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COMPARATIVE VEGETATIVE ANATOMICAL STUDY OF THE GENUS *CALOPHYLLUM* L. (CLUSIACEAE) IN SRI LANKA

S. Somaratne and Tissa R. Heart*
Department of Botany, The Open University of Sri Lanka, Nawala, Nugegoda, Sri Lanka.
*Faculty of Applied Sciences II, The Rajarata University of Sri Lanka Polgolla, Sri Lanka.

ABSTRACT

Vegetative anatomical studies of 13 species of *Calophyllum* L. taxa described by earlier workers to be present in Sri Lanka were carried out. A detailed anatomical investigation of the lamina, petiole, young stem and secondary xylem was carried out and the results were analysed by using statistical techniques. The present study revealed that there are only 12 *Calophyllum* species in Sri Lanka and that recognition of the varieties of earlier workers is not supported by comparative anatomy. Further, with the results of anatomical, morphological and ecological studies combined with statistical analysis, an evolutionary relationship between the non-endemic and endemic taxa has been suggested. The study revealed that species centring the non-endemic *Calophyllum inophyllum* form the less specialised basic stock from which two lines of specialization emerged. A species complex allied to the endemic *C. walkeri* forms the montane group and the species associated with non-endemic *C. calaba* form an intermediate group of taxa.

Key words
Calophyllum, comparative vegetative anatomy, principle components analysis and cluster analysis.

INTRODUCTION

The pre-Linnaean history of the Sri Lankan *Calophyllum* species could be traced back to the period of the Dutch occupation of Sri Lanka. Hermann (1717) described two species, which belong to the present genus and named them using Sinhala vernacular names such as Domba, Doba and Tomba for *Calophyllum inophyllum* L., and Hinkina and Kina minor for *Calophyllum calaba* L. Subsequently, Burmann (1737) based on lamina, floral and fruit characters named the above mentioned two species in Latin as *Nux zeylanica rotunda exitiosa, folio glabro spendente* for *C. inophyllum* L. and *Inophyllum flore quadrifido* for *C. calaba* L. Going by the lamina shape Linnaeus (1747) in his Flora Zeylanica decided the two taxa as *Calophyllum foliis ovatibus* (= present day *C. inophyllum* L.) and *Calophyllum ovatis obtusis* (= present day *C. calaba* L.). These two names were later cited as the first reference in the protologues of *C. inophyllum* and *C. calaba* in the first edition of *Species Plantarum* (Linnaeus, 1753). Further he placed an asterisk at the end of Sri Lankan elements in his protologue. Stearn (1957) is of the opinion that Linnaeus' placement of an asterisk at the end of a reference denoted that it contained a good description.

Thwaites (1858) agreed with Choisy (1823, 1824) in maintaining three sections based on the presence, absence and the number of sepals and recognised three sections within the genus, namely

Section I	-	calyx with 4 sepals
Section II	-	calyx with 2 sepals and
Section III	-	calyx without sepals.

Thwaites recognised 10 Sri Lankan species as belonging to Section I, Section II and Section III as shown in Table 1. Thwaites included the endemic montane species *C. walkeri* Wight with 4 sepals and 8 petals within Section I while the seven species characterised by 4 sepals and 4 petals, *C. decipiens* Wight (= *C. thwaitesii* Planch. & Triana) with two varieties, *C. trapezifolium* Thw., *C. cuneifolium* Thw., *C. inophyllum* L., *C. tomentosum* Wight, *C. bracteatum* Thw. and *C. cordato-oblongum* Thw. were included in Section II. Based on the lack of petals and the presence of 4 sepals, the third section was recognised to include the two species *C. moonii* Wight and *C. burmanni* Wight.

Table 1
Classification system of the genus according to Thwaites (1858, 1864).

Section I Sepals 4, Petals 8

C. walkeri Wight

Section II Sepals 4, Petals 4

C. decipiens Wight (var. α & ß)
(= *C. thwaitesii* Planch. & Triana)
C. trapezifolium Thw.
C. cuneifolium Thw.
C. inophyllum L.
C. tomentosum Thw.
C. bracteatum Thw.
C. cordato-oblongum Thw.

Section III Sepals 4, Petals 0

C. moonii Wight
C. burmanni Wight

Trimen (1893; 1931) using floral characters specially the number of petals, morphological characters of leaf, indumentum hairs and fruit, enumerated the Sri Lankan species. He recognised 11 taxa out of which 6 were endemic. Further, he did not recognise *C. calaba* L., but instead recognised the two species *C. burmanni* of Wight and *C. pulcherrimum* of Wallich. In this respect Trimen's treatment of the genus differed from that of Thwaites.

Worthington (1959) gave a brief account of the Sri Lankan species of the genus. He recognised 10 species out of which as indicated by Trimen (1893) six taxa are endemic. However, on fruit characters he distinguished *C. calaba* L., and *C. pulcherrimum* Wall. According to Worthington the tipped fruit of *C. calaba* L., is different from that of *C. pulcherrimum* Wall.

Kostermans (1980) in his revision of the genus reported 11 species out of which 10 species are endemic. In this revision, Kostermans (1976) included a new endemic species, *Calophyllum zeylanicum*, which he described in his earlier paper, and believed that it was closely related to *C. cuneifolium* Thw. Further, based on the characters of leaf and inflorescence, he distinguished *C. zeylanicum* from *C. trapezifolium* Thw. However, Kostermans did not agree with the classification of *C. thwaitesii* Planch. & Triana into α and β varieties and as such included them within the *C. thwaitesii*. According to Kostermans, there is no difference between *C. calaba* L. and *C. pulcherrimum* Wall. But the latter is only a juvenile stage of the former. Stevens (1980), enumerating the Old World *Calophyllum* species, recognised 11 taxa from Sri Lanka. Further, considering the circumscription and infraspecific variation of *C. calaba* L., he described two new varieties *C. calaba* var. *calaba* and *C. calaba* var. *worthingtonii* from Sri Lanka. Stevens, recognising the fact that there is confusion with respect to *C. thwaitesii* Planch. & Triana, described a new species *C. vergin* to include *C. thwaitesii* var. β and incorporated *C. zeylanicum* of Kostermans within *C. trapezifolium* Thw.

According to Kostermans (1980) and Stevens (1980) seven species are distributed throughout the Wet zone and the Intermediate zone as shown in Table 2 and Fig. 1. *C. walkeri* Wight, *C. trapezifolium* Thw. and *C. cuneifolium* Thw. are restricted to high altitudes and are referred to as montane species. Further, according to Kostermans (1976), *C. cordato-oblongum* Thw. is a rare species restricted to the southern parts of the island, especially to Kanneliya-Dediyagala-Nakiyadeniya Forest Reserve (KDN) of Galle District. The only non-endemic *C. inophyllum* L. is known to be naturally distributed as a mangrove associate. *C. calaba* L., which Kostermans (1980) considered as endemic to Sri Lanka, thrives well in the Wet zone as well as in the coastal parts of the Dry zone and thalawa (savannah) (Fig. 1). It is evident from the above discussion that the species limits of the genus have not been solved. This investigation makes an attempt to understand the delimitation of species of the genus in Sri Lanka. In addition, these observations have been compared to reports from other genera of the family for a proper understanding of the interrelationships among the genera within the family. Further, ecological trends, if any, of the genus are also considered for the completeness of the investigation.

MATERIALS AND METHODS

Wood samples of the Sri Lankan species reported by Kostermans (1980) and Stevens (1980) as belonging to the genus were collected in the field along with voucher herbarium specimens in triplicate. Wood samples and a set of herbarium specimens were deposited in the herbarium of the Botany Division, The Open University of Sri Lanka, Nawala, Nugegoda and a set of specimens will be deposited at the National Herbarium, Royal Botanical Gardens, Peradeniya (PDA). The voucher specimens were identified by consulting Kostermans(1980), Trimen (1893), and other literature

Table 2
***Calophyllum* species reported from Sri Lanka by Kostermans (1980) and P. F. Stevens (1980)**

Species	Endemicity		Distribution	
Calophyllum bracteatum Thw.	*		+	
Calophyllum calaba var. *calaba* P. F. Stevens				α
Calophyllum calaba var. *worthingtonii* P. F. Stevens		+		
Calophyllum cordato-oblongum Thw.	*		+	
Calophyllum cuneifolium Thw.	*		x	
Calophyllum inophyllum L.				ß
Calophyllum moonii Wight		*		+
Calophyllum thwaitesii Planch. & Triana	*		+	
Calophyllum tomentosum Wight	*		+	
Calophyllum trapezifolium Thw.	*		x	
Calophyllum vergin P. F. Stevens		*		+
Calophyllum walkeri Wight		*		x
Calophyllum zeylanicum Kosterm.		*		x

* = Endemic species
α = Pan-islandic species
ß = Mangrove associates
+ = Species in Wet and Intermediate zones
x = Montane species

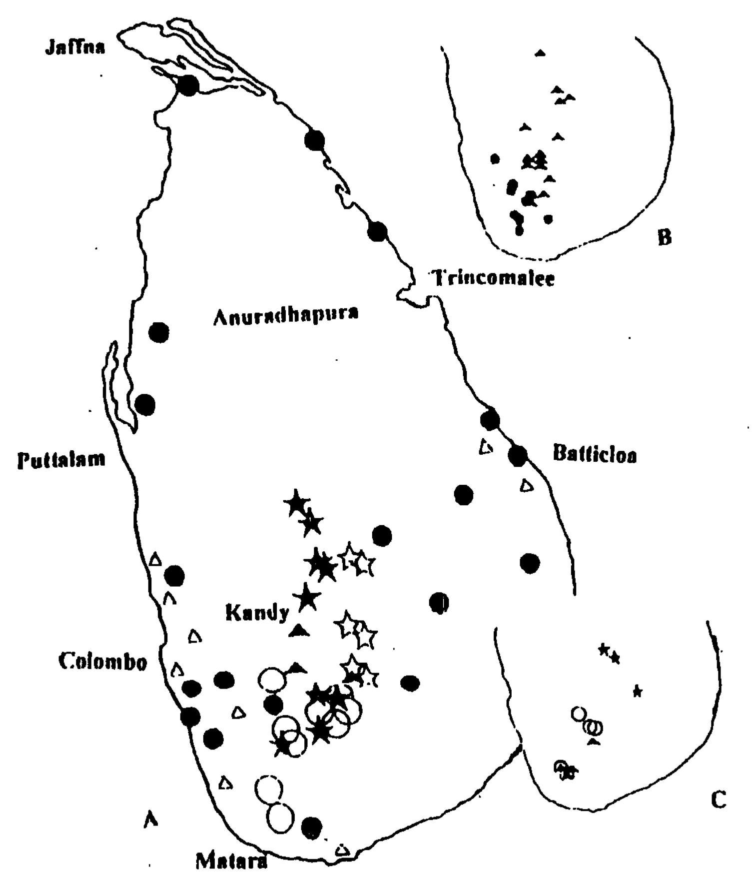

Fig. 1. Distribution of *Calophyllum* in Sri Lanka (after P. F. Stevens, 1980). (A) *C. tomentosum* (solid star), *C. walkeri* (open star), *C. thwaitesii* (solid triangles), *C. bracteatum* (open circles), *C. calaba* var. *calaba* (solid circles), *C. inophyllum* (open triangle), *C. cordato-oblongum* (open square) (B) *C. trapezifolium* (solid triangles), *C. calaba* var. *worthingtonii* (solid circles) (C) *C. cuneifolium* (solid stars), *C. vergin* (open circles), *C. moonii* (triangles).

pertaining to the taxon under consideration and also by comparing herbarium specimens deposited at the Royal Botanical Garden, Peradeniya.

Wood samples were taken from straight branches to avoid inclusion of tension or pressure wood. An attempt was made to collect mature wood with maximum diameter whenever possible. Samples of wood were cut into pieces of ca. $2cm^3$ and placed in a 50% ethyl alcohol until such time as they were sectioned in the microtome.

Transverse, tangential and radial wood sections were taken using a sliding microtome (E. Leitz Wetzlar, Germany) at thickness ranging from 10-15µm. However, in certain instances where sectioning was impossible at 15µm, thickness of the sections were increased up to 20µm. These sections were stained in a safranin and Fast Green FCF series (Sass, 1958) and mounted in Canada balsam. Maceration were made by splitting the wood into slivers of matchstick size and softening in a solution of 1:4:5: 30% hydrogen peroxide, distilled water and glacial acetic acid and changing the solution a number of times till the slivers were soft enough to macerate. The slivers were then washed in running water to remove the macerating fluid, macerated, stained in safranin and mounted in Canada balsam after using an alcohol dehydration series (Johansen, 1940).

Samples of young stem, leaves and nodes were preserved in the field by using FAA (2:10:1:7, 40% Formalin, 95% ethyl alcohol, glacial acetic acid and distilled water). Once in the laboratory these samples were rinsed in running water for over 24 hrs to wash out the FAA and were used for the preparation of microscopic slides.

Portions of young stems (1cm in length) for sectioning were taken from the middle of the first internode. Preliminary observations indicated that there were variations in structure of vascular bands of midribs from proximal to the distal ends of leaves. On this basis portions of midribs (ca. 1cm in length) were selected approximately at the middle of each leaf for the purpose of comparative anatomical study. Portions of lamina (ca. 5mm x 5mm) for paradermal sectioning were taken from the middle of the lamina of each leaf selected. The total lengths of petiole were used for serial sectioning. All the samples selected were then passed through an ethyl alcohol dehydration series followed by embedding in wax (Johanson, 1940). These samples together with petioles and nodes were sectioned using a Reichert - Jung rotary microtome at thickness ranging from 10-15µm. Cross sections of lamina were taken at right angles to the secondary veins. Sections thus obtained were stained in Safranin-Fast Green FCF and mounted in Canada balsam (Johansen, 1940).

Wood anatomical characters to be measured were selected following Record and Chattaway (1932), Tippo (1964) and Metcalf & Chalk (1989). Measurements of cell lengths (i.e. vessel element length, fibre length etc.) of wood were taken from macerated material. Total lengths of vessel elements including the tails were measured in compliance with Chalk & Chattaway (1936). Fibre lengths to vessel length ratios were calculated according to Baretta-Kuipers (1976).

Measurements of tangential vessel diameter, vessel wall thickness and vessel frequencies were made by using the cross sections prepared. Measurements of vessel frequencies were based on fifteen counts in an area of 19.7mm^2 field of view. Individual pores were counted following Wheeler (1986). Ray heights and ray frequencies were measured using tangential wood sections. The latter was measured following the method outlined by the International Association of Wood Anatomists (IAWA) Committee (1989). Descriptions of ray types in this study are in accordance with Kribs (1935) and the Committee on Nomenclature of Wood Anatomists (1957). Relative cell sizes and vessel frequencies are in accordance with Chattaway (1932), Chalk (1938) and the "Committee on the Standardisation of Terms of Cell Size" (1937).

Terminology used for features of wood is based on the List of Microscopic Features for Hard Wood Identification (IAWA Committee, 1989). The code words used in the study for wood anatomical characters are given in Table 3.

Table 3

Code words used in this study for Wood anatomical characters.

Character Code	Explanation
Quantitative characters	
VE	Vessel element length in µm
VD	Vessel diameter in µm
VWT	Vessel wall thickness in µm
VDF	Vessel distribution frequency as Vessels/mm^2
FL	Fibre length in µm
FD	Fibre diameter in µm
TL	Imperforate Tracheary element length in µm
TD	Imperforate Tracheary element Diameter in µm
RM	Ray frequency as Rays/mm
RH	Ray height in µm
FVRAT	Fibre length/Vessel length ratio
Qualitative characters	
WOOD	Storiedness of wood
GRI	Absence or Presence of Growth rings
POR	Pore distribution
SOLP	Shape of solitary pore
EWT	Vessel end-wall type
VEA	Vessel arrangement as seen in cross section of wood
PRPP	Perforation plate of vessel element
LIVR	Type of intervascular pits
PVR	Type of vessel-ray pits
RTY	Ray type
PDIS	Axial parenchyma distribution
CRYS	Occurrence and place of crystals
RHIS	Ray histology
RDOMI	Dominance of ray cell type
TYLO	Absence or presence of tyloses

Quantitative and qualitative data of anatomical features of the lamina were obtained from cross sections except for stomatal measurements, which were taken from paradermal sections. Guard cell measurements were taken parallel to the aperture and perpendicular to the aperture. Classification of crystal types is based on Esau (1983), Chattaway (1955, 1956) and Metcalf & Chalk (1989). Heights to width ratios of palisade cells were calculated according to Keating (1984). Midrib bundle angle was measured following Stevens (1974). Statistical data were derived from at least 10 measurements of each sample. Stomatal classification and terminology used are in accordance with Wilkinson (1979). The code words used are listed in Table 4.

Table 4
Code words used in this study for Lamina anatomical characters.

Character code	Explanation
Quantitative characters	
VF	Vein Frequency /mm
BAN	Vascular Bundle angle of midrib in degrees
DD	Secretory Duct Diameter in μm
LA	Lamina thickness in μm
ADE	Adaxial Epidermal height in μm
CAD	Adaxial Cuticular thickness in μm
CAB	Abaxial Cuticular thickness in μm
ABE	Abaxial Epidermal height in μm
LME	Lamina Mesophyll thickness in μm
PAL	Height of Palisade tissue in μm
SPO	Thickness of Spongy tissue in μm
BPA	Breadth of Palisade cell in μm
HPA	Height of Palisade cell in μm
SC	Diameter of guard cell (perpendicular to aperture) in μm
CWT	Diameter of guard cell (parallel to aperture) in μm
Qualitative characters	
MCV	Midrib sclerenchyma in an inverted 'V' shape
DD	Duct position in lamina
DT	Duct transcurrent or not
SBC	Sclerenchyma below duct
HYP	Presence or absence of hypodermis

Ecological data such as Mean Annual Rainfall (hereafter referred to as RFA) and Mean Annual Temperature (hereafter referred to as MAT) were obtained from the Meteorological Department, Bauddhaloka Mawatha, Colombo. Altitudes were measured using an altimeter supplemented with Topographic sheets of the Survey Department (1992). Agro-ecological zones identified are based on the soil map of Sri Lanka (Irrigation Department, 1988).

METHOD OF ANALYSIS

For simple statistical analysis (i.e. analysis of variance, simple linear regression, etc.) Snedecor and Cochran (1967) were consulted. The sample mean and 95% confidence intervals of quantitative wood and lamina anatomical characters are calculated and tabulated along with the species descriptions and the mean ranges of species with minimum and maximum confidence intervals are noted within parentheses. Preliminary statistical analysis of variations within the taxa under consideration was performed. Pearson product movement correlation coefficients (r) were calculated for the data matrix of quantitative characters. Simple regression analyses were carried out with ecological parameters and quantitative anatomical characters to detect any dependencies between the two. Further, regressed scores were used in Components Analysis to obtain scatter plots. The collected specimens were assigned to the Agro-ecological categories according to the soil map of Sri Lanka (Irrigation Department, 1988), and arranged into groups of decreasing moisture availability. Subsequently, mean vessel element lengths and agro-ecological zones were used to derive scatter plots. In addition, multivariate analysis adopted by Sneath & Sokal (1973), Gauch (1986) and Burley & Miller (1989) was employed in the present study.

Principle Components Analysis (PCA) and Cluster Analysis (CA) were attempted in order to examine the taxonomic patterns of the data and to generate a classification system. During both courses of PCA and CA each species was coded by a number and acronym respectively. The code numbers and acronyms used for each species in the study are listed in Table 5. Prior to the analysis raw data obtained were converted to standard scores, i.e. Z-values. Phenetic similarity / dissimilarity was used to obtain phenogram, following Bass *et al.* (1988) and Hedren (1990 b).

Mesomorphy indices and conductivity values (Carlquist & De Buhr, 1977) and conductance (Carlquist Hoekman, 1985) for each specimen were calculated as follows;

$$\text{Mesomorphy} = \frac{\text{Vessel length x Vessel diameter}}{\text{Vessels per mm}^2}$$

$$\text{Conductance} = \frac{\text{(Vessel diameter) x 0.0001}}{\text{Vessels per mm}^2}$$

All anatomical observations were made using an Olympus, PM - 10AD Light Microscope while statistical computations were done using a Commodore PC 10 - III (Model PC 10C / PC 20 C) with the aid of the statistical package SPSS/PC+™, Version 4.0 (SPSS Inc., 1990).

Table 5
Species, Code numbers and Acronyms used in the study.

Species	Code	Acronym
Calophyllum bracteatum	1	CCBRAC
C. calaba var. *calaba*	2	CCALAB
C. calaba var. *worthingtonii*	22	CCALAW
C. cordato-oblongum	3	CCORDA
C. cuneifolium	4	CCUNEF
C. inophyllum	5	CINOPH
C. moonii	6	CMOONI
C. thwaitesii	7	CTHAIT
C. tomentosum	8	CTOMEN
C. trapezifolium	9	CTRAPE
C. vergin	10	CVERGI
C. walkeri	11	CWALKE
C. zeylanicum	12	CCEYLA

DISCUSSION

The anatomy of the lamina, petiole and node, young stem and wood is discussed in relation to its possible taxonomic significance among the Sri Lankan taxa of the genus *Calophyllum*.

Lamina

Vesque (1893) is of the opinion that presence of hypodermis, sclerides below secretory ducts and degree of sclerosis of epidermal cells are important in delimiting taxa within the genus. The systematic or diagnostic value of lamina anatomy, i.e., epidermal - hypodermal complex, and the variations of midrib vascular bundle structure of *Calophyllum* species have been emphasised by Stevens (1974). Further, Stevens (1974) pointed out that anatomical study of leaves might cause problems due to the inclusion of juvenile leaves. In this present study care was exercised to avoid the inclusion of immature leaves. D'Arcy and Keating (1979) working with sterile materials from herbarium specimens showed the importance of lamina anatomical characters in the identification of taxa within the genus *Calophyllum*.

The leaves of all species considered are dorsiventral except for *C. walkeri* Wight, which is isobilateral and could be distinguished easily from the rest of the species. Abaxial and adaxial epidermises of all the taxa considered are uniseriate and comprise rectangular to square cells in cross section. The outer cell walls of the epidermal cells are well cuticularized with cuticular ridges extending between anticlinal walls (Fig. 2b). This feature, common to all the species considered, could be used to characterise the genus as a whole. A unique, uni-seriate sclerosed columnar-shaped hypodermis was present in *C. walkeri* (Fig. 2c), which could be used to distinguish the species from the rest of the taxa. Some characteristic transcurrent secretory ducts are

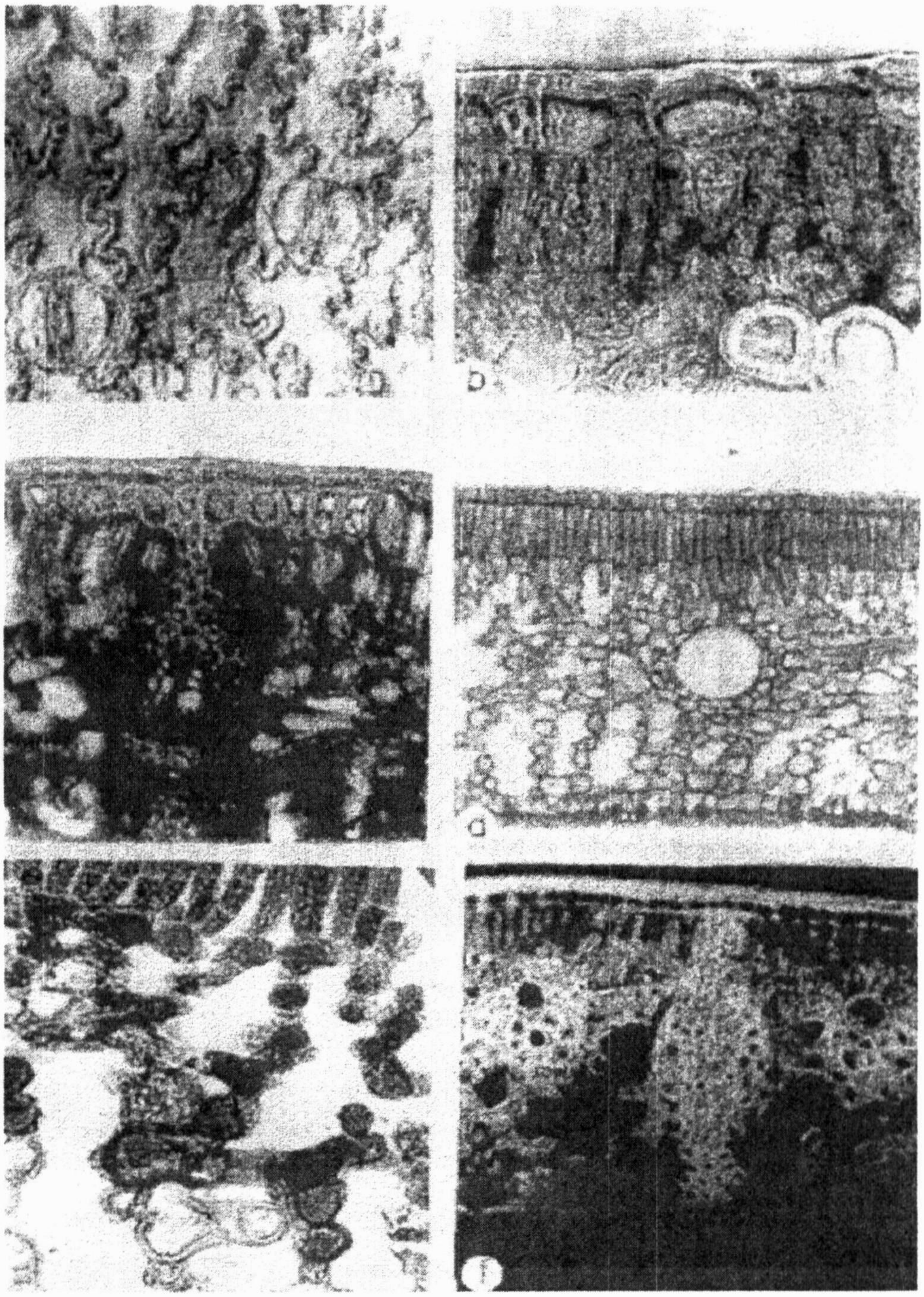

Fig. 2 Lamina anatomical features of the genus *Calophyllum*. **a)**. Paradermal section of *Calophyllum inophyllum* L., showing anomocytic stomata x 1320. **b)**. Portion of transverse section of lamina of *C. thwaitesii* showing cuticlular ridges running between epidermal cell walls x 1320. **c)**. Cross section of lamina of *Calophyllum walkeri* Wight showing unique, sclerosed, columnar-shaped hypodermis x 660. **d)**. Cross section of lamina of *Calophyllum inophyllum* x 660 showing well developed palisade and loosely arranged spongy cell layers, a feature common to all the Sri Lankan taxa considered x 660. **e)**. Portion of transverse section of lamina of *Calophyllum trapezifolium*, showing sclerides in spongy tissue x 1320. **f)**. Portion of transverse section of lamina of *Calophyllum thwaitesii*, showing secretory ducts placed close to adaxial epidermis (under phase contrast) x 660.

present in the lamina of *C. trapezifolium* and on this basis it could be distinguished from the rest.

The mesophylls of all the species considered are well developed into palisade and loosely arranged spongy cell layers (Fig. 2d). However, on the basis of the number of layers of palisade present, the following three groups could be distinguished:

- 1- layered in *C. bracteatum, C. calaba, C. cordato-oblongum, C. thwaitesii, C. vergin* and *C. zeylanicum*
- 2- layered in *C. inophyllum, C. moonii, C. trapezifolium*, and *C. tomentosum*
- 3-layered in *C. cuneifolium* and *C. walkeri.*

Brachysclerides and macroslcerides (Fig. 2e) were present in the mesophylls of all the Sri Lankan species except in the mesophylls of the three lowland species namely *C. calaba, C. inophyllum* and *C. bracteatum.* On this basis the three widely distributed lowland species mentioned could be separated from the rest of the Sri Lankan taxa.

D'Arcy & Keating (1979) dealing with the secretory duct in the lamina stated, "The duct lumen may be medially placed between the epidermal surfaces or it may be high (close to the adaxial epidermis). Duct size and placement appear to be systematically valuable characters". On this basis the Sri Lankan species could be categorised into two groups:

- secretory ducts placed high towards the adaxial surface, *C. cuneifolium, C. moonii, C. thwaitesii, C. trapezifolium, C. vergin* and *C. zeylanicum* (Fig. 2f)

- secretory ducts placed medially, *C. bracteatum, C. calaba, C. cordato-oblongum, C. inophyllum, C. tomentosum* and *C. walkeri* (Fig. 2a).

Secretory ducts associated with extraxylary Fibres have been reported to be present within the taxa of *Calophyllum* by workers such as Vesque (1893) and D'Arcy & Keating (1979). Such Fibres were observed in all the Sri Lankan species except for *C. inophyllum*, which is a non-endemic, restricted to the lowlands of the island. This character could be used to distinguish the latter from the rest of the Sri Lankan species.

D'Arcy and Keating (1979) pointed out that the shape of the vascular bundles and their associated structures are important in the delimitation of taxa within the genus *Calophyllum*. For instance, they have shown that the presence or absence of inverted V-shaped sclerenchyma fibre tissues adaxial to the vascular bundle and presence or absence of large-lumened cells below the vascular bundle are of importance in distinguishing *Calophyllum* species.

On these lines the present study clearly shows that the position/association of extraxylary sclerenchyma Fibre tissues on the adaxial side of the midrib vascular bundle could be used to separate the Sri Lankan taxa of the genus into three main basic groups as shown in Fig. 3:

Group I In this group, the extraxylary sclerenchyma fibre tissues are placed above the xylem at the ends of the arc formed by the vascular tissue as shown in Fig. 3a. This group would include only *C. inophyllum*.

Group II In this group, the extraxylary sclerenchyma fibre tissues are placed directly towards the adaxial surface at the distal ends of the vascular tissue as shown in Fig. 3b. This group thus would include species such as *C. bracteatum*, *C. calaba*, *C. tomentosum*, *C. walkeri* and *C. cordato-oblongum*.

Group III In this group, the extraxylary sclerenchyma fibre tissues are placed directly above, towards the adaxial surface of the vascular bundle and not associated with the latter as shown in Fib. 3c. This group according to the present study would include species such as *C. cuneifolium*, *C. moonii*, *C. thwaitesii*, *C. trapezifolium*, *C. vergin* and *C. zeylanicum*.

Out of the three groups, Group I, which includes *C. inophyllum*, according to Vesque (1893) and Stevens (1980) widely distributed throughout the tropics, could be considered as the basic stock of the *Calophyllum* taxa of Sri Lanka. It is interesting to note that out of the 12 species recognised in Sri Lanka, according to Stevens (1980) *C. inophyllum* and *C. calaba* are not endemic. According to Kostermans (1980), *C. inophyllum* is endemic but restricted to the coastal belt while *C. calaba* is endemic and distributed throughout the island. The fact that *C. inophyllum* could be the first introduction to Sri Lanka will be further discussed in the latter stages of the discussion.

Group II includes the species mentioned earlier, and, except for *C. walkeri*, have been collected in the low montane forests (Werner, 1982). *Calophyllum walkeri* stands out in this respect for the reason that it has been reported only from the upper montane forests.

In Group III, all the species except for *C. moonii* are restricted to the upper montane forests (Werner, 1982). Again *C. moonii* stands out in this respect for the reason that it has been collected and reported to be restricted to the Wet zone Kanneliya (KDN) area (Fig. 1).

Based on these facts, it is possible that there has been a gradual shift through time and evolution of the extraxylary sclerenchyma fibre tissues seen in *C. inophyllum* associated with xylem strands, towards the adaxial surface within the endemic species, probably due to mechanical and ecological reasons. On this basis *Calophyllum walkeri*, which is an endemic could probably be considered as an intermediate between the low montane forms and the upper montane forms. However, it is difficult to interpret the position of the endemic *C. moonii* for it is reported to be restricted to the wet zone Kanneliya area. Whether this is a secondary or specific adaptation in evolution needs further studies. Also, detailed studies beyond the scope of the present study are needed before any conclusions are made. Also, it would be interesting to study the extraxylary fibre lengths to find out whether there are significant variations.

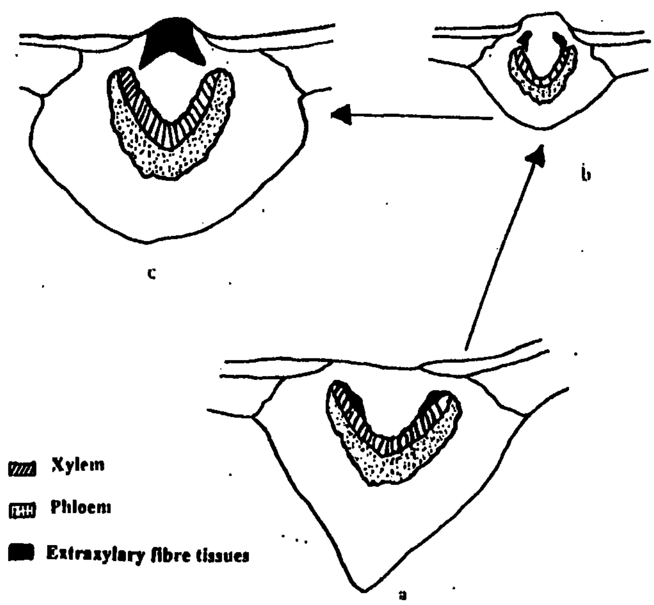

Fig. 3. Three basic groups recognized in the study based on position/ association of extra xylary sclerenchyma fiber tissue within midrib vascular bundle: a) Calophyllum *inophyllum* b) *Calophyllum tomentosum* c) Calophyllum moonii

D'Arcy and Keating (1979) described the adaxial surface of the midrib using terms such as convex, truncated, grooved, etc. Using such terms the Sri Lankan species could be further divided into sub-groups and the species recognised as follows:

Group I recognised with only *C. inophyllum* would stand as it is.

Group II recognised in the study could be broadly divided into two sub-groups as listed below.

a) A group with extraxylary fibre tissues (large-lumened Fibres *sensu* D'Arcy & Keating 1979) as shown in Fig. 3c, on the abaxial surfaces of midrib vascular bundle which include *C. bracteatum*, *C. calaba*, *C. tomentosum* and *C. walkeri*. Further, based on the shape of the adaxial surface of the midrib sub-group recognised could be further divided as follows:

a1) A group with convex adaxial surface, which would include *C. bracteatum* and *C. calaba*. Further, these two species could be separated easily on the basis of vascular bundle angle as follows:

A species with midrib vascular bundle angle 94^o–95^o that would include *C. bracteatum*.

A species with midrib vascular bundle angle 60^o–82^o that would include *C. calaba*.

a2) A group with grooved or flattened adaxial surface, which would include *C. tomentosum* and *C. walkeri*. Further, based on the adaxial surface of the midrib these two species could be separated as follows:

A species with grooved adaxial surface, which would include *C. tomentosum*.

A species with flattened adaxial surface, which include *C. walkeri*.

b) A species without extraxylary fibres below the midrib vascular bundle, which would include *C. cordato-oblongum*.

Group III recognised in the study could be divided into two sub-groups as follows:

a) A group of species with truncated adaxial surfaces, which would include *C. cuneifolium*, *C. trapezifolium* and *C. zeylanicum*. Further, based on the shape of the adaxial surface of the midrib the sub-group recognised above could be further divided as follows:

a1) with rounded abaxial surface which would include *C. cuneifolium*

a2) with angled abaxial surface which would include *C. trapezifolium* and *C. zeylanicum*. Further, on the basis of the presence/absence of transcurrent secretory ducts in the mesophylls of lamina, these two taxa could be separated as follows:

A species with transcurrent secretory ducts in the mesophyll, which would include *C. trapezifolium*

A species without transcurrent secretory ducts in the mesophyll, which would include *C. zeylanicum*

b) A group of species with convex adaxial surfaces, which would include *C. moonii*, *C. thwaitesii* and *C. vergin*. Further, on the basis of adaxial surfaces of midribs, group recognised above could be divided as follows;

b1) A species with 3-angled abaxial surface, which would include *C. moonii*

b2) A group of species with rounded abaxial surface, which would include *C. thwaitesii* and *C. vergin*. Based on the leaf anatomy, these two taxa cannot be separated. The results of the present study agreed with the idea of Kostermans (1980) that the above mentioned species are the same. The present study does not agree with Stevens (1980) who recognised the above two taxa based on the shape of the young stem and the length of the apical bud of dried herbarium specimens.

Young Stem

Metcalf and Chalk (1950) and Metcalf (1979) pointed out the importance of anatomical characters such as type of vascular bundle, origin of periderm and nature of cork cells, presence/absence of pericycle and endodermis, presence/absence of extraxylary Fibres, degree of sclerosis of pith parenchyma cells, etc., of the young stem for the purpose of systematic studies. Stevens (1980) used various terms to describe the outlines of stems in cross sectional view. However, considering the outline shape of the young stem in cross sectional view, Sri Lankan *Calophyllum* species could be divided into 2 groups, *viz*.

Group I. Species with 4-angled stems: *C. bracteatum, C. calaba, C. cuneifolium, C. thwaitesii, C. tomentosum, C. vergin* and *C. inophyllum*.

Group II. Species with oval-shaped stems: *C. cordato-oblongum, C. moonii, C. walkeri, C. trapezifolium* and *C. zeylanicum*.

Sclerosed parenchyma cells in pith were observed in species *C. bracteatum, C. cordato-oblongum, C. thwaitesii, C. trapezifolium,* and *C. vergin* and they were absent in *C. calaba, C. cuneifolium, C. inophyllum, C. moonii, C. tomentosum, C. walkeri* and *C. zeylanicum*. Whether this character could be used to separate the taxa within the genus would need further investigations, which are beyond the scope of the present study. According to Metcalf and Chalk (1950), presence/absence and if present the location, and types of crystals in the young stems are of importance in delimiting the taxa within families and genera. Because of the absence of crystals in the pith *C. calaba* (including both var. *calaba* and var. *worthingtonii)* and *C. tomentosum* could be separated from the rest of the species of the genus. Further, considering the type of crystals, *C. moonii* and *C. thwaitesii* stand out having rhombohedral crystals in the pith and cortex. Apart from the above facts, occurrence and type of crystals are of limited value in separating species within the genus *Calophyllum*.

Even though, on the basis of presence/absence of crystals, two groups could be recognised within the genus, species cannot be separated on the basis of the type of crystals as far as the Sri Lankan species are concerned. Thus, these characters have limited value in delimitation within the genus *Calophyllum*.

Wood

The potential use of wood anatomical diversity for taxonomic purposes was suggested by many workers such as Kieser in 1815, De Candolle in 1818, and Hartig in 1859 during the nineteenth century (Bass, 1982). According to Dickison (1975) among the authors who thoroughly investigated wood anatomical characters and their applicability in systematic studies were Sanio in 1863, Radlkofer in 1885 and Vesque in 1881. From early times considerable development in the study of wood anatomy for the interpretation of systematic, phylogenetic and ecological studies has been achieved (Bailey, 1957; Bass, 1982). Dickison (1975) summarised the various wood anatomical characters and listed the characters useful for systematic purposes. There has been a considerable amount of work during recent decades on wood anatomy with application and evaluation of values of many anatomical features in taxonomy (Record, 1934; Bailey 1953, 1957). Further, the significance of comparative anatomy and morphology and their application in the study of Sri Lankan Gesneriaceae has been emphasised by Herat and Theobald (1979). Herat & Theobald (1977) dealing with the Sri Lankan fraction of Theaceae have shown the significance of wood and vegetative anatomy in recognising the genera and species within the family.

Literature on wood anatomy of *Calophyllum* is scanty. Gamble (1881) Janssonius (1952) described the wood anatomy of the genera within Clusiaceae. They described vessel distribution, ray histology and other microscopic wood anatomical features in relation to hard wood identification. Solereder (1908) and Metcalf & Chalk (1950) gave general descriptions of wood, young stem and lamina anatomy of the genus under consideration. General wood anatomical descriptions of individual species could be gleaned form the works of Paula (1974), Green (1932), Vestal (1937) and Mennega (1948). Tieghem (1884) dealt with the secretory canals of the genus in his study on Clusiaceae, Hypericaece, Ternstroemiaceae and Dipterocarpaceae. However, it is clear that previous work, except for that of Vestal and Tieghem, on the genus has been mainly on various properties of wood, which had not been used for species delimitation within the genus.

The present study shows that wood anatomical characters are homogenous and the diagnostic value of such characters is of limited value in the delimitation of the species concerned. However, characters such as vessel diameter, vessel element length, vessel distribution frequency, fibre length and diameter and imperforate tracheary element length with some reservations could be used separately or together to identify some of the species under consideration. For instance *C.cordato-oblongum* Thw. could be separated from the rest of the species by their comparatively long vessel members and oblique end-walls with high caudate vessel endings.

The presence or absence of growth rings in the tropical woods could be regarded as a controversial subject (Mariaux, 1967). However, the occurrence of such is reported to depend on environmental factors such as photoperiod, temperature and availability of water (Vliet, 1979, 1981; Fahn *et. al.*, 1985; Chalk 1979). On this basis growth rings of some degree of distinctness were observed in six species as listed below:

- Upper montane species - *C. cuneifolium, C. trapezifolium, C. walkeri; C. bracteatum*
- Low montane species - *C. vergin, C. thwaitesii*
- Lowland species - *C. moonii.*

However, such rings were absent in the rest of the *Calophyllum* species. The presence of growth rings restricted to a few endemic species only is of interest and requires further ecological and anatomical studies before any conclusions can bereached. All the woods examined are non-storied and diffused porous. Solitary pores are oval or circular in outline. The pores are distributed in diagonal or rarely radial patterns in all the species examined. These characters mentioned could be used to distinguish the genus as a whole. Tangential vessel diameters ranged from 55.6 - 157.2 µm. *C. bracteatum, C. calaba, C. tomentosum* and *C. zeylanicum* stand out from the rest of the species concerned in having mean diameters greater than 100.0 µm. *Calophyllum inophyllum* is prominent for its lowest value with respect to the tangential vessel diameter (55.6 µm). However, Aloni & Zimmermann (1983) are of the opinion that the vessel size and density (i.e. vessel frequency) vary along the plant axis, and as such have limited value in species delimitation.

The formation of tyloses has been considered as an indication of evolutionary primitiveness of angiosperms (Bonsen & Kucera, 1990). Thin walled tyloses were observed in the lowland species *C. inophyllum, C. cordato-oblongum, C moonii, C. calaba* and low montane species such as *C. tomentosum, C. thwaitesii* and *C. vergin.* Based on these facts, lowland species could be considered as the most primitive taxa within the genus and montane species as the advanced taxa. This idea further agrees with the findings of the present study with respect to the evolutionary trends observed in sclerenchyma fibre association with the vascular bundle of the midrib of the leaves.

Principle component analysis (PCS) and cluster analysis (CA)

Based on midrib anatomical characters, it was possible to show an evolutionary trend within the taxa concerned. Further, it was possible to show evolutionary trends from the possible widely distributed ancestral forms such as *C. inophyllum* and *C. calaba*, which occur in the lowlands of Sri Lanka to the endemic upper montane taxa through the intermediate low montane endemic species. Similar relationships, to a certain extent, have been established possibly with limited scope considering the presence of tails in the vessel. With respect to other anatomical characters considered individually under the heading of petiole, young stem and wood, it was seen that they are of limited value in the elimitation of taxa within the genus, but could be of value in the characterisation of the genus as whole.

The basic idea of employing statistical methods using all the possible anatomical and ecological features considered was to find out whether possible relationships between taxa and the degree of specialisation could be understood. The following discussion deals with the outcomes of such a study. Multivariate analyses have been extensively used in the field of biology. Further, Sneath and Sokal (1973) have shown the importance of multivariate statistical techniques in Numerical Taxonomy. Based on these techniques Jansen et al. (1979), Jacobseni (1979), Khidir and Wright (1982), Robertse et al. (1980), Khordhopani and Ingrouille (1991) and Hedren (1990 a) successfully solved the problems using allozymic variations in *Capsicum*, variations between *Allium cernuum* and *Allium stellatum*, systematics of Graminae, wood anatomy of South African *Acacia*, morphological patterns in *Acacia* in Sudan and the African complex of *Justicia striata* respectively. On this basis mean values of quantitative anatomical data of wood and lamina were subjected to a PCA in order to obtain several scatter diagrams. These scatter diagrams were used to interpret taxonomic pattern reflected by the above data. Further, Cluster analysis was attempted to generate a classification system.

PCA and CA of quantitative wood anatomical characters

Components loading of each wood anatomical character along the components are given in Table 6. The characters such as vessel element length, fibre length and tracheary element length could be considered as heavily loaded along the first principle component axis (Table 6). On the other hand fibre diameter and tracheary diameter showed heavy loading along the second component axis while the variables such as vessel diameter, vessel distribution frequency and rays per mm are heavily loaded along the third component axis. Since the loading of variables along the 4th and 5th component axis is less and it could be considered negligible. The resulting scatter plots

(Fig. 4) revealed that there is no particular grouping pattern of specimens and thus wood anatomical features considered in the study are of limited value in the delimitation of the genus *Calophyllum* in Sri Lanka. The result obtained in the cluster analysis also supported the above conclusion (*cf.* Fig. 4 and Fig. 5).

PCA and CA of lamina anatomical characters

Component loading of lamina anatomical characters given in Table 7 clearly indicate that variables such as mean lamina thickness, mean palisade thickness and mean mesophyll thickness are highly contributive to the variation in the data set considered. Component scores derived from PCA produced scatter plots (Fig.6 and 7). Based on these findings, three main groups within the genus could be recognised and could be correlated with ecological conditions. For instance, on this basis montane species *C. walkeri*, *C. cuneifolium*, *C. trapezifolium*, and *C. zeylanicum*, intermediate species *C. thwaitesii*, *C. tomentosum* and *C bracteatum* and lowland species. *C. inophyllum*, *C. calaba*, *C. moonii* and *C cordato-oblongum* could be recognised as groups.

Table 6

Principle component analysis of 10 wood anatomical characters of *Calophyllum* species. Table entries are coefficients for each character for the first five principle component. The per cent variation is the amount of the variation in the multivariate date set explained by each component.

	PC 1	PC 2	PC 3	PC 4	PC 5
%variability explained	21.6	20.0	15.2	11.0	8.0
Cumulative %	21.6	41.7	56.8	67.9	75.9
VE	.78322	-.18822	.15779	.25896	.08207
VD	-.09276	-.13111	-.78866	.09034	-.06941
VWT	-.04736	.11248	-.08385	.95219	-.09670
VDF	.23362	-.19928	.73918	.00067	-.37098
FL	.68446	.24102	-.22514	-.06051	.19256
FD	.08620	.91276	-.00360	.04879	.10123
TL	.79870	.09551	.09957	-.23529	.05126
TD	.01483	.88015	-.02641	.06399	-.16505
RM	-.19629	-.06543	.71689	-.00773	.21913
RH	.26443	-.07738	.09430	-.10578	.87937

Hair structure of the indumentum led Vesque (1893) to believe that *C. tomentosum* and *C. bracteatum* were closely related. However, a scatter plot of the first Principle component axis along the second Principle component axis separates *C. tomentosum* (Group A in Fig 6) into an isolated position supporting the view of Stevens (1980) that the relationships of the taxa are obscure. Analysis showed that *C. calaba* and *C. inophyllum* are closely related to *C. tomentosum* on lamina anatomical basis. Specimens of *C. calaba* clustered into two groups (Fig. 7B1 and B2) and thus support the idea of Stevens (1980) that *C. calaba* consists of two varieties *C. calaba* var. *calaba* and *C. calaba* var. *worthingtonii*. However the present findings do not support the idea of Kostermans (1980) who stated that variations within *C. calaba* specimens from different localities are due to variations in maturity of the specimens. The third Group, which centred around *C. walkeri* includes species such as *C. cuneifolium, C. trapezifolium* and *C, zeylanicum* (Group C in Fig. 6 and Fig. 7). These species are closely related to each other. This agrees with the idea of Stevens (1980) who stated that these species are interrelated on morphological grounds. Even though *C. zeylanicum* shows close relationships to *C. trapezifolium*, in a limited amount of specimens considered, the real relationships are yet to be known. The present findings support Kosterman's (1980) view that *C. zeylanicum* is a distinct species, which, Stevens (1980) believed to be the same as *C. trapezifolium*. Stevens (1980) recognised *C. thwaitesii* var β of Planchon and Triana as a new species, *C. vergin*. Kostermans (1980) however, does not agree with Stevens (1980). Working with a limited number of specimens it is seen that *C. vergin* of Stevens (1980) falls between the second and the third group (Group B and C in Fig. 7).

Table 7

Principle component analysis of 15 lamina anatomical characters of *Calophyllum* species. Table'entries are coefficients for each character for the first five principle component. The per cent variation is the amount of the variation in the multivariate date set explained by each component.

	PC 1	PC 2	PC 3	PC4	PC 5
% variability explained	23.6	15.7	11.0	10.5	8.6
Cumulative %	23.6	39.2	50.2	60.7	69.4
VF	.04372	-.05105	.18309	-.19801	.78969
BAN	.02977	.16956	.55454	.0573	-.22721
DD	.13797	-.04863	.19612	.66269	-.24615
LA	.88099	-.19902	-.12039	.24170	.04315
ADE	.07100	.75784	-.14115	.01653	-.08738
CAD	.03672	-.17189	.79513	.00680	.8054
CAB	-.24282	-.06975	.83508	-.04749	.6626
ABE	.09659	.67810	.00678	.50189	.2683
LME	.86297	-.37826	-.05099	.11809	.03077
PAL	.76756	.10561	-.01282	-.05893	.08392
SPO	.37795	-.68156	-.15647	.1881	-.7756
BPA	.20396	.31133	-.06101	-.60159	-.43868
HPA	.79101	.32924	-.0709	-.16758	.05924
SC	.47988	.07717	-.14076	.18895	.67014
CWT	.03110	.32607	-.30965	.63250	.05099

On morphological grounds Stevens (1980) pointed out that *C. bracteatum* is closely related to *C. calaba*. Results of the present investigation support the idea of Stevens (1980) as seen in Group B in Fig. 7. The montane taxa are still well separated in PCA on regressed scores of lamina anatomical features with respect to altitude. These findings reveal that the influence of altitude is negligible on the grouping pattern of species. However, further studies are needed before conclusive decisions are made. Cluster analysis of lamina anatomical features agrees with the PCA as seen in Fig. 8. The PCA and CA of lamina anatomical characters revealed that taxa within the genus could be separated into groups such as lowland, intermediate and montane groups.

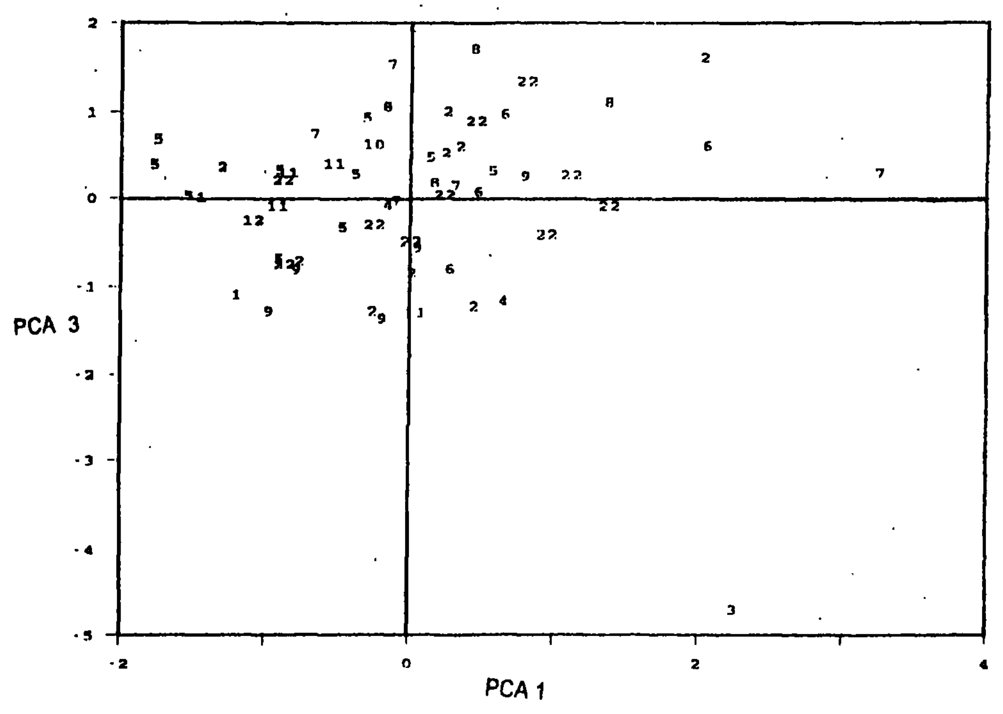

Fig. 4. PCA scatter diagram produced by plotting the first PC against the second PC for wood anatomical characters. Figures in the plot follow the species code in Table. 5. Percentage of trace along the First PC = 21.6% and along the second PC =20.0%; Total =41.6%.

PCA and CA of combined wood and lamina anatomical characters

The grouping pattern of species in scatter plots derived from PCA analysis of wood and lamina anatomical data do not differ from those derived from lamina anatomical characters alone as pointed out earlier (Fig. 6 and 7). This could be due to the heavy loading of the lamina anatomical features over wood anatomical features within components (*cf.* Table 6 and 7). Combined anatomical features in CA produced a phenogram (Fig. 8), which agrees with the results of PCA. Based on these lines, agreeing with Whitmore (1973), anatomically the Sri Lankan species could be placed in a series between two groups to include *C. walkeri, C. cuneifolium, C. zeylanicum* and *C. trapezifolium* into one group and the rest in a second group.

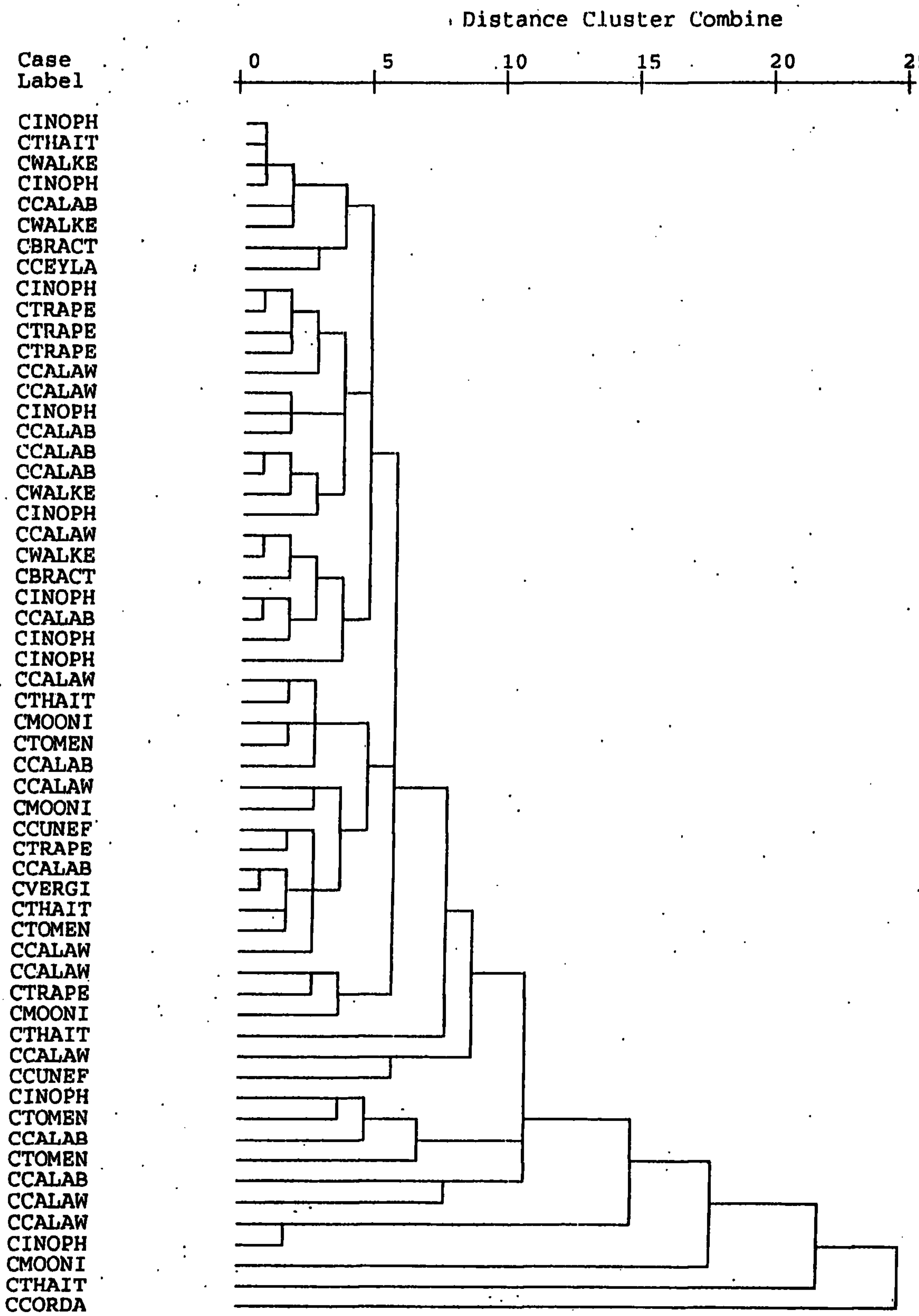

Fig. 5. Phenogram based on the Euclidean distance matrix including 10 wood anatomical characters of 58 specimens. Acronyms of specimens follows Table 5.

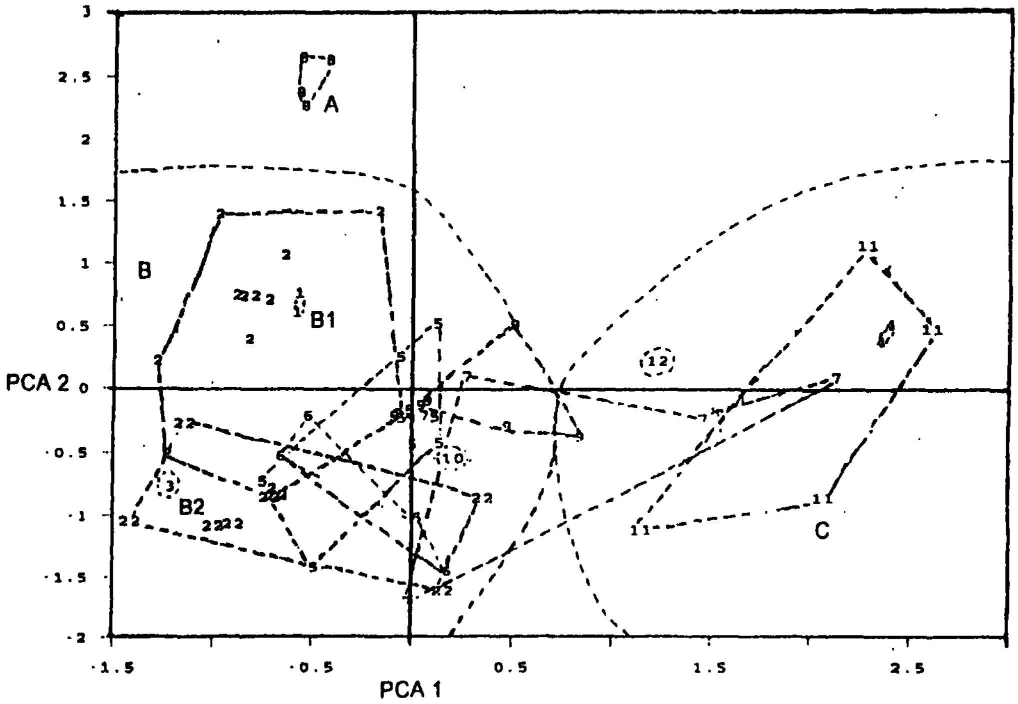

Fig. 6. PCA scatter diagram produced by plotting the first PC against the second PC for lamina anatomical characters. Figures in plot follow the species codes in Table 5. Percentage of trace along the first PC = 23.5% and along the second PC = 15.7%; Total =39.2%.

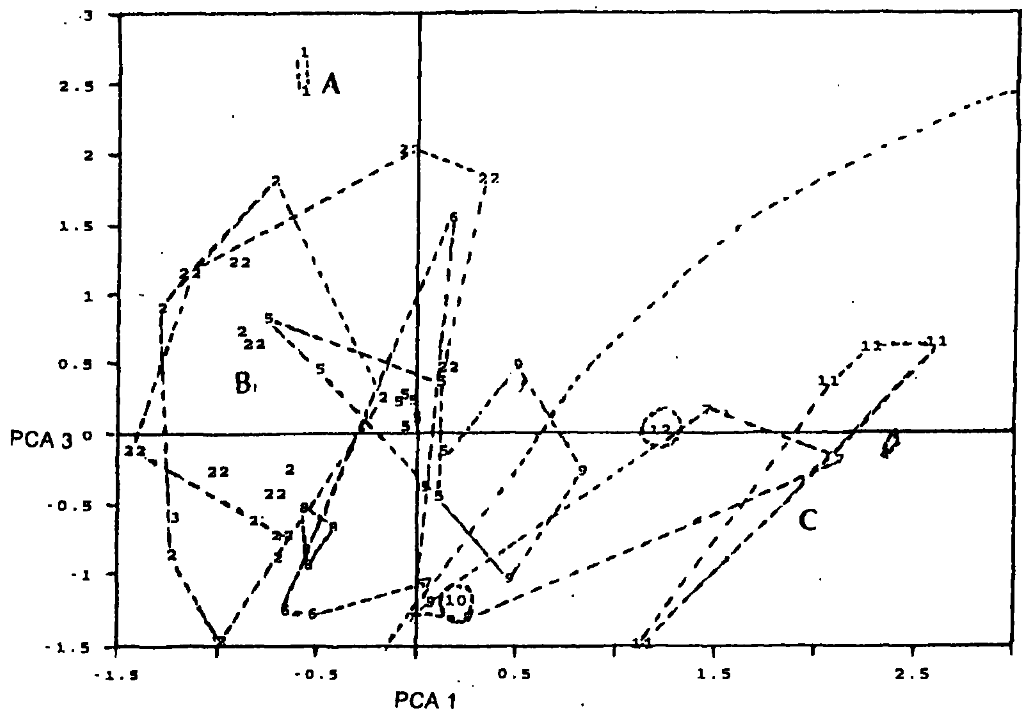

Fig. 7. PCA scatter diagram produced by plotting the first PC against the third PC for lamina anatomical characters. Figures in plot follow the species codes in Table 5. Percentage of trace along the first PC = 23.5% and along the third PC = 11.0%; Total =34.5%.

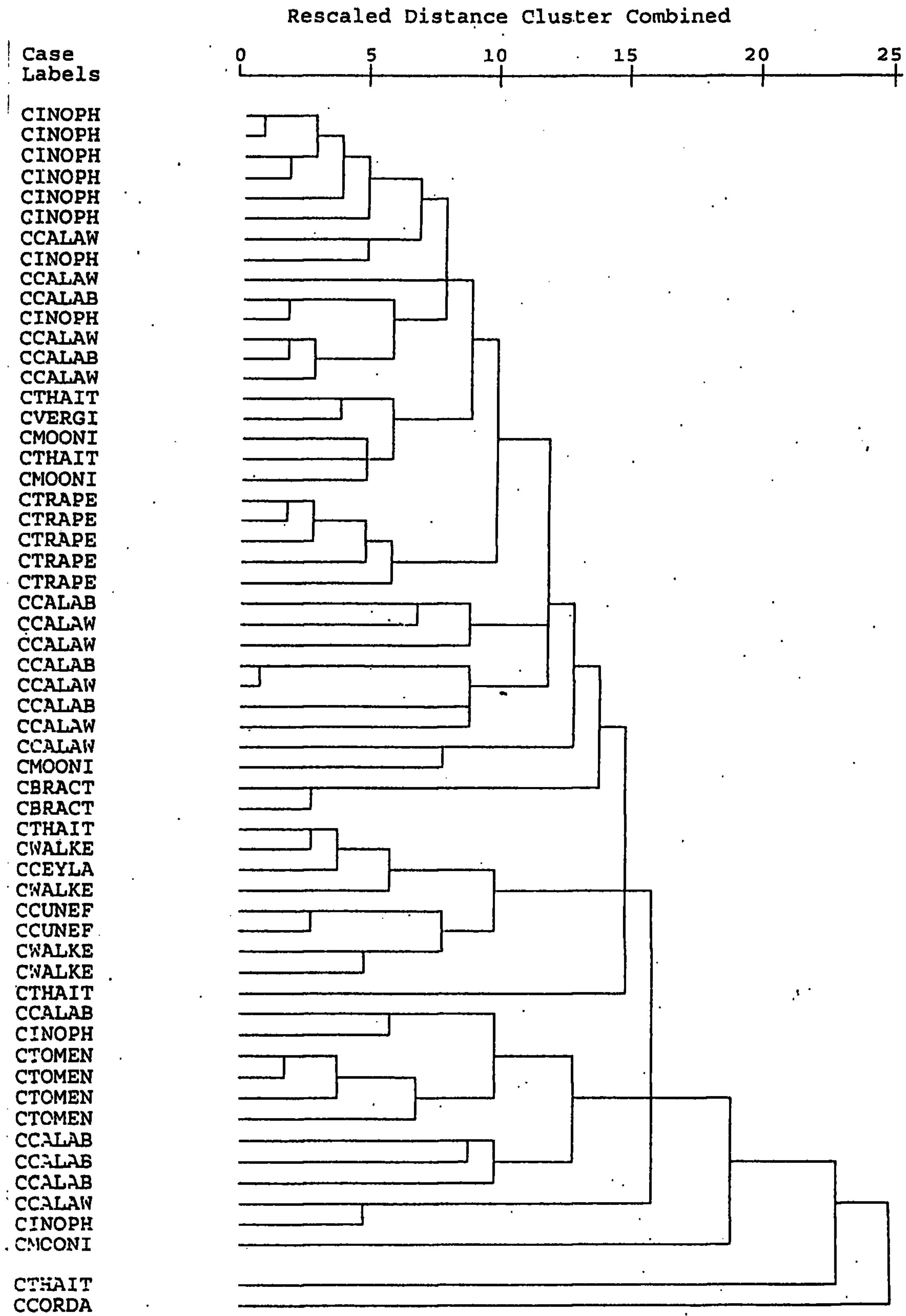

Fig. 8. Phenogram based on the Euclidean distance matrix including 15 quantitative lamina anatomical characters of 58 specimens. Acronyms of species follow Table 5.

Statistical correlation

Oever *et al.* (1981) and Carlquist and Hoekman (1985) working with Symplocaceae and Staphyleaceae respectively showed significant correlation among wood anatomical characters such as vessel wall thickness to vessel diameter and vessel diameter to distribution frequency; vessel element length to Fibre length and imperforate tracheary element length and vessel element length to ray height. The present study supports the above workers on the basis that correlation between vessel element lengths, fibre lengths and imperforate tracheary element length showed highly significant correlation coefficients ($P<0.001$). This study revealed that the correlation coefficient between height of ray and vessel element length; vessel diameter and Fibre diameter are significant at $P<0.01$. As far as the Sri Lankan species are concerned no work has been done on such correlation. However, such correlation could be of great value in selection of diagnostic characters. Further work is needed on these lines of study to generalize the relationships among the wood anatomical features.

Graff and Baas (1974) working on *Calophyllum* have shown that there is no significant correlation between altitude and wood anatomical characters except for fibre diameter. which has significant correlation with altitude. The present findings of the study are in agreement with Graff and Baas (1974). Further, the present observations with respect to correlation between Fibre diameter and altitude with the significant positive correlation coefficient at $P<0.001$ could be interpreted in terms of mechanical strength of wood which is required to resist twisting and tensile forces caused by wind action at higher altitude.

It is evident from the results that lamina anatomical characters such as thickness of lamina highly correlated with the altitude. Such correlation illustrates the adaptability of taxa within the genus *Calophyllum* to xeric and mesic conditions. Further, epidermal thickness of lamina showed significant positive correlation with mean annual rainfall patterns ($P<0.001$). Further investigations on this aspect are required before conclusive interpretations are made.

CONCLUSION

According to the vegetative anatomical studies carried out on Calophyllum L. taxa of Sri Lanka revealed that there were only 12 species. The varieties that had been recognized by earlier workers cannot be accepted. Based on anatomical, morphological and ecological studies combined with statistical analyses revealed that species centring the non-endemic *Calophyllum inophyllum* forms the less specialized basic stock from which two lines of specialization emerged. On the other hand, it is evident that the non-endemic *C. culaba* complex forms an intermediate group from which the other taxa represented in Sri Lanka evolved.

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