart. (P.M.)	*concolor* Burret (M)
exilis Griff.(I,P.M.)	*kunstleri* Becc. (P.M.)	*debilis* Becc. (M)
filispadix Becc. (P)	*leptopus* (Griff.) Mart. (P.M.)	*echinometra* Becc. (I)
javensis Blume (I,P.M.,P)	*loheriana* Becc. (P)	*ferox* Becc. (I)
laevigatus Mart. (P.M.)	*melanochaetes* Blume (I)	*flagellaris* Miq. (P.M.)
manan Miq. (I, P.M.)	*mollis* Merrill (P)	*furcata* Becc. (M)
marginatus (Blume) Mart. (P)	*verticillaris* (Griff.) Mart. (P.M.)	*furtadoana* Dransfield (M)
merrillii Becc. (P)		*hispida* Becc. (P.M.)
microcarpus Becc. (P)		*jala* Dransfield (M)
microsphaerion Becc. (P)		*laciniosa* Mart. (P)
mindorensis Becc. (P)		*lanceolata* Dransfield (P.M.)
optimus Becc. (I)		*merrillii* Becc. (P)
ornatus Blume (I, P.M.)		*paucijuga* Becc. (M)
reyesianus Becc. (P)		*rigida* Blume (I, P.M.)
scabridulus Becc. (P.M.)		*robusta* Blume (I)
scipionum Lour. (I,P.M.)		*rostrata* Becc. (P.M.)
simphysiphus Mart. (P)		*scaphigeroides* Becc. (P)
trachycoleus Becc. (I,P.M.)		*scortechinii* Becc. (P.M.)
		tenuissima Becc. (P.M.)

(I) = Indonesia; (P.M.)= Peninsular Malaysia; (M)= East-Malaysia; (P)= Phillippines

In the **central corpus,** the first rows of vascular bundles are arranged in a circle. The vascular bundle itself consists of one metaxylem vessel (diameter approximately 300-350 μm, but only 150-170 μm in *C. javensis* and *C. exilisis*), and two lateral phloem fields about four to six sieve tubes each. The sieve tubes are arranged like a string of pearls. The surrounding fibre sheath is extensive and strongly lignified (Figure 2). The ground tissue conforms to type A -an interwoven network of large and generally thin-walled parenchyma cells resembling a jigsaw puzzle (Figure 3). Longitudinally individual cells are arranged in tidy rows of exact vertical orientation, like "coins in a pile" (Figure 4).

Daemonorops: The species investigated are listed in Table 1. The **epidermis** cells measure 20-30 μm in radial direction. With hardly any tangential size variation present two cell forms can be described: either square with a ratio 1:1 or rectangular with 1:2.5. The somewhat elongated cells resemble the slender epidermis cells of *Calamus.* The outer tangential wall of both cell types is twice as thick

as the radial walls. Reddish cell contents are located in the lumens of some species. The peripheral zone consists of six to ten rows of parenchyma cells. The outer ones are closely spaced and more heavily lignified than the following cells. The last inner cells are partly sclerified (Figure 5). In the central corpus the outer vascular bundles form a ring, whereas the following ones are diffusely arranged. The vascular bundles consist of one metaxylem vessel (diameter approximately 220-270 μm) and two lateral phloem fields with about four to five sieve tubes in a single row (Figure 6). As an exception, in *D. angustifolia* two forms of sieve tube arrangements are present, either in a row or in a cluster (Figure 7). The fibre sheath of thick-walled and strongly lignified cells surrounds the metaxylem vessel like a horseshoe. The ground tissue corresponds to type B (Figure 8). In longitudinal section the cells appear as "coins in a pile" (see Figure 4).

Korthalsia: The species investigated are listed in Table 1. The epidermis cells measure 12-35 μm radially. In cross section they appear square to rectangular. The ratio between tangential and radial cell dimension is approximately 1:2. Smaller epidermis cells besides the larger ones exist in the species *K. rigida, K. echinometra, K. laciniosa, K. jala* and *K. flagellaris.* These compact and square cells appear quite irregular with no difference in thickness. As many as ten parenchyma cell rows form the pripheral zone. They are isodiametric, and strongly lignified and sclerified towards the central corpus. The outermost vascular bundles of the central corpus form a "yellow cap" as sclereids as part of the first fibre sheath (Figure 9). The other vascular bundles do not exhibit this feature. The vascular bundle has one metaxylem vessel (diameter approximately 180-280 μm) and two phloem fields with about five sieve tubes each. The fibre sheath consists of thick-walled cells (Figure 10).

The **ground-tissue** conforms to type B (Figure 10), with the "coins pile" pattern longitudinally (see Figure 4). Based on the foregoing description, a dichotomic key can be developed for the three genera, as presented in Figure 11:

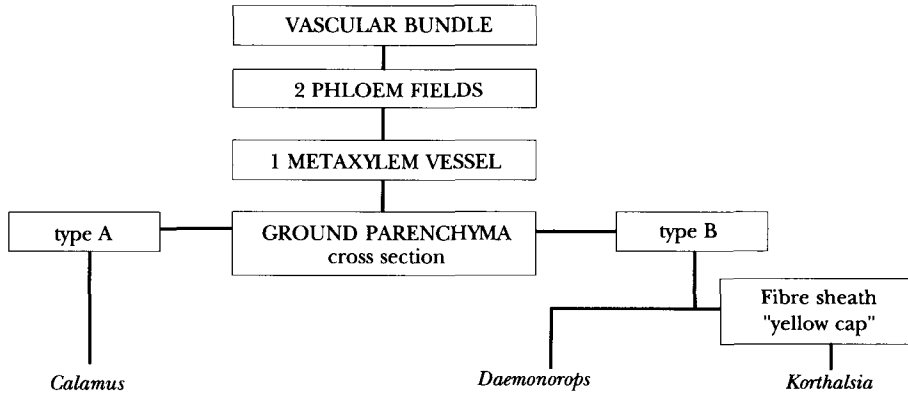

Figure 11. Identification key for the three rattan genera from Southeast Asia based on anatomical structures of the stem

Ongoing investigations indicate that most of the structural features outlined above also appear to be suitable for a differentiation of the other genera of the Calamoideae. The extent to which the epidermis can be used as a supporting diagnostic feature will have to be clarified. Siripatanadilok (1983) regarded it as an important taxonomic character.

Structural features of rattan were also described by Siripatanadilok (1974) and Teoh (1978) and regarded by Siripatanadilok as possible criteria for diagnostic purposes. Teoh pointed to the composition of the vascular bundle and the special feature "yellow cap" as criteria for differentiation. Based on these two features, she divided the eight genera investigated into three groups. Both authors did not consider the different types of ground tissue and the arrangement of the parenchyma in the peripheral zone as additional criteria. However, only a combination of all these characters permits a reliable identification of the genera.

For the $Si0_2$-particles described in this paper the term "stegmata" has been used by Tomlinson (1961). This term had apparently been introduced by Mettenius (1864) with its meaning as "Deckzelle". Both authors have obviously applied the same term for different cells. Tomlinson has also used the term "mucilage canals" for the large duct-like structures present in the rattans. In the present investigation on dead material no secretions of mucilage have been recognized. Raphides were observed in what appears as strands of individual cells.

Summary

The anatomical structure of rattan was observed in 53 species from the three genera *Calamus, Daemonorops* and *Korthalsia.* A differentiation between the periphery and central corpus is apparent. Structural elements are epidermis cells, parenchyma cells of the peripheral zone, the vascular bundles consisting of phloem, xylem, fibre and parenchyma sheath, embedded in the ground parenchyma as well as special structures. An anatomical differentiation and, consequently, an identification of the genera investigated is possible by a combination of these diagnostic features. It could be used for taxonomic purposes.

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Forest Products Research and Development Institute, College, Laguna, Philippines; and

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