ANATOMY AND IDENTIFICATION OF FIVE INDIGENOUS RATTAN SPECIES OF GHANA

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ABSTRACT

*Stem anatomy of **Calamus deeratus, Eremospatha hookeri, Eremospatha macrocarpa, Laccosperma acutiflorum** and **Laccosperma secundiflorum** growing naturally in Ghana were investigated to explore the possibility of using anatomical features to distinguish between them. Although the anatomy of all the stems of the five species investigated exhibited a common monocotyledonous structure, they differed considerably in many of their anatomical features. Anatomical features of taxonomic and diagnostic significance at genus level included: the number of metaxylem vessels and phloem fields in a vascular bundle and type of ground parenchyma. However, the most important anatomical features to distinguish species are the epidermal cell size and shape. A combination of several anatomical features is used to develop a tentative identification key to the five rattan species occurring naturally in Ghana.*

Keywords: Ghana, indigenous, rattan, anatomy, identification

INTRODUCTION

Rattan is a collective term commonly used for spiny palms belonging to the subfamily Calamoideae of the family palmae. This subfamily comprises 13 genera with more than 600 species (Uhl & Dransfield, 1987). Ten genera with their species occur in the Southeast Asian region and four genera of 19 species occur in West and Central Africa. Of these four genera: *Laccosperma, Eremospatha, Oncocalamus* are endemic to West and Central Africa region while *Calamus* is a native to both regions (Dransfield, 1981; Sunderland, 1997a).

In contrast to the situation in Southeast Asia, knowledge of Africa rattans is still not sufficiently known in spite of the very important role they play in the local economy of most West and Central African countries. Consequently, the rattan industry is beset with many problems including misidentification of semi-processed rattan stems. Once the rattan stems are cut and leaf sheaths removed, identification of the stems become difficult. Many rattan collectors group stems on the basis of stem diameter irrespective of botanical names. The local names often vary from one locality to another and sometimes the same name is given to more than one species. In addition, poor harvesting techniques and over-exploitation from its natural habitat without cultivation have led to scarcity of economic rattan supply, especially *Eremospatha spp,* the species of highest demand in Ghana. Hence, the future of the rattan industry upon which many rural people depend appears to be threatened (Oteng-Amoako & Obiri-Darko, 2000).

Cultivation of rattans remains the best option for sustaining the industry. Hence basic comprehensive research is needed to identify taxonomical, ecological, physical and anatomical properties that would help in conservation of genetic resources and lead to efficient utilization of rattans in Ghana. The primary objective of this study therefore was to determine anatomical properties of five indigenous rattan species of Ghana to aid in identification of their stems.

MATERIALS AND METHODS

A total of five species, comprising three genera, viz: *Calamus, Eremospatha* and *Laccosperma* were used in this study. Samples were collected from three different forest types of Ghana viz.: Wet

Evergreen (WE), Moist Evergreen (ME) and Moist Semideciduous (MSD) (Table 1). Within a forest type, five matured stems were collected randomly from different growing clumps for each species. Due to difficulty in collection, the entire length of stems could not be obtained. Herbarium samples for the taxonomic identity of the samples were also collected and kept in the herbarium of Forestry Research Institute of Ghana (FORIG).

For anatomical investigations, a sample of 1cm length at mid internode level was removed from the second basal, middle and top internodes positions from each of the collected stem. It was then subdivided into two diameter flanks. One flank was used for microtomy and the other for maceration (Fig. 1). The sample for microtomy was softened by first saturating it with water and later keeping it in a mixture of ethanol and glycerol (1:1) in labeled containers for an average period of about 20-30 days.

Five each of transverse and longitudinal sections of 15-25µm thickness were then made for each of the 165-microtomy samples on a sliding microtome. The sections were first washed in water and then stained in 1% safranin in 50% ethanol solution for about 10-20 minutes. After staining, they were washed in water and then dehydrated in increasing concentration of ethanol: 30, 50, 70, 85, 90 and 100% before being mounted in Canada Balsam. The sections were kept in xylene and creosote solution (1:1) and then xylene for hardening. All prepared slides were dried at 60°C overnight. The prepared slides of various species were then examined under light microscope following a prepared checklist of anatomical features.

TABLE 1

Species and Samples Used for the Study

Name of Species	Number of Stems				Mean Diameter (mm)	Mean Length (m)
	WE	ME	MSD	Total		
Calamus deeratus G. Mann and H. Wendl.	5	5	5	15	11.9	16.08
**Eremospatha hookeri* G. Mann and H. Wendl.	5	-	-	5	13	18.1
Eremospatha macrocarpa G. Mann and H. Wendl.	5	5	5	15	11	19.7
**Laccosperma acutiflorum* (Becc.) J. Dranst	5	-	-	5	28	10.3
Laccosperma secundiflorum P. Beauv	5	5	5	15	25.5	9.9

*Species not readily available at MSD and ME

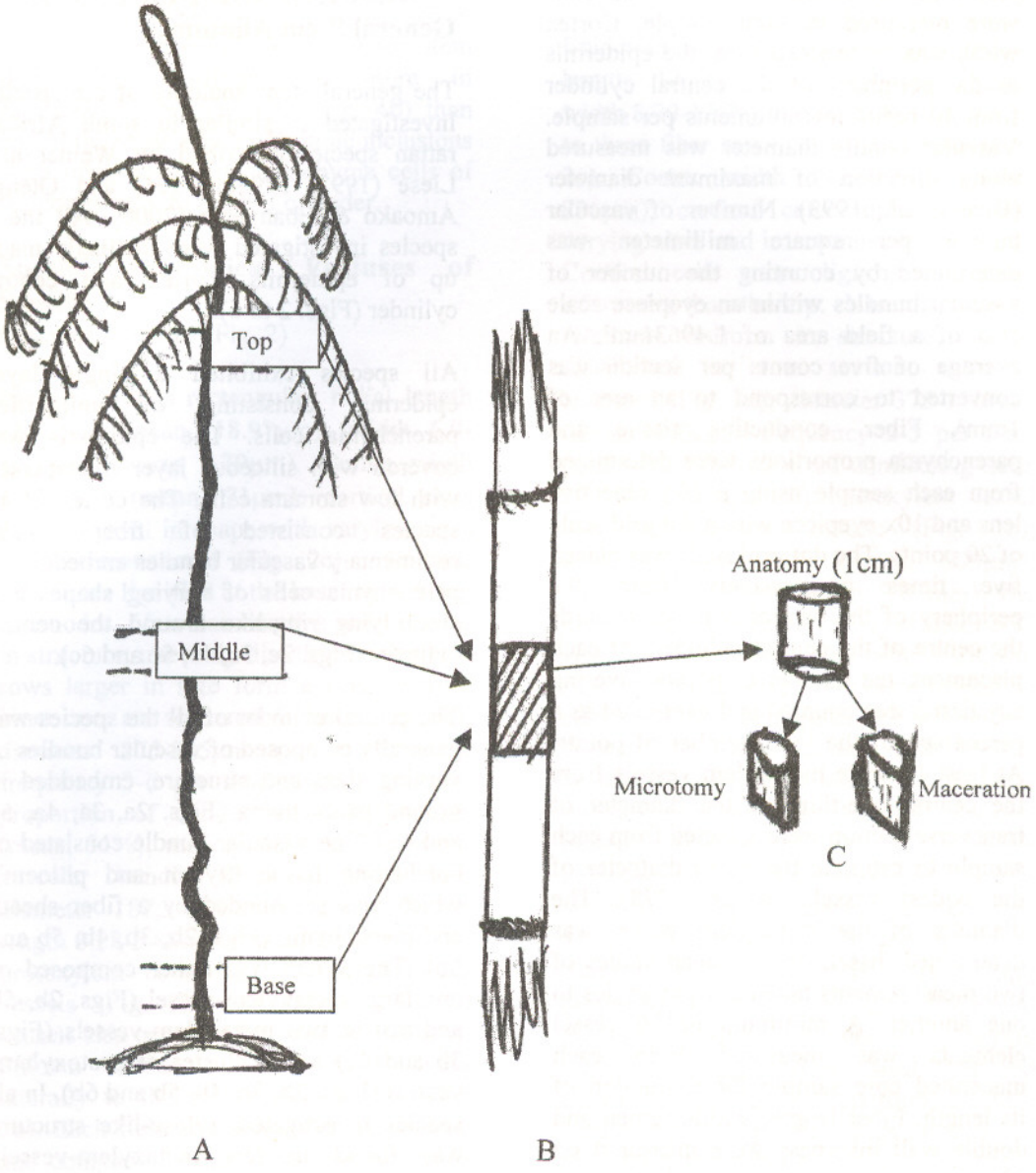

Fig. 1 Schematic diagram of (a) Rattan stem showing three levels: second basal, middle and top internodes (b) Internode showing mid portion (shaded portion) and (c) 1cm length sample and diameter flanks for microtomy and maceration.

From each of the samples for maceration, two splits of matchstick size were taken from the core and the periphery portions and kept in separate vials containing mixtures of 6% hydrogen peroxide and 97% acetic acid. The specimens were then incubated at 60°C for 3 days to obtain complete macerations. The macerated cells were rinsed with water and mounted temporarily in dilute glycerol for measurements of cell dimensions.

Measurements were made with an eyepiece scale of 100 divisions after the micrometer value has been determined for all the objective lenses. The radial length

and width of at least 20 epidermal cells were measured in each sample. Cortex width was determined from the epidermis to the periphery of the central cylinder from 10 radial measurements per sample. Vascular bundle diameter was measured along direction of maximum diameter (Bhat *et al.*, 1993). Number of vascular bundle per square millimeter was determined by counting the number of vascular bundles within an eyepiece scale grid of a field area of 1.4963mm². An average of five counts per section was converted to correspond to an area of 1mm². Fiber, conducting tissue and parenchyma proportions were determined from each sample using a 10x objective lens and 10x eyepiece with a dot grid scale of 20 points. The dot grid scale was placed five times progressively from the periphery of the central cylinder towards the centre of the central cylinder. At each placement, the number of points covering any tissue was counted and expressed as a percentage of the total number of points. At least 10 wide metaxylem vessels from the central one-third of the diameter of transverse section were selected from each sample to estimate the mean diameter of the widest vessels (Klotz, 1978). The diameter of the metaxylem vessel was determined, based on the mean values of two measurements taken at right angles to one another. A minimum of 10 vessel elements was measured from each macerated core sample for estimation of its length. Fiber length, width, lumen and double wall thickness were measured on 50 complete and straight fibers per macerated sample. Terminology for description followed the recommendations of IAWA Committee (1989) and Weiner and Liese (1990). Photomicrographs were made using a photomicroscope at Wood Anatomy Laboratory of FORIG. The development and printing of the micrographs were however done by private photo-laboratory in Kumasi. Qualitative anatomical features were presented in descriptive form with photomicrographs. StatView Software (1999 version) was used to perform all statistical analysis.

RESULTS AND DISCUSSION

General Stem Anatomy

The general stem anatomy of the species investigated is similar to some African rattan species described by Weiner and Liese (1992, 1993b, 1994) and Oteng-Amoako & Ebanyenle (2000). All the 5 species investigated were distinctly made up of epidermis, cortex and central cylinder (Figs. 2 to 6).

All species exhibited a single layer epidermis consisting of unlignified parenchyma cells. The epidermis was covered with siliceous layer interspersed with few stomata cells. The cortex of all species consisted of fiber bands, rudimentary vascular bundles embedded in parenchyma cells of varying shapes and sizes lying ring-like around the central cylinder (Figs. 2c, 3c, 4c, 5c and 6c).

The central cylinder of all the species was generally composed of vascular bundles of varying sizes and structure, embedded in ground parenchyma (Figs. 2a, 3a, 4a, 5a and 6a). The vascular bundle consisted of conducting tissue (xylem and phloem), which was surrounded by a fiber sheath and parenchyma (Figs. 2b, 3b, 4b, 5b and 6b). The xylem was either composed of one large metaxylem-vessel (Figs. 2b, 5b and 6b) or two metaxylem-vessels (Figs. 3b and 4b) and a cluster of protoxylem-vessels (Figs. 2b, 3b, 4b, 5b and 6b). In all species investigated, tylose-like structure was found in few metaxylem-vessels (Figs. 2b and 3b). The phloem consisted of different number of sieve tubes with companion cells (Figs. 2b, 3b, 4b, 5b and 6b). Raphides were occasionally seen in the tube-like structure in cross and longitudinal sections of ground parenchyma (Fig. 2d). Liese & Weiner (1989) and Weiner & Liese (1994) also reported of the occurrence of raphides in the cortex of some Asian and African rattan species respectively. Numerous silica bodies were found in small parenchyma cells of the cortex in the interface between the fiber sheath and the adjoining ground parenchyma (Figs. 2b, 3b, 4b, 5b and 6b). This compares well

with other findings of Weiner & Liese (1992, 1994). The silica bodies seemed to be more at the basal portion of the stem than other portions and more in *Eremospatha macrocarpa* (Fig. 4b) than any of the other species.Organic inclusions were often found in parenchyma cells of the cortex and the central cylinder.

Special Anatomical Features of Species

Calamus deeratus (Fig. 2)

*Epiderm*al cells rectangular; radial length 15-26μm (mean, 18.97μm); width 6.9-11.16μm (mean 9.29μm). Cortex width 45-113μm (mean, 71μm); cortical cells round to oval in shape with varying sizes and interconnected (Fig. 2c). Cortical cells more lignified at the basal than at top internodes. Vascular bundles not uniform in structure; unevenly distributed; first two rows larger in size form a ring; smaller inner ones are scattered diffused (Fig. 2a); diameter 275-825μm (mean, 516μm); frequency 3-6 per mm² (mean, 4 mm²); proportion of conducting cells 18-43% (mean, 31%); metaxylem vessel, one per vascular bundle (Figs. 2a and 2b); diameter 187.5-375μm (mean, 278μm); length 0.28-5.88mm (mean, 2.87mm). The protoxylem consisted of a cluster 2-6 vessels (Fig. 2b) but cluster of 6-10 vessels also occur in few vascular bundles. Phloem double stranded fields lying laterally to the metaxylem vessels (Fig. 2b). Each field contained 4-6 sieve tubes and companion cells. The fiber sheath is relatively extensive in peripheral and basal vascular bundles than inner and top internodes vascular bundles (Fig. 2a). Fiber length 0.60-4.2mm (mean, 1.94mm); width 5.8-34.8μm (mean, 17.89μm); lumen 2.9-29μm (mean, 9.01μm); wall thickness 1.45-20.3μm (mean, 8.88) and proportion 8-35% (mean, 23%). Ground parenchyma oval to round and sometimes weakly branched; more lignified at basal than top internodes. Proportion of parenchyma 32-65% (mean, 47%); type 'A' (Figs. 2d and 2e) (Weiner & Liese, 1990).

Eremospatha hookeri (Fig. 3)

Epidermal cells rectangular (Fig.2); radial length 12.76-19.72μm (mean15.78μm); width 8.70-12.76μm (mean 10.75μm), two to three fiber rows below epidermis (Fig. 2c). Cortex width 113-733μm (mean, 236μm); cortical cells round to oval; varying sizes and interconnected (Fig. 3c). Cortical cells more lignified at basal internodes than the top. Vascular bundles relatively uniform in structure; evenly distributed in cross section; diffusely arranged (Fig. 3a); diameter 375-1000μm (mean, 680μm); frequency 2-5 per mm² (mean 3). Proportion of conducting cells 27-50% (mean, 40%). Metaxylem vessel two per vascular bundle (Fig. 3b); diameter 200-438μm (mean, 309μm); length 0.13-4.90mm (mean, 2.87mm). The protoxylem consisted of a cluster 2-10 vessels (Figs. 3a and 3b). Single phloem field with 4-12 sieve tubes and companion cells (Fig. 3b) lie opposite to protoxylem vessels. The fiber sheath was slightly broader in peripheral and basal vascular bundles than the inner and top vascular bundles (Fig.3a). Fiber length 0.77-2.63mm (mean, 1.57mm); width 8.7-40.6μm (mean, 22.20μm); lumen 2.9-34.8μm (mean, 12.08μm); wall thickness 2.9-26.1μm (mean, 10.12μm) and proportion 10-48% (mean, 23%). Ground parenchyma round to oval with varying sizes; more lignified at basal than top internodes (Fig.2a); proportion 23-52% (mean, 38%); type 'B' (Figs.3d and 3e) (Weiner & Liese, 1990).

Eremospatha macrocarpa (Fig. 4)

Epidermal cells approximately square (Fig. 4c); radial length 9.86-16.82μm (mean, 13.01μm); width 9-13.34μm (mean, 11.27μm); two to three fiber rows directly below epidermis (Fig. 4c). Cortex width 73-373μm (mean, 172μm) (Fig. 4a); cortical cells round to oval with varying sizes; interconnected; more lignified at basal internodes than top (Fig. 4c). Vascular bundles relatively uniform in structure; evenly distributed; diffusely arranged (Fig. 4a); diameter 50-1150μm

(mean, 687μm); frequency 2-5 per mm²; (mean, 3); proportion of conducting cells 29-52% (mean, 40%). Metaxylem vessel two per vascular bundle (Figs. 4a and 4b); diameter 150-437.5μm (mean, 305μm); length 0.84-4.62mm (mean, 2.50mm). The protoxylem consisted of a cluster of 2 to 10 small vessels (Figs. 4a and 4b); phloem single field lying opposite to protoxylem vessels; field contained a cluster of 4-12 sieve tubes and companion cells (4b). The fiber sheath slightly broader in peripheral and basal vascular bundles than inner and top vascular bundles (Fig. 4a). Fiber length 0.175-2.63mm (mean, 1.32mm); width 8.7- 43.5μm (mean, 19.93μm); lumen 1.45-37.7μm (mean, 10.27μm); wall thickness 2.9-26.1μm (mean, 9.65μm) and proportion 10-48.33% (mean, 26%). Ground parenchyma round to oval with uniform sizes; more lignified at basal than top internodes (Fig. 4a); proportion 20-57% (mean, 33%); type 'C' (Figs. 4d and 4e) (Weiner & Liese, 1990).

Laccosperma acutiflorum (Fig. 5)

Epidermal cells rectangular; radial length 13.9-19.72μm (mean 16.01μm); width 8.0-13.34μm (mean, 11.29μm); two to three fiber or sclereids below epidermis (Fig. 5c). Cortex width 105-477.5μm (mean, 265μm); cortical cells round, oval and occasionally radially elongated (rectangular); varying sizes; more lignified at the basal internodes than top internodes; not connected; intercellular spaces present (Fig. 5c). Vascular bundles not uniform in structure; not evenly distributed; larger peripheral vascular bundles "patch-work-like" arranged; inner smaller ones diffusely scattered; diameter 312.5-937.5μm (mean, 646μm); frequency 2-4 per mm² (mean, 3). Proportion of conducting cells 17-32% (mean, 24%). Metaxylem vessel one per vascular bundle (Figs. 5a and 5b); diameter 213-363μm (mean, 276μm); length 0.70-4.20mm (mean, 2.25mm). The protoxylem consisted of a cluster of 1-6 vessels. Phloem single field; field contained a cluster of 4-10 sieve tubes. The fiber sheath is very extensive at the peripheral vascular bundles than inner vascular bundles. The fiber sheath of inner vascular bundles are 'horse-shoe shaped' (Fig. 5a); fiber length 0.87-4.73mm (mean, 2.40mm); width 8.7-34.8μm (mean, 18.03μm); lumen 1.45-23.2μm (mean, 5.40μm); wall thickness 2.9-31.9μm (mean, 12.63μm) and proportion 23-42% (mean, 30%). Ground parenchyma round, oval and sometimes radially elongated (rectangular); more lignified at basal than top internodes; proportion 38-57% (mean, 46%); type 'B' (Figs. 5d and 5e) (Weiner & Liese, 1990).

Laccosperma secundiflorum (Fig. 6)

Epidermal cells approximately square; radial length 11.6-19μm (mean, 14.63μm); width 8.7-14.5μm (mean, 11.95μm); two to three sclereids or fiber rows below epidermal cells (Fig. 6c). Cortex width 80-822.5μm (mean, 258μm); cortical cells round, oval and occasionally radially elongated (rectangular) of varying sizes; more lignified at the basal internodes than upper internodes; not connected; intercellular spaces present (Fig. 6c). Vascular bundles not uniform in structure; not evenly distributed; larger peripheral vascular bundles ''patch-work-like arranged''; inner ones diffusely scattered (Fig. 6a), diameter 287.5-1250μm (mean, 658μm); frequency 2-4 (mean, 3). Proportion of conducting tissue 12-33% (mean, 23%). Metaxylem vessel one per vascular bundle (Fig. 6a and 6b); diameter 175-375μm(mean, 266μm); length 0.91-5495mm (mean, 2.16mm). The protoxylem consisted of a cluster of 1-6 vessels. Phloem single field; field contained a cluster of 4-10 sieve tubes. The fiber sheath very extensive in the peripheral vascular bundles than inner vascular bundles. The fiber sheath of inner vascular bundles is "horse-shoe shaped"(Fig. 6a). Fiber length 0.42-6.65mm (mean, 2.80mm); width 7.250-43.5μm (mean, 19.01μm); lumen 0.29-26μm (mean, 6.58μm); wall thickness 2.9-37.7μm (mean, 12.42μm) and proportion 15-45% (mean, 29%). Ground parenchyma round to oval and sometimes

radially elongated (rectangular); more lignified at basal than top internodes; proportion 35-63% (mean, 48%); type 'C' (Figs. 6d and 6e) (Weiner & Liese, 1990).

Stem Anatomy and Identification

Although all the stems of *C. deeratus* (Fig. 2), *E. hookeri* (Fig. 3), *E. macrocarpa* (Fig. 4), *L. acutiflorum* (Fig. 5), and *L. secundiflorum* (Fig. 6) exhibited general monocotyledonous structure (Tomlinson, 1961), they differed considerably in many of the anatomical features.

A comparison of the anatomical variation in the stems of the species showed that the most promising features for separating rattan genera in Ghana are: number of metaxylem vessels, phloem fields per vascular bundle and type of ground parenchyma (Figs. 2 to 6). Weiner and Liese (1988; 1990; 1993a; 1994) also used these features for generic separation of some Asian and African rattans. Presence or absence of fiber rows below epidermal cells, nature of fiber sheath of vascular bundles at the periphery of the central cylinder can be used as supporting features for rattan genera separation in Ghana rattans. For example at the generic level *Calamus sp.* differed from *Eremospatha spp.* and *Laccosperma spp.* by having: - one metaxylem vessel with double phloem field strand lying laterally to it (Figs. 2a and 2b); ground parenchyma type 'A' (Figs. 2d and 2e), and the absence of fiber rows below epidermal cells (Fig. 2c). On the other hand, *Eremospatha* spp. differed from *Laccosperma* spp. and *Calamus deeratus* by exhibiting two metaxylem vessels per vascular bundle and having relatively uniform size and distribution of vascular bundles (Figs. 3 and 4). However, *Laccosperma spp.* differed from *C. deeratus*, and *Eremospatha spp.* by showing a vascular bundle consisting of one metaxylem vessel and a single cluster of phloem field lying opposite to the protoxylem vessels; the "patch-work-like" arrangement of the vascular bundles and the relatively extensive fiber sheath in vascular bundles at the periphery of the central cylinder (Figs. 5 and 6)

The investigation also suggests that identification of species within the same genus could be possible using the type of ground parenchyma, the shape and size of epidermal cells. Siripatanadilok (1983); Weiner & Liese, (1993b); Bhat *et al.*, (1993) and Mathew & Bhat 1997 also observed that epidermal cell size and shape could be useful in identification of rattans at the species level. For example *E. macrocarp*a differed from *E. hookeri* by having relatively square shaped epidermal cells and ground parenchyma type 'C' (Fig.4), whereas *E. hookeri* has rectangular shaped epidermal cells and ground parenchyma type 'B' (Fig. 3). Although *L. acutiflorum* and *L. secundiflorum* both exhibited rectangular epidermal cells, the former had longer radial epidermal cell length than the latter. In addition, *L. acutiflorum* exhibited ground parenchyma type 'B' (Fig. 5) whereas *L. secundiflorum* exhibited ground parenchyma type 'C' (Fig. 6).

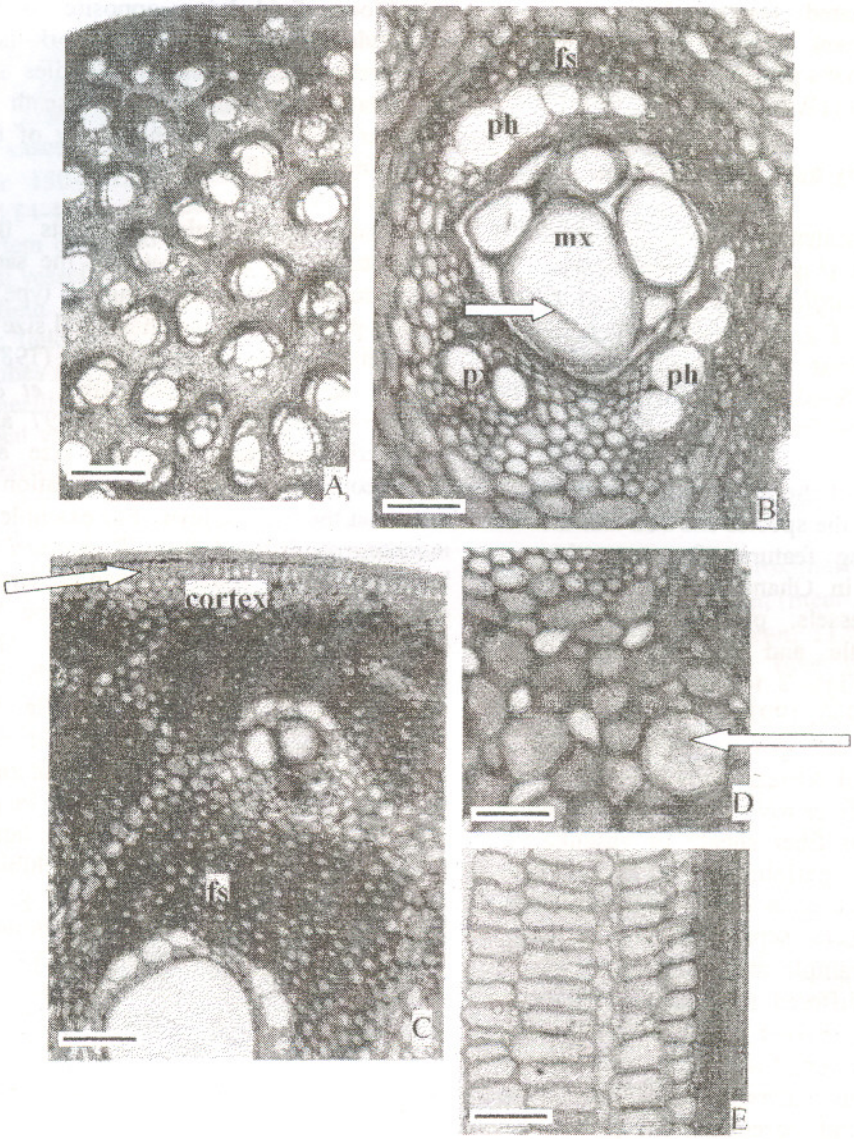

Fig. 2: *Calamus deeratus* (a) cross-section. Scale bar =25µm; (b) vascular bundle: fiber sheath (fs); double stranded phloem (ph); one metaxylem vessel (mx) with tylose-like structure (arrowed) and protoxylem vessels (px). Scale bar =100µm; (c) periphery: rectangular epidermal cells (arrowed) and round to oval cortical cells. Scale bar =100µm;(d) cross section, ground parenchyma type 'A'; tube-like structure containing raphides (arrowed) and (e) longitudinal section, ground parenchyma, ' like stacks of coins'. Scale bar= 100 µm.

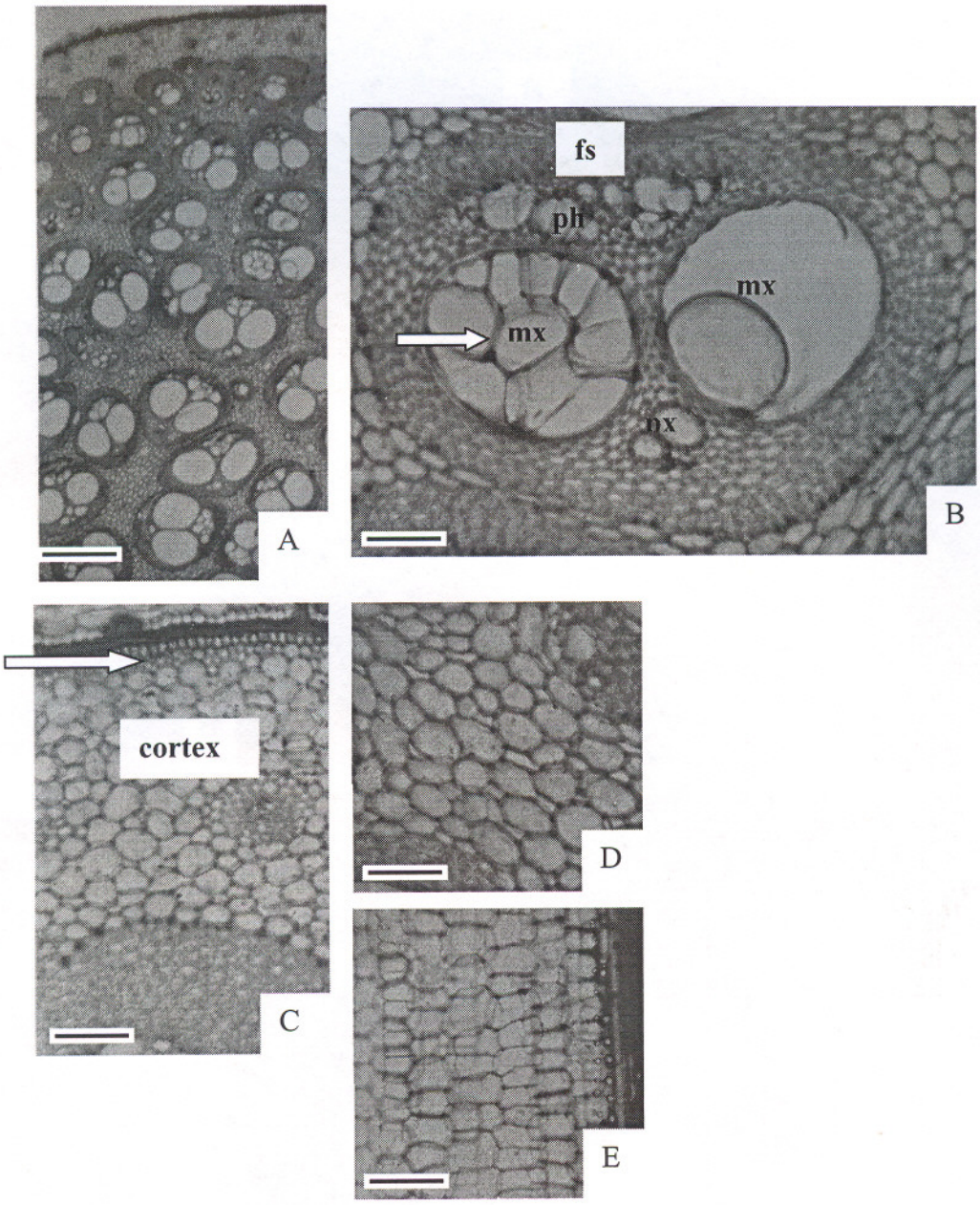

Fig. 3: *Eremospatha hookeri* (a) cross-section. Scale bar =25μm; (b) vascular bundle showing single phloem field, two metaxylem vessels with tylose-like structure (arrowed) and protoxylem vessels. Scale bar =100μm; (c) periphery showing the rectangular epidermal and round to oval cortical cells. Scale bar =100μm; (d) cross section, ground parenchyma type 'B'; (e) longitudinal section, ground parenchyma; 'like stacks of coins' Scale bar =100μm.

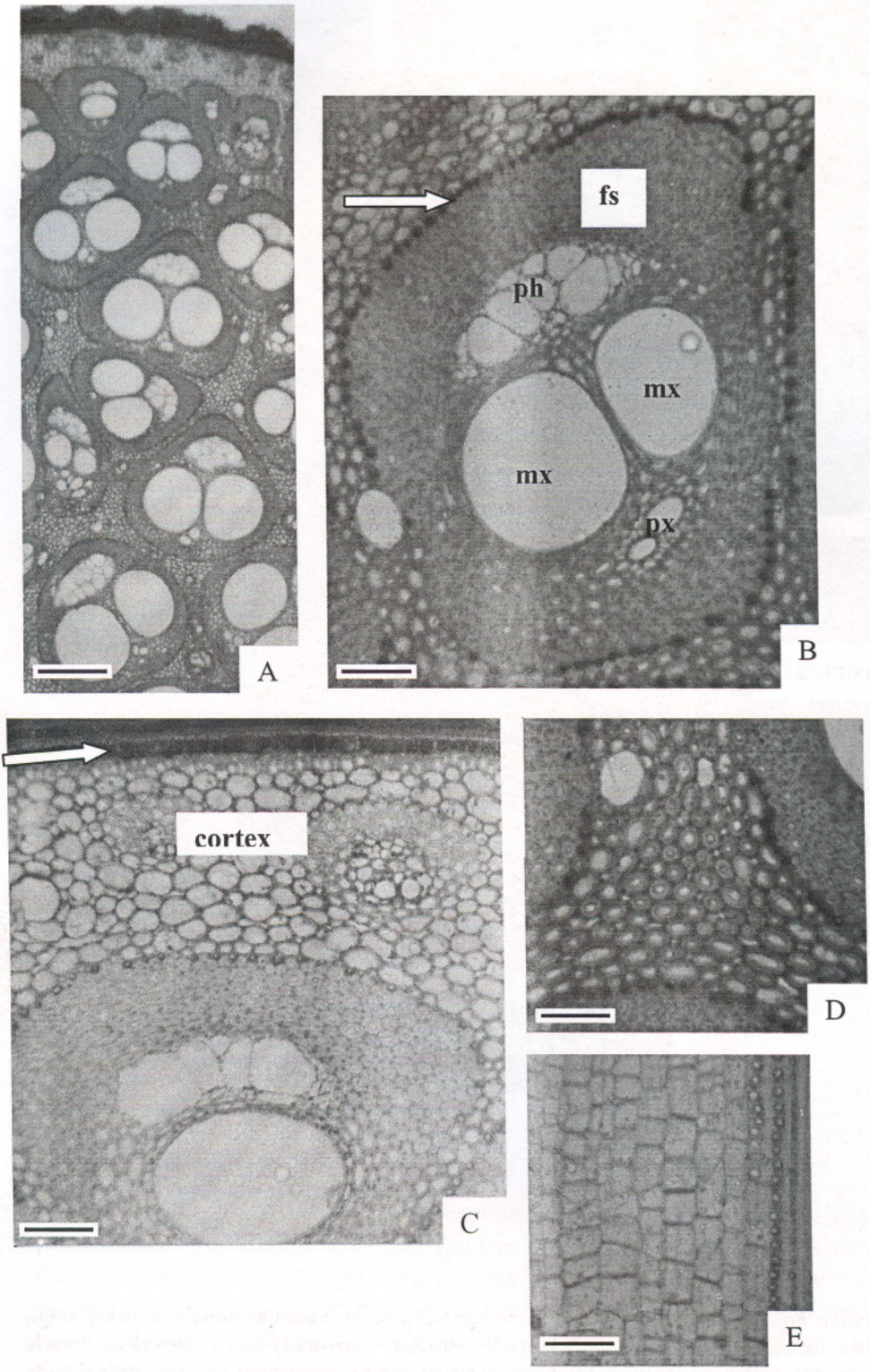

Fig. 4: *Eremospatha macrocarpa* (a) cross-section, Scale bar =25µm. (b) vascular bundle showing silica (arrowed); single phloem field, two metaxylem vessels and protoxylem vessels. Scale bar =100µm; (c) periphery showing square epidermal (arrowed) and round to oval cortical cells. Scale bar =100µm; (d) cross section, ground parenchyma type 'C' and (e) longitudinal section, ground parenchyma; 'short and elongated cells oriented perpendicular to each other'. Scale bar =100µm.

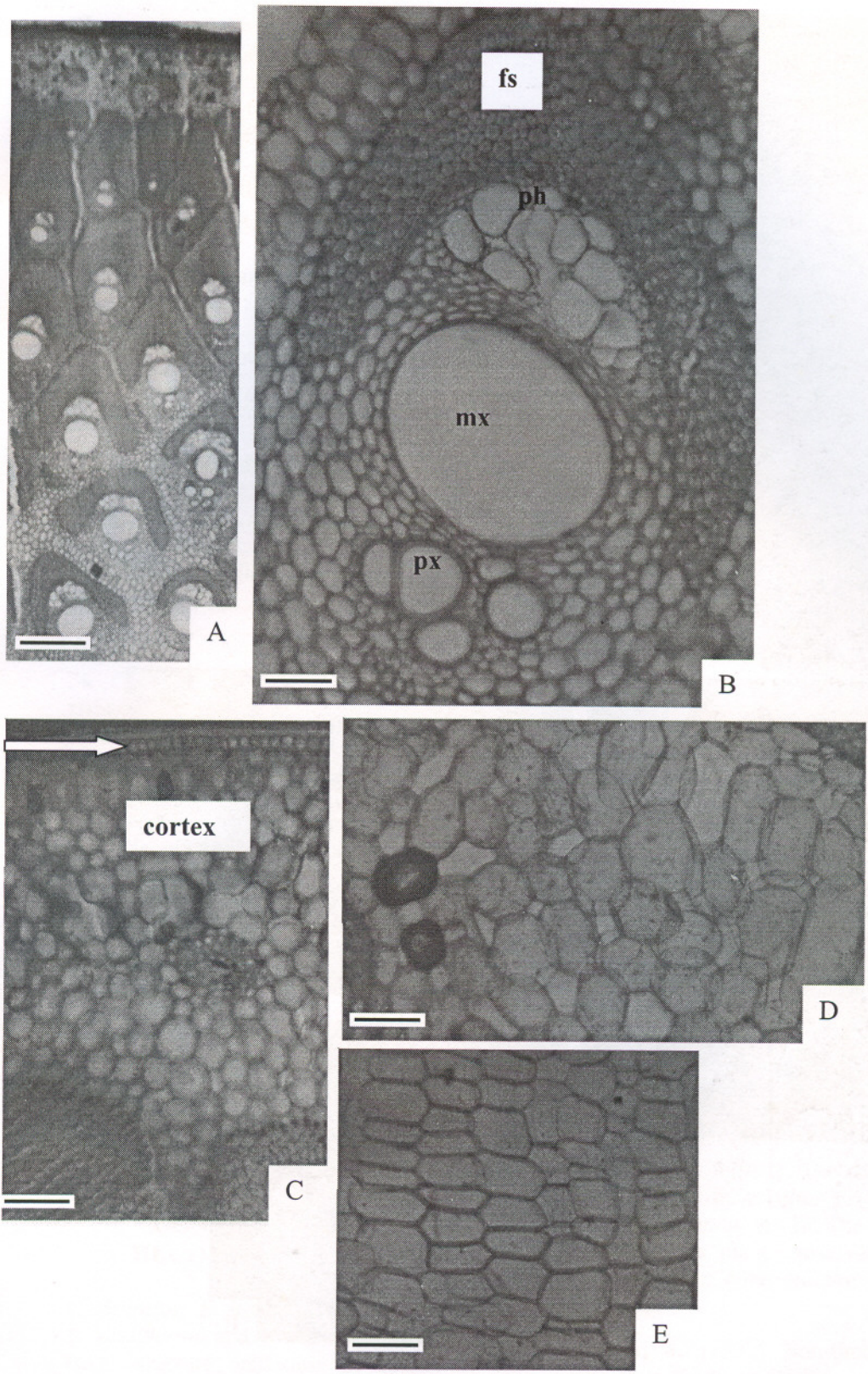

Fig. 5: *Laccosperma acutiflorum* (a) cross-section. Scale bar =25µm. (b) vascular bundle showing single phloem field, one metaxylem vessel and protoxylem vessels. Scale bar =100µm; (c) periphery showing rectangular epidermal (arrowed) and round to oval cortical cells. Scale bar =100µm; (d) cross section, ground parenchyma type 'B'; (e) longitudinal section, ground parenchyma, like 'stacks of coins' Scale bar =100µm.

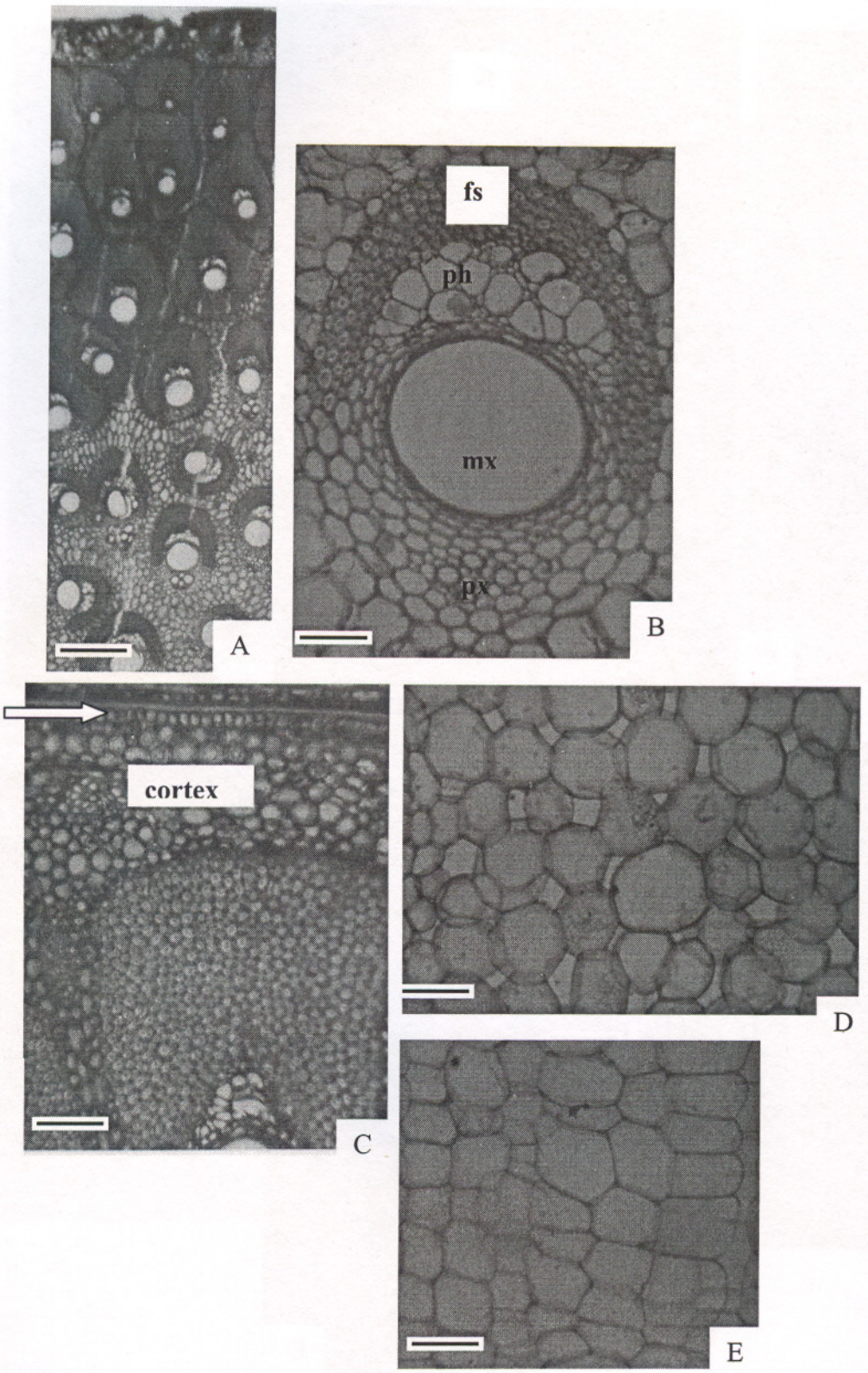

Fig. 6: *Laccosperma secundiflorum*: (a) cross-section. Scale bar =25µm (b) vascular bundle showing single phloem field, one metaxylem vessel and protoxylem vessels. Scale bar =100µm; (c) periphery showing approximately square epidermal (arrowed) and round to oval cortical cells. Scale bar =100µm; (d) cross section, ground parenchyma type 'C' and (e) longitudinal section, ground parenchyma; 'short and elongated cells oriented perpendicular to each other'. Scale bar =100µm.

These diagnostic anatomical features identified at the generic and species level have been used to present a tentative identification key to the five rattan species of Ghana as follows:

Tentative Identification Key to Five Rattan Species Occurring in Ghana

1a. Vascular bundle with one phloem field.....
.....2
b. Vascular bundle with two phloem fields.....
.....*C. deeratus*

2a. Vascular bundle with one metaxylem vessel.....
.....3

b. Vascular bundle with two metaxylem vessel.....
.....4

3a. Ground parenchyma type 'C'; mean radial epidermal cell length up to 15µm...
.....
.....*L.secundiflorum*

b. Ground parenchyma type 'C'; mean radial epidermal cell length up to 16µ...
.....
..... *L. acutilforum*

4a. Ground parenchyma type 'B'; mean radial epidermal cell length up to 16µm
.....
.....*E. hookeri*

b. Ground parenchyma type 'C 'mean radial epidermal cell length up to 13µm.....
.....*E. macrocarpa*

CONCLUSION AND RECOMMENDATIONS

C. deeratus; E. hookeri; E. macrocarpa; L. acutiflorum and *L. secundiflorum* have basic structure consisting of epidermis, cortex and vascular bundles embedded in ground parenchyma similar to other Asian rattans. However all the species investigated have fiber rows below the epidermis with exception of *C. deeratus*. This feature therefore distinguishes endemic African rattans from Asian rattans.

Anatomical features of taxonomic and diagnostic significance at genus level include: the number of metaxylem vessels and phloem fields in a vascular bundle and type of ground parenchyma. It is possible to use these features to differentiate between the five rattan species of Ghana.

It is recommended that, the anatomical properties of stems of *L. laeve*, which is currently not being utilized in Ghana, should be undertaken to identify diagnostic features to aid in its identification and possible use.

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