The role of leaf anatomy and tannins in litter decay in a tropical stream

El rol de la anatomia foliar y de los tanninos en la descomposición de la hojarasca en un arroyo tropical

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Abstract: Leaf litter of nine tree species occurring in the riparian forest of Quebrada Negra, a first-order tropical lowland stream in Costa Rica, was analysed by plant-anatomical and phytochemical methods in order to test the hypothesis that r-strategists invest less in defence against herbivores and pathogens than K-strategists and therefore have faster aquatic decay rates. In a parallel study on leaf litter decomposition, detritus formation and colonisation by macroinvertebrates, Tschelaut (2008b) showed clear differences between plant material originating from 4 species of trees common in the riparian forest of the stream. The 4 species represented different life history strategies of trees, fast growing pioneer species (for simplicity called r-strategists) (*Acalypha diversifolia* – Euphorbiaceae, *Cecropia obtusifolia* – Cecropiaceae, *Tetrathylacium macrophyllum* – Flacourtiaceae) and a slow growing climax species (K-strategists) (*Sloanea medusula* – Elaeocarpaceae). In addition to these 4 plants, 5 additional species were analysed in the present study in order to obtain a broader presentation of defence mechanisms. The plants differ in their anatomical structures and content of tannins of the leaves. Anatomical differences were quantified by 13 parameters, e.g. proportion of sclerenchyma and collenchyma, on anatomical sections and combined in a "toughness index". The results are set relation to the observed decay rates. The comparison shows that the amount of tannins influences the litter decay during the first days whereas anatomical structures play the main role during the whole decomposition process.

Key words: litter decomposition, tannins, plant anatomy, toughness.

Resumen: La hojarasca de 9 especies de árboles presentes en el bosque ribereño de Quebrada Negra, un arroyo de primer orden en los trópicos bajos de Costa Rica, fueron analizadas por métodos anatómicos y fitoquímicos, para probar la hipótesis que los estrategas r invierten menos en la defensa contra herbívoros y patógenos que los estrategas k, por lo tanto, tienen tasas de descomposición más rápidas en el agua. En un estudio paralelo sobre descomposición de hojarasca, formación de detritus y colonización por macroinvertebrados, Tschelaut et al. (2008b), mostró claras diferencias entre el material vegetal proveniente de 4 especies frecuentes de árboles en el bosque ribereño del arroyo. Las 4 especies representaban diferentes estrategias de historia de vida, especies pioneras de rápido crecimiento (por simplicidad llamadas estrategas r), (*Acalypha diversifolia* – Euphorbiaceae, *Cecropia obtusifolia* – Cecropiaceae, *Tetrathylacium macrophyllum* – Flacourtiaceae), y especies con un climax de lento crecimiento (estrategas K) (*Sloanea medusula* – Elaeocarpaceae). Además de estas 4 plantas, 5 especies adicionales fueron analizadas en el presente estudio, para obtener una representación más amplia de los mecanismos de defensa. Las plantas difieren en su estructura anatómica y contenido de taninos en las hojas. Las diferencias anatómicas fueron cuantificadas por 13 parámetros, por ejemplo, proporción de esclerénquima y colénquima, en cortes anatómicos y combinados en un "Indice de dureza" (Toughness Index). Los resultados establecen relaciones para las tasas de descomposición observadas. La comparación muestra que la cantidad de taninos afecta la descomposición de la hojarasca durante los primeros días, no obstante las estructuras anatómicas juegan un rol principal durante todo el proceso de descomposición.

Palabras clave: descomposición de la hojarasca, taninos, anatomía vegetal, dureza.

Introduction

In low order streams, allochthonous material generally plays a major role as the energetic basis of the stream ecosystem. Allochthonous material, mainly in the form of leaf litter, develops a biofilm of bacteria and fungi which forms a major food source for invertebrates and detritivorous fish. The shredding of plant material by certain macrozoobenthic species transforms coarse particulate organic material (CPOM) to fine particulate organic material (FPOM) and enhances the microbial breakdown and decomposition processes (Vannote et

Stapfia **88**, zugleich Kataloge der oberösterreichischen Landesmuseen Neue Serie **80** (2008): 467-484

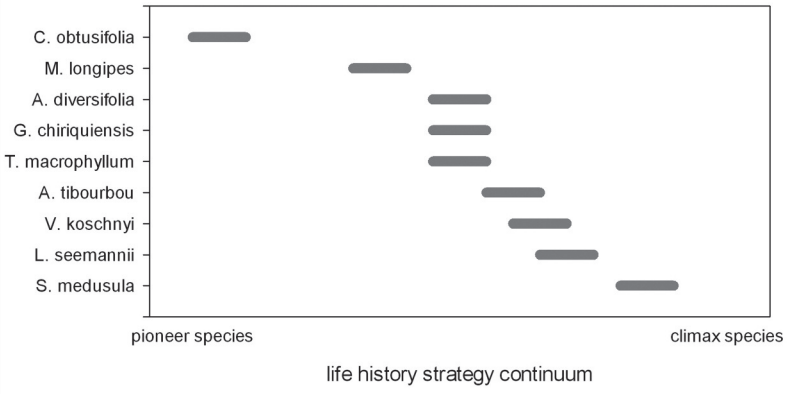

Fig. 1: Position in the life history strategy continuum of the nine species arranged according to a selected range of criteria proposed by WHITMORE (1993);

Table 1: Values of daily litter decomposition rate (k) from four different types of leaf packs (*Acalypha diversifolia*, *Cecropia obtusifolia*, *Tetrathylacium macrophyllum*, *Sloanea medusula*) exposed in the Q. Negra for 28 days, obtained from the slopes of regression equations, between log W_t and t (TSCHELAUT 2005).

taxon	W_0	W_t	t	k
Acalypha diversifolia (Euphorbiaceae)	3,16	1,045	28	0,0395
Cecropia obtusifolia (Cecropiaceae)	3,295	1,71	28	0,0234
Tetrathylacium macrophyllum (Flacourtiaceae)	3,175	0,88	28	0,0458
Sloanea medusula (Elaeocarpaceae)	4,67	3,73	24	0,0093

al. 1980). Tannins and anatomical leaf structures are effective defence mechanisms against herbivores. It can be assumed that leaf palatability and litter decomposition are strongly correlated (e.g. SCHÄDLER et al. 2003).

Leaf litter decay rates of different trees from the riparian forest of Quebrada Negra selected after their life history strategy were analysed by TSCHELAUT et al. (2008b). The four exposed plant species can be either put into the "fast" or "slow" decomposition group according to the classification of PETERSEN & CUMMINS (1974). *Acalypha diversifolia* (with a decay rate of 0.040), *Cecropia obtusifolia* (0.023) and *Tetrathylacium macrophyllum* (0.046) can be assigned to the "fast" group. *Sloanea medusula* (0.009) can be placed into the "slow" group. During the experiment duration of 28 days *A. diversifolia* and *T. macrophyllum* lost about 70% of initial dry weight and *C. obtusifolia* about 45%. *S. medusula* lost less than 20% of initial dry weight during 24 days of exposure in the river (Table 1).

In order to test the hypothesis that fast-growing pioneer species make a smaller investment in defence against herbivores and pathogens than slow-growing plants (climax species) and therefore pioneer species have faster decay rates, five more species (*Apeiba tibourbou* – Tiliaceae, *Guatteria chiriquiensis* – Annonaceae, *Luehea seemannii* – Tiliaceae, *Myriocarpa longipes* – Urticaceae, *Virola koschnyi* – Myristicaceae) were analysed in the present study with respect to anatomical features of the leaves (combined to a "toughness index") and their tannin content. Overall anatomical and chemical defence mechanisms related this findings to the leaf litter decomposition rates were obtained by TSCHELAUT et al. (2008b).

Material and Methods

Study site and selection of species

The plant material was collected at the Biological Field Station La Gamba, Costa Rica, located at the Piedras Blancas National Park (8°27'–8°41'N and 83°15'–83°45'W), especially in the riparian forest of Quebrada Negra, a first-order lowland rainforest stream. It has its source in the primary forest (~180 m above sea level) of the Esquinas rainforest (TSCHELAUT et al. 2008a).

In addition to four plants used by TSCHELAUT et al. (2008b) in their litter decomposition experiments, five more species were analysed in order to obtain a broader representation of the tree vegetation in the gallery forest along the river Quebrada Negra. The selected nine species were ordinated along a life history strategy continuum defined according to criteria proposed by WHITMORE (1993), e.g. growth rate, reproduction, spread of fruits and seeds, quality of wood, requirement of light and lifetime. The continuum ranges from fast-growing pioneer plants to slow growing climax species. For simplicity the terms r- and K-strategists are used (MACARTHUR & WILSON 1967).

Figure 1 outlines the position of the selected species in a life history strategy continuum. *Acalypha diversifolia*, *Cecropia obtusifolia*, *Guatteria chiriquiensis*, *Myriocarpa longipes* and *Tetrathylacium macrophyllum* are termed r-strategists, while *Apeiba tibourbou*, *Luehea seemannii*, *Sloanea medusula* and *Virola koschnyi* are K-strategists, although the distinction is not sharp.

Leaf material of the nine species was collected in February 2005. Attention was given to collect leaves near to abscission. In case of *Virola koschnyi*, which casts its leaves in dry seasons, only relatively young leaves could be harvested.

Leaf anatomy

Material was fixed in 30% ethanol. Five regions of the leaf were processed by anatomical sectioning techniques and the sections were stained for further analysis. The safranin-astra blue coloration and the auramin-mucicarmin coloration are tests to distinguish between lignified and non-lignified cell structures (BRAUNE et al. 1999; Färbevorschriften Firma CHROMA 1962). Furthermore the lignin-specific phloroglucin-HCl test and the lipid-specific Pearse reaction were applied. All plant

anatomical analysis were carried out at the Department of Ecophysiology and Functional Anatomy of Plants, University of Vienna.

With the stained cross-section preparations, the attempt was made to quantify and summarise the plant anatomical parameters considered to represent defence strategies for herbivore predation. The nine following measurable parameters were combined in a "toughness index" (TI):

- mean relative leaf dry weight
- area of the mid-vein
- size of epidermal cells
- cuticle formation and cuticle ridges
- amount of side-veins
- lignifications in the mid-vein
- lignifications in the leaf blade
- amount of sclerenchyma in the mid-vein
- amount of collenchyma in the mid-vein

In order to calculate the "toughness index" the individual results were converted into relative values from 0 (lowest toughness value) to 100 (highest toughness value). The scale finally was divided into three equal sections and allocated a factor from one to three. The index is the sum of the results from each section in order to obtain a single robust value.

Measurements of tannins

To quantify the amount of tannins in plant tissue, two common methods were applied. Total phenolics on fresh and dried leaf samples were measured by the Folin Denis Method, first described by SWAIN & HILLIS (1959) and modified by MARTIN & MARTIN (1982), HAGERMAN (1988), COLEY & BARONE (1996) and HÄTTENSCHWILER et al. (2003). The Folin Denis Assay detects hydrolysable and condensed tannins and also non-tannin phenolics and other readily oxidisable materials such as ascorbate (HAGERMAN & BUTLER 1991). The determination of condensed tannins was carried out by the butanol HCl method (PORTER et al. 1986) on dried leaf material. For both assays, aqueous acetone was used as extraction solvent. Acetone is an effective solvent which inhibits interaction between tannin and proteins and thus prevents tannin from binding to leaf proteins during homogenisation (discussed in HAGERMAN 1988). Fresh samples were processed at the Biological Field Station La Gamba, Costa Rica. Dry samples were analysed at the Department of Chemical Ecology and Ecosystem Science, University of Vienna. At least five samples were analysed for each parameter. The statistical analysis of data was performed with the software Statgraphics Plus for Windows 5.0. Statistical significance was tested with one-way ANOVA and Scheffe or LSD test ($p<0,05$).

Results

Leaf anatomy

C. obtusifolia show long unicellular hairs on the underside of the leaf blade and cone-shaped and glandular hairs on the upper side. The cross sections of the mid-vein vascular bundles show dispersed lignified structures. Sclerenchyma cells and crystals are rare, secretion canals frequent. The side-veins in the leaf blade are surrounded by a bundle sheath, which protrude to epidermal cells (see Plate 1).

M. longipes (Urticaceae) exhibits great variation in the leaf size between sun and shade leaves. The upper surface of the leaf blade is glabrous but warty. One of the typical characteristics of Urticaceae are cystoliths as supplements of the epidermal cell wall incrusted with calcium carbonate. Sclerenchyma cells are absent. The vascular bundles occur in form of a ring. In the parenchyma, secretion canals and crystals are frequent. Side-veins have a prominent bundle sheath without sclerenchyma cells (see Plate 2).

T. macrophyllum (Flacourtiaceae) are glabrous on the top. The underside is covered with unicellular hairs. The mid-vein shows pronounced lignified structures that occupy almost half of the mid-vein area. Sclerenchyma cells form a circle several cells wide around the vascular bundles. The spongy parenchyma in the leaf blade is loose and contains large gas spaces (see Plate 3).

G. chiriquiensis (Annonaceae) leaves are covered by hairs on both sides. Especially on the veins the hairs grow very densely. The cross section of the mid-vein shows a ring-shaped sclerenchyma around the vascular bundles. The species contains crystals in large epidermal cells which is a typical feature for the Annonaceae. We found some special cells with lipid stores. The side-veins have prominent bundle sheets of sclerenchyma cells that do not reach the epidermis (see Plate 4).

A. diversifolia (Euphorbiaceae) has long hairs along the veins on the underside of the leaves. Older leaves are heavily covered with epiphylls. Some solitary cells of sclerenchyma lie outside the vascular bundles. Crystals are very frequent in epidermal cells, a typical attribute for many species of the Euphorbiaceae, although they occur only rarely in the parenchyma and phloem. Large crystals form a distinctive feature of the leaf blade (see Plate 5).

A. tibourbou (Tiliaceae) leaves are barely covered with hairs on the upper side, whereas on the underside, dense stellate hairs without ramification occur. The cross sections of the mid-veins show a ring-shaped vascular bundle with a prominent bundle sheet of sclerenchyma cells. In the parenchyma outside the vascular

bundle, secretion canals and crystals were found. Beneath the epidermal cells of the leaf blade, large idioblasts are frequent (see Plate 6).

V. koschnyi (Myristicaceae) shows a significant heart-shaped leaf node. On both sides as well as on the veins, star-like hairs can be found. Anatomical sections of the mid-veins show a ring-shaped xylem. Phloem is arranged in small groups surrounded by parenchyma cells. In the outer parenchyma, a disconnected ring of sclerenchyma cells occurs. Further sclerenchyma surrounded by phloem can be found in the inner parenchyma. Cells walls of the parenchyma are partially lignified as shown by fluorescence microscopy. Epidermal cells of the leaf blade are homogeneous in dimension and form. Cells of the palisade mesophyll have an isodiametric form. Among the palisade mesophyll, larger cells with crystals occur. The spongy mesophyll of leaves of *V. koschnyi* is relatively dense. Between palisade and spongy mesophyll idioblasts which have a positive reaction with gentian violet are conspicuous (see Plate 7).

L. seemannii (Tiliaceae) leaves are clearly characterised by their brown underside which is covered by a tomentum of hairs whereas the upper side is rarely pubescent. The vascular bundle of the mid-vein surrounded by a prominent ring of sclerenchyma is made up of two semicircles of which the smaller upper semicircle is inversely orientated. Crystals are abundant in parenchyma cells near the prominent sclerenchyma. Sections of the leaf blade show the tomentum of multi-cellular hairs on the bottom side. Lignified structures occur in vascular bundles of side-veins. Cell walls of mesophyll cells are partially lignified. Bleached and uncoloured sections show that the mesophyll of the leaf blade is extremely densely packed. The cross section of leaf blade has a characteristic undulating form. At the constrictions, lignified structures occur which reach from the upper to the lower epidermis. Above the lignified structures, large idioblasts can be found which contain cubic crystals. As discussed in leaves of *A. tibourbou* big idioblasts beneath the epidermal cells of the leaf blade are frequent (see Plate 8).

S. medusula (Elaeocarpaceae) has large leaves which carry hairs on the veins on both sides. The upper side of the leaf blade feels furry. Anatomical cuts show the relation between the extremely prominent mid vein and the blade. Vascular bundles form a ring with three horizontal strata of xylem and phloem in the centre of the ring. The upper layer is inversely orientated. A dominant element of stability is the extensive development of collenchyma. In the collenchyma and parenchyma cells, cubic and columnar crystals occur. In the xylem of the horizontal bundles, secretion canals of various sizes occur. In the leaf blade, the high number of side-veins with bundle sheets that reach up to the upper epidermis is conspicuous. Analyses by fluorescence microscopy showed that stomatal cells of *S. medusula* are lignified. Sometimes secretion canals are embedded in the mesophyll of the leaf blade (see Plate 9).

Table 2: Summary of lignified structures, amount of sclerenchyma and collenchyma in per cent of the mid-vein area.

species	lignified structures	amount of sclerenchyma	amount of collenchyma
C. obtusifolia	13,1 %	0,2 %	12,5 %
M. longipes	5,2 %	0 %	16,3 %
A. diversifolia	18,6 %	2,9 %	8,9 %
G. chiriquiensis	26,2 %	12,9 %	0 %
T. macrophyllum	43,6 %	16,6 %	8,2 %
A. tibourbou	29,3 %	8,5 %	10,9 %
V. koschnyi	42,4 %	6,5 %	2,3 %
L. seemannii	43,6 %	21,3 %	6,9 %
S. medusula	45,2 %	8,3 %	16,5 %

Toughness index

The proportions of the various mechanical stabilising elements in the mid-vein of the leaves of the nine species compared are given in table 2.

The characteristics of toughness for each species are shown in fig. 2 and 3.

The toughness index (TI) values calculated for the nine species ranged from 18 to 36. The highest values among the K-strategists were found for *L. seemannii* and *S. medusula* (36) followed by *A. tibourbou* (27) and *V. koschnyi* (21). Among the r-strategists the range was 17 to 23 (*T. macrophyllum:* 23, *C. obtusifolia*: 22, *A. diversifolia:* 21, *M. longipes:* 18 , *G. chiriquiensis:* 17). The difference between the two groups is statistically significant.

A correlation coefficient between toughness index and litter decay rates of *A. diversifolia*, *C. obtusifolia*, *S. medusula* and *T. macrophyllum* is $R^2 = 0{,}632$.

Content of tannins

The Folin Denis method for overall tannins on fresh leaves of the nine species indicated concentrations of 1.9% to 45.9% tannic acid equivalents on dry weight (TAE). Among the K- strategists the highest amounts were measured for *V. koschnyi* with values up to 45.9% TAE (s = 10.6, n = 10), whereas the lowest level was found in leaves of *L. seemannii* with 13.7% TAE (s = 1.9, n = 10). The lowest levels for r-strategists were found in *G. chiriquiensis* with 1.88% TAE (s = 0.01, n = 15) and *M. longipes* with 2.30% TAE (s = 2.4, n = 10). Among the r-strategists, a maximum level of tannins was found

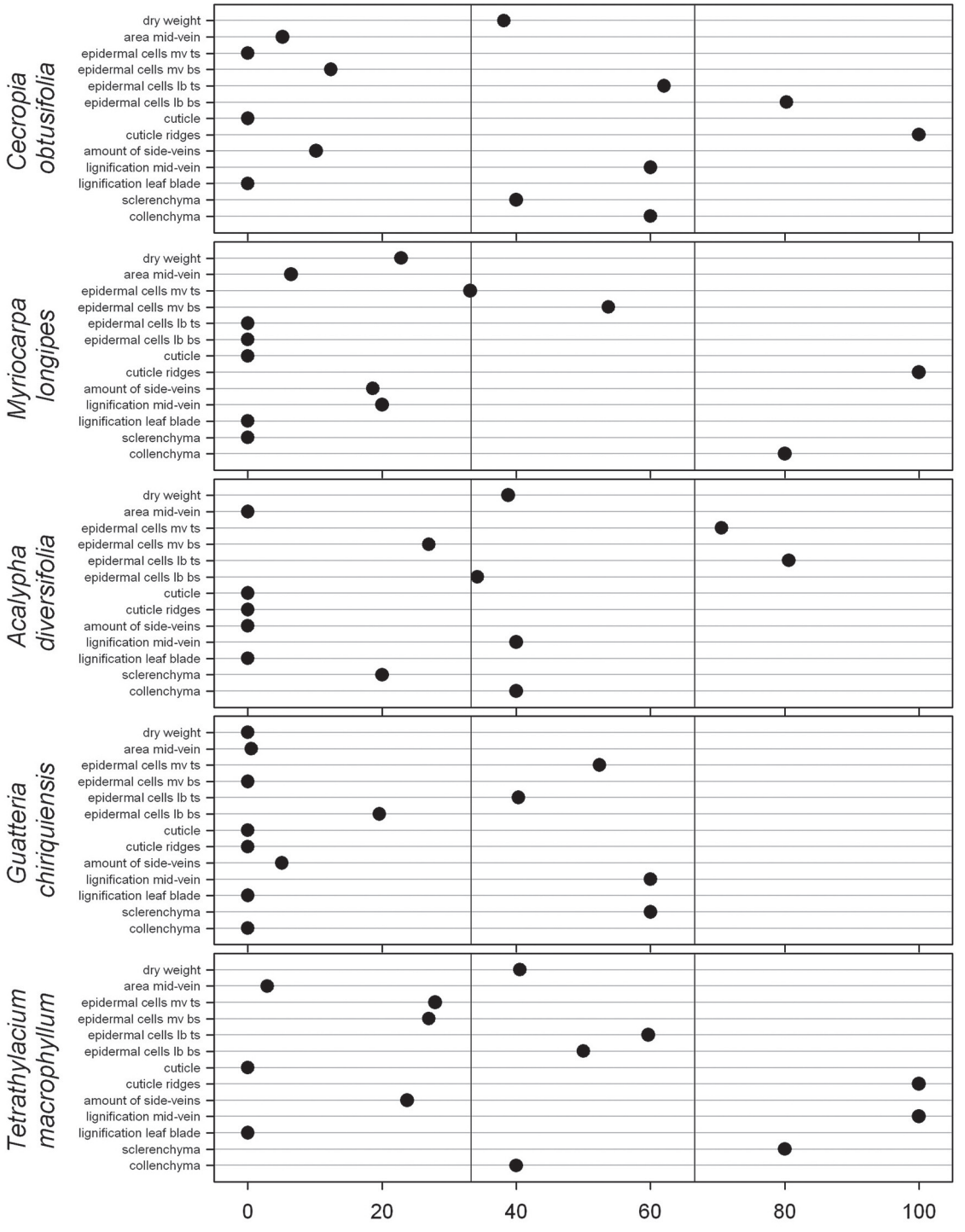

Fig. 2: Summary of the characteristic anatomical parameters for the fast growing species. All measured values were assigned from cero to 100. Scale is divided into three equal ranges for calculation of the "Toughness Index".

in leaves of *C. obtusifolia* with 20.6% TAE (s = 2.5, n = 10). The difference between the two groups is statistically significant.

In comparison to measurements on fresh material the same method was carried out on dry material. The amounts of TAE range between 1.6% and 51.2%. The highest level was found in leaves of *V. koschnyi* with 51.2% TAE (s = 12.0, n = 10) followed by *S. medusula* with 36.0% TAE (s = 17.9, n = 10). Similar to the analysis on fresh material *M. longipes* and *G. chiriquiensis* showed the lowest levels (1.6% TAE, s = 1.7, n = 10 and 4.9% TAE, s = 1.9, n = 5, respectively). Again, a difference between the two life history groups is statistically significant.

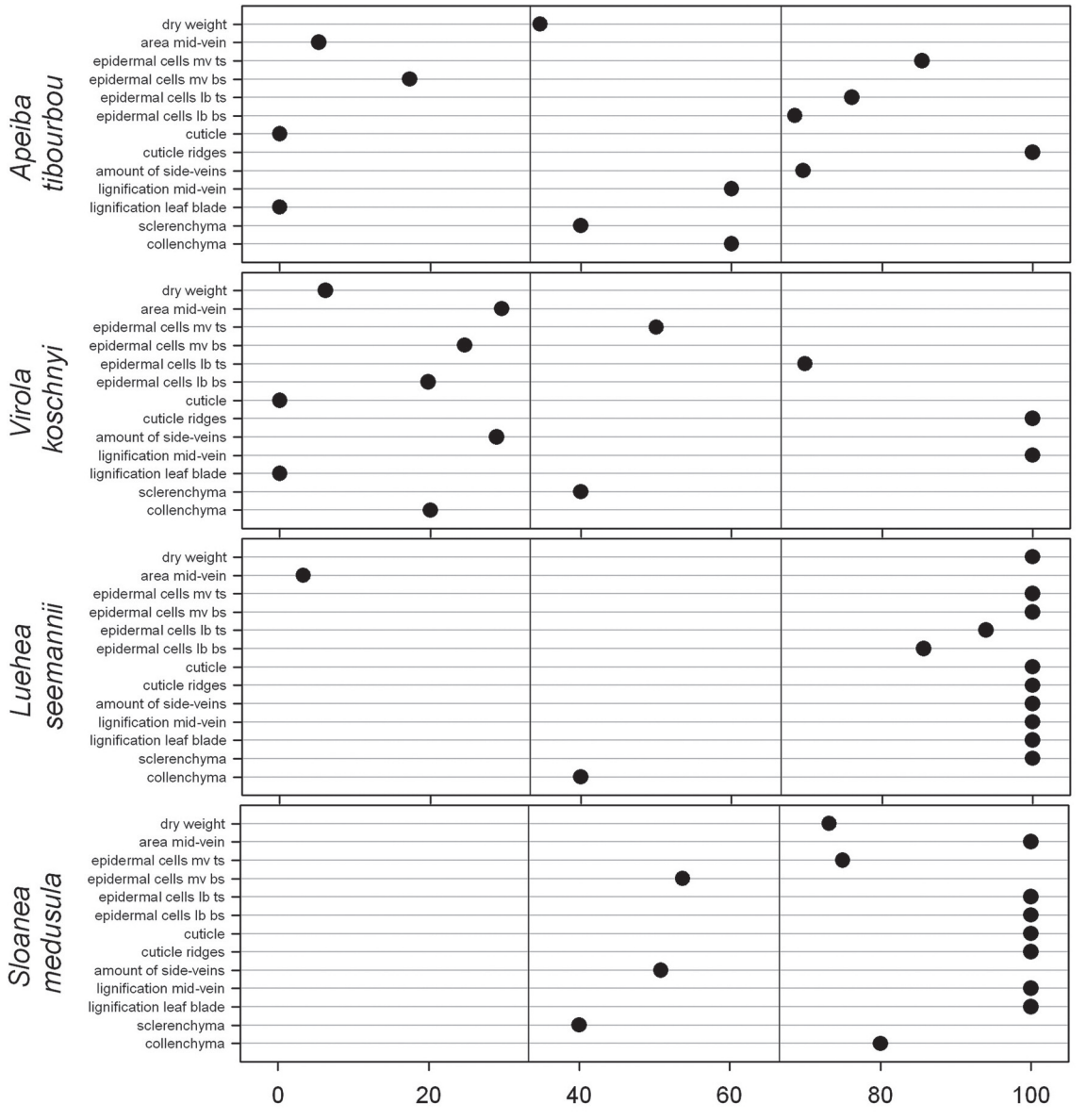

Fig. 3: Summary of the characteristic anatomical parameters for the slow growing species. All measured values were assigned from cero to 100. Scale is divided into three equal ranges for calculation of the "Toughness Index".

Table 3: Results of tannin measurements; All over tannins (%TAE) measured by the Folin Denis method on dry and fresh samples an condensed tannins (%CE) by the Butanol HCl method on dry material.

	TAE fresh	TAE dry	CE dry
C. obtusifolia	20,6 ± 2,5	17,7 ± 3,9	11,3 ± 2,3
M. longipes	2,3 ± 2,4	1,6 ± 1,7	0,5 ± 0,8
A. diversifolia	10,6 ± 2,5	27,8 ± 9,4	1,9 ± 0,3
G. chiriquiensis	1,9 ± 0,6	4,9 ± 1,3	0,6 ± 0,3
T. macrophyllum	8,4 ± 1,9	7,3 ± 2,1	0,1 ± 0,1
A. tibourbou	19,6 ± 6,5	27,4 ± 12,7	0,0 ± 0,0
V. koschnyi	45,9 ± 10,6	51,2 ± 12,0	13,3 ± 1,5
L. seemannii	13,7 ± 1,9	9,6 ± 3,4	5,9 ± 0,2
S. medusula	25,8 ± 4,4	36,0 ± 17,9	7,4 ± 0,7

A different pattern was obtained when using the butanol HCl method with dry leaf material. The highest concentrations on condensed tannins were found in leaves of *V. koschnyi* with 13.3% cyanidin equivalents on dry weight (CE) (s = 1.5, n = 10) closely followed by *C. obtusifolia* with 11.3% CE (s = 2.3, n = 10). The next range is formed by *S. medusula* (7.4% CE, s = 0.7, n = 10) and *L. seemannii* (5.9% CE, s = 0.2, n = 10). The amounts of condensed tannins in *A. diversifolia*, *G. chiriquiensis* and *M. longipes* are clearly lower. In the case of *T. macrophyllum* and *A. tibourbou*, single values were below detection level (Table 3).

A comparison between decay rates of *A. diversifolia*, *C. obtusifolia*, *S. medusula* and *T. macrophyllum* and the overall tannin levels measured by Folin Denis on fresh

material shows a very high correlation (R^2 = 0.988), whereas the condensed tannins measured by buthanol HCl method have a weaker correlation (R^2 = 0.614).

Analyses of the tannin levels during decomposition show that overall tannins as well as condensed tannins are leached or decomposed after at least 25 days of exposure in the river. Tannins of *S. medusula* leached or decomposed more slowly than those of *A. diversifolia*, *C. obtusifolia* and *T. macrophyllum*, in which the tannins were already gone after 14 days of exposure in the river.

Discussion

Anatomical parameters are difficult to quantify and compare, because morphology and leaf anatomy are highly diverse. The toughness of leaves has been an issue of many studies but there is no consistent definition of this term and method for assessments. CHOONG (1996) used the epidermis, thick walled cells beneath and the cuticle, WRIGHT & WESTOBY (2002) the concentration of cell wall, vascular tissue, fibre or sclerenchyma in a leaf as parameters for toughness. It was one of the objectives of our study to quantify and combine a broad range of plant anatomical characteristics that are thought to be important in leaf litter decay in an "toughness index" (TI). Our index shows a statistical significant difference between plants of different life history strategies and correlates well with aquatic decay rates.

Tannin levels after the Folin Denis assay showed a clear (R^2 = 0.828) correlation between the values measured on dry and fresh material. This means that a rough estimate on tannin levels can also be obtained form dry material extractions. Tannin levels measured by the Folin Denis assay (TAE) on fresh material vary from 1.9% to 45.9% TAE. BALDWIN & SCHULTZ (1988) found values of overall tannins with the same assay under 17%, whereas COLEY & BARONE (1996) cite an average value of 6.9% from a literature search. Few citations describe higher overall tannin values, e.g. CAMPBELL & FUCHSHUBER (1995) up to 35% and SWAIN (1965) up to 60%. These differences may be caused by variations in extraction and purification of extracts. A statistical outlier is *V. koschnyi*, which shows the highest overall tannin value. This may be caused by the relative young leaves of this species used for the measurements (see COLEY & AIDE 1991).

Condensed tannins (CE) are assumed to be the main factor regulating decomposition processes in rivers and streams (CAMPBELL & FUCHSHUBER 1995). COLEY & BARONE (1996) found values of condensed tannins in mature leafs of tropical species at about 5.5% CE and HÄTTENSCHWILER et al. (2003) values up to 14%. In this study levels of CE ranged from 0% to 13.3%. *V. koschnyi*

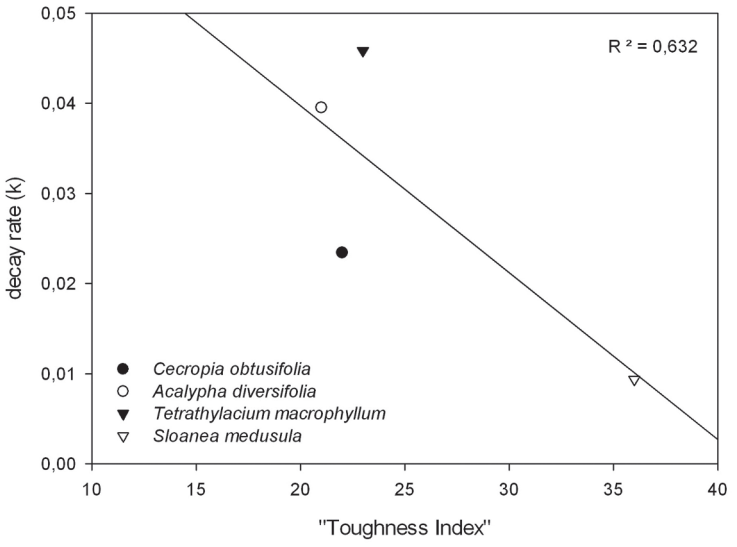

Fig. 4: Correlation between „Toughness Index" and decay rate.

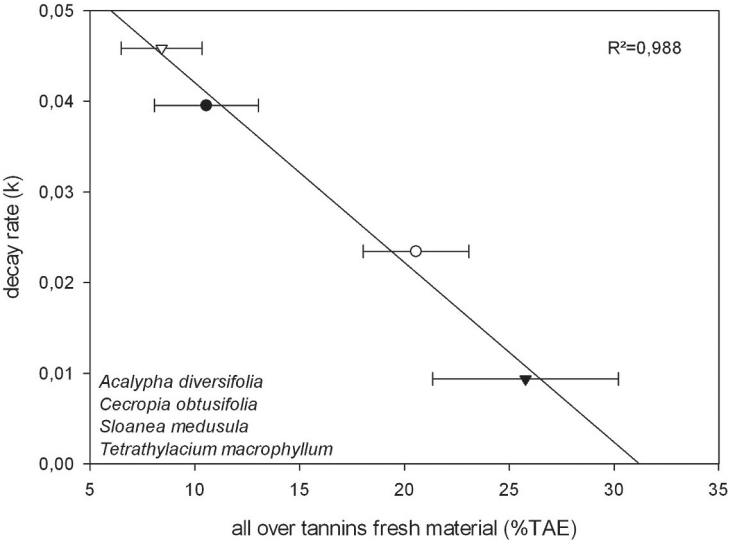

Fig. 5: Correlation between all over tannins and decay rate.

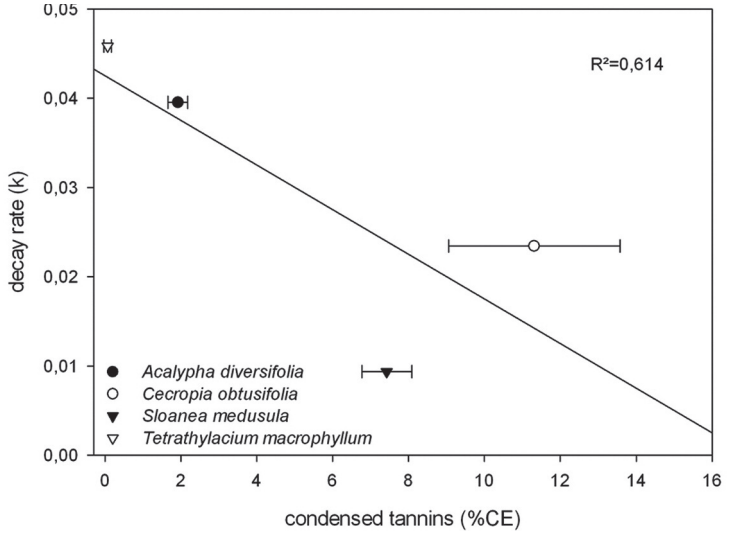

Fig. 6: Correlation between condensed tannins and decay rate.

again shows the highest levels, closely followed by C. *obtusifolia*. Leaves of the r-strategist C. *obtusifolia* are often directly exposed to the sun. COLEY & KURSAR (1996) demonstrate that carbon based defence mechanisms like tannins can be increased by sun light in different tropical plants. For *T. macrophyllum* and *A. tibourbou*, condensed tannins could not be detected, possibly as a result of the drying process. Despite these inconsistencies all three methods, Folin Denis assay on dry and fresh material and buthanol HCl method, show a statistical significant difference between the two distinguished life history strategy groups. In our results, the correlation between decay rates and overall tannins is stronger than with condensed tannins. This is in contrast to results of STOUT (1989), FIELD & LETTINGA (1992) and CAMPBELL & FUCHSHUBER (1995).

In summary, a statistically significant difference between life history strategy groups of plants are found both in tannin levels and in anatomical parameters and thus a classification according to life history strategies can be used as a predictor for aquatic decomposition of aquatic leaf litter decay in tropical rivers. Our results are consistent with the observation of SMITH et al. (1998) that the first stages of leaf litter decay are mainly controlled by chemical compounds whereas later on mechanical structures become more important.

Acknowledgements

We thank T. Weissenhofer for the logistic support during the collection of plant material in La Gamba.

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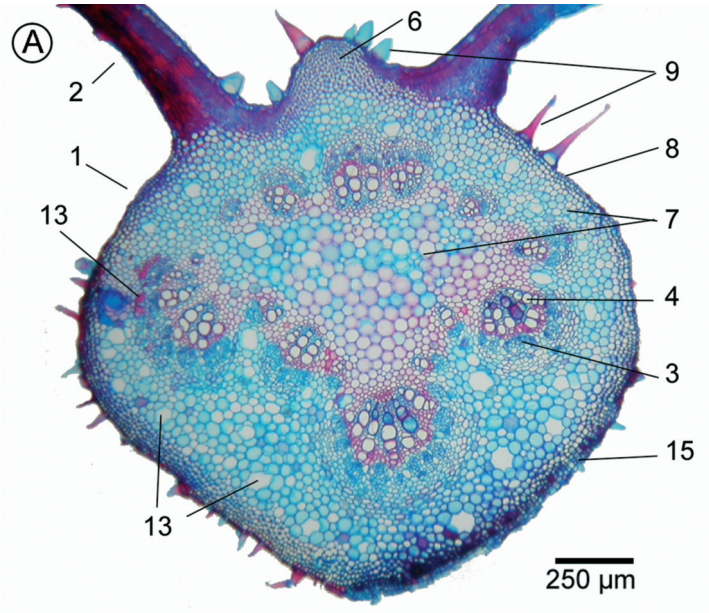
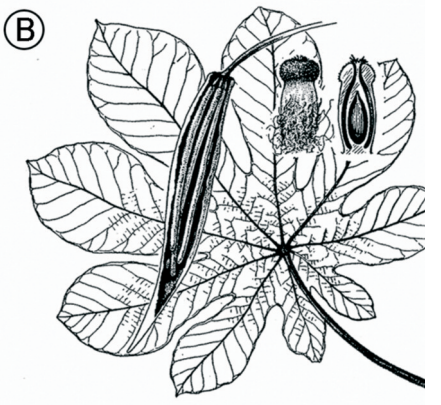
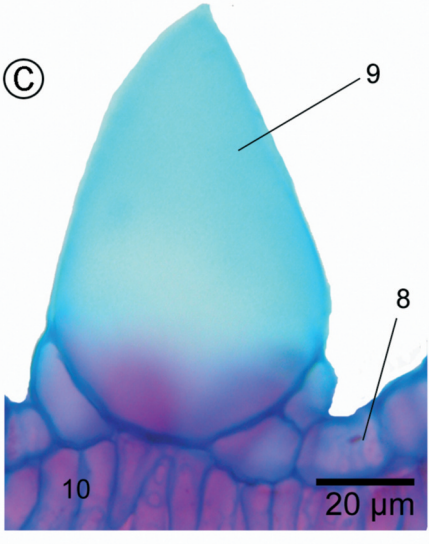
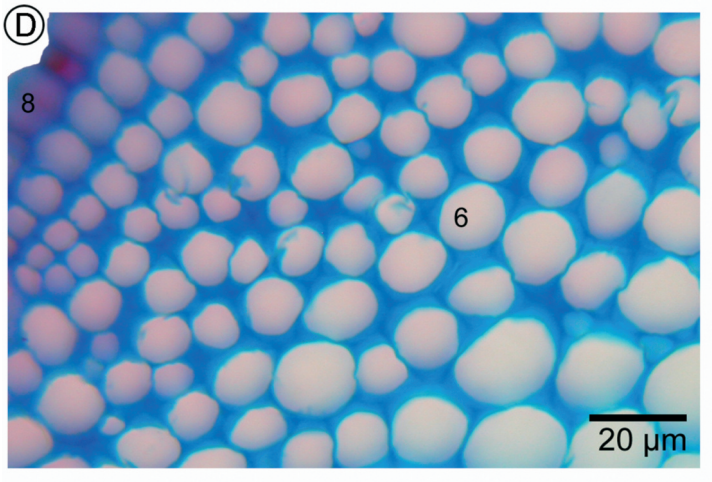
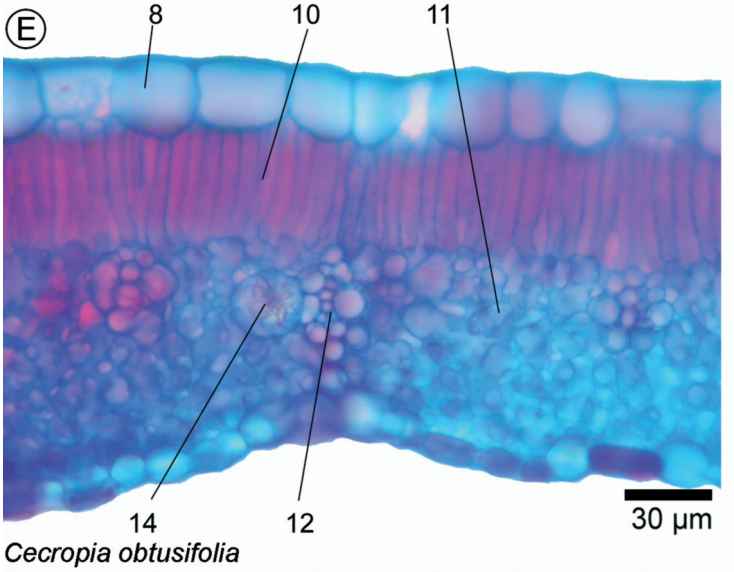
Plate 1:
Cecropia obtusifolia









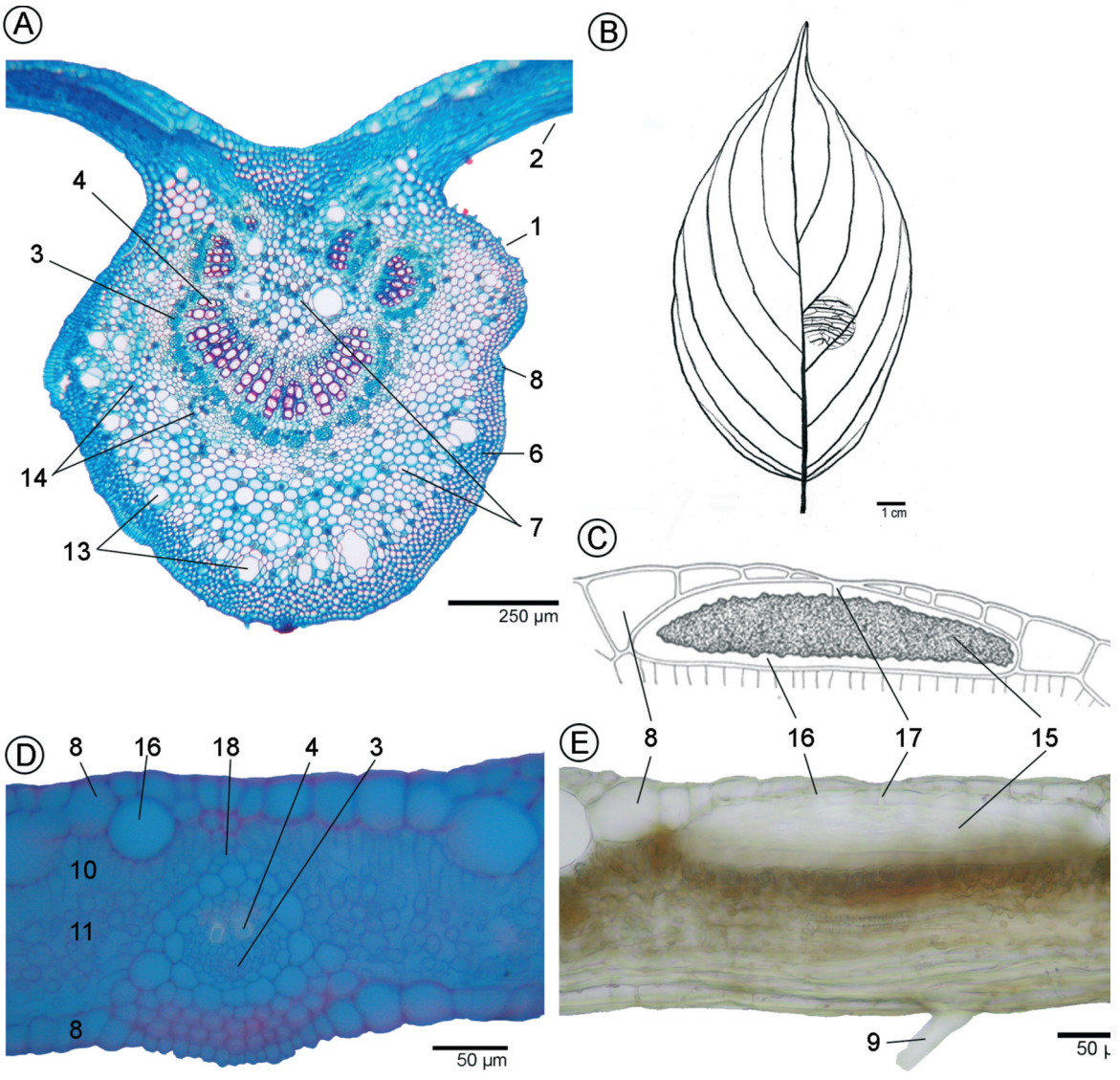


1 mid vein
2 leaf blade
3 phloem
4 xylem
5 sclerenchyma
6 kollenchyma
7 parenchyma
8 epidermis
9 hair
10 palisade mesophyll
11 spongy mesophyll
12 vascular bundle
13 secretion canal
14 crystal
15 periderm

Cecropia obtusifolia

A mid vein (bleached, safranin - astrablue)
B draft of the leaf (WEBER et al. 2001)
C hair (leaf blade) (bleached, safranin - astrablue)
D part of the mid vein (bleached, safranin - astrablue)
E leaf blade (bleached, safranin - astrablue)

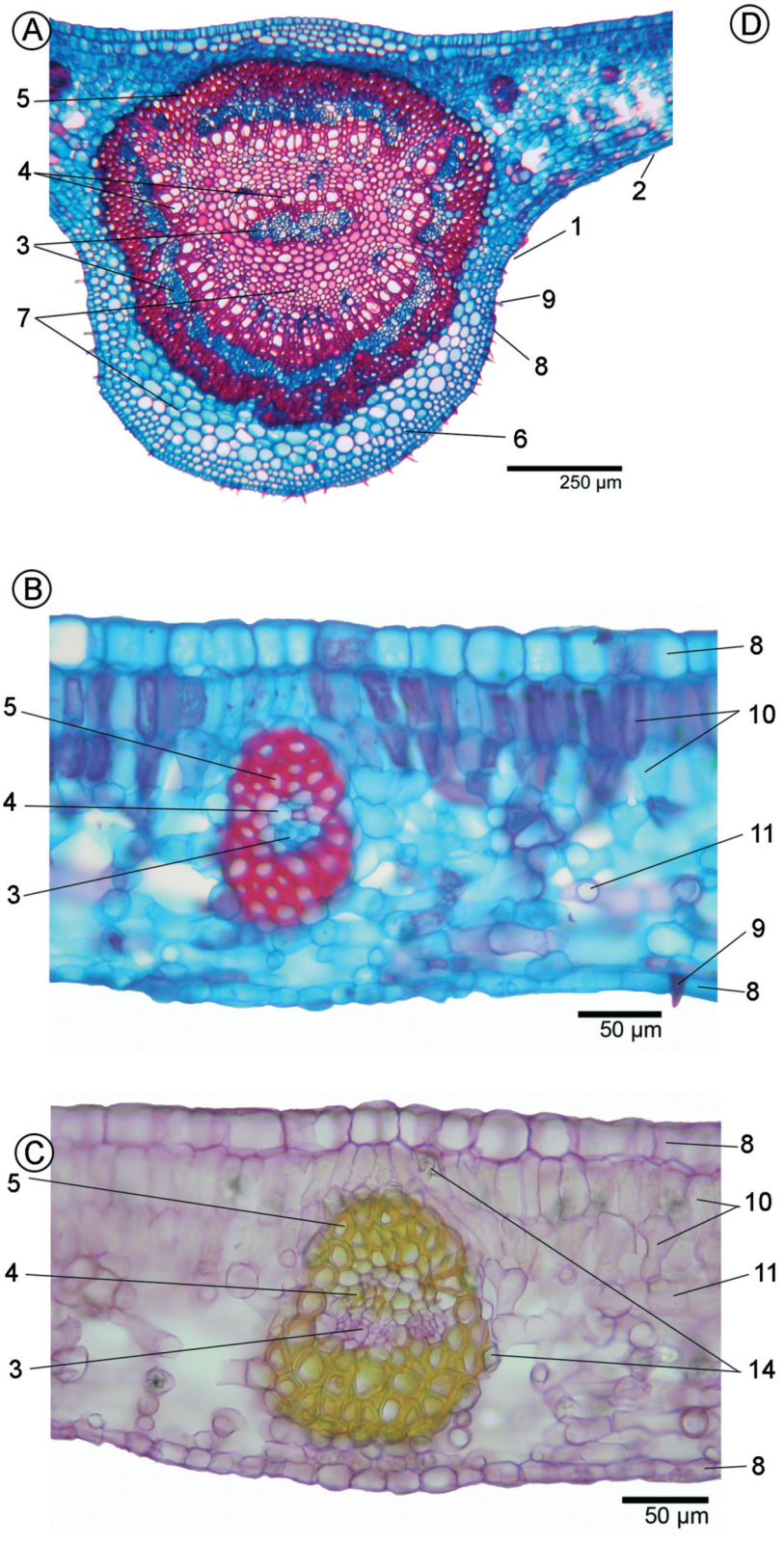
Plate 2:
Myriocarpa longipes



1 mid vein
2 leaf blade
3 phloem
4 xylem
5 sclerenchyma
6 kollenchyma
7 parenchyma
8 epidermis
9 hair
10 palisade mesophyll
11 spongy mesophyll
12 vascular bundle
13 secretion cana
14 crystal
15 cystolith
16 lithocyst
17 connection
18 bundle sheat

Myriocarpa longipes
A mid vein (bleached, safranin - astrablue)
B draft of the leaf
C draft of a cystolith (Napp-Zinn 1973)
D leaf blade (bleached, auramin - mucicarmin, fluorescence microscopy)
E leaf blade (unbleached, unstained)
F leaf blade (bleached, safranin - astrablue)

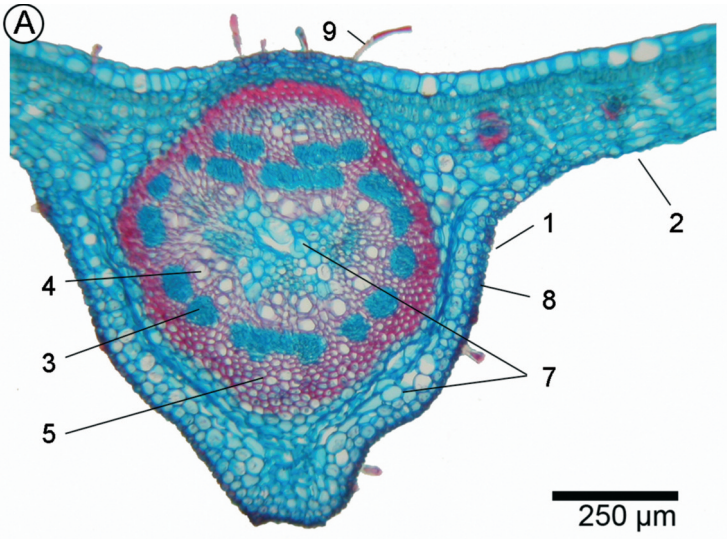
Plate 3:
Tetrathylacium macrophyllum



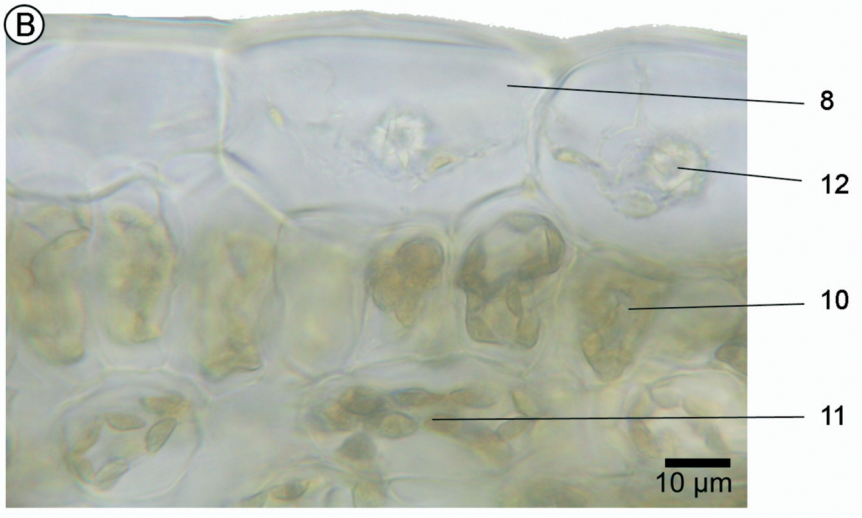
1 mid vein
2 leaf blade
3 phloem
4 xylem
5 sclerenchyma
6 kollenchyma
7 parenchyma
8 epidermis
9 hair
10 palisade mesophyll
11 spongy mesophyll
12 vascular bundle
13 secretion canal
14 crystal

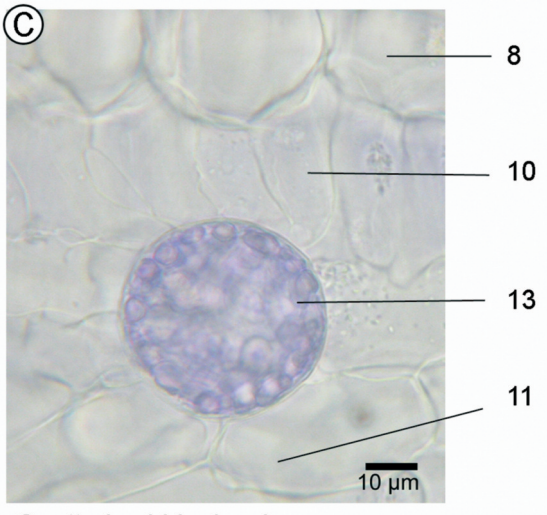
Tetrathylacium macrophyllum
A mid vein (bleached, safranin - astrablue)
B leaf blade (bleached, safranin - astrablue)
C leaf blade (bleached, auramin - mucicarmin)
D draft of the leaf

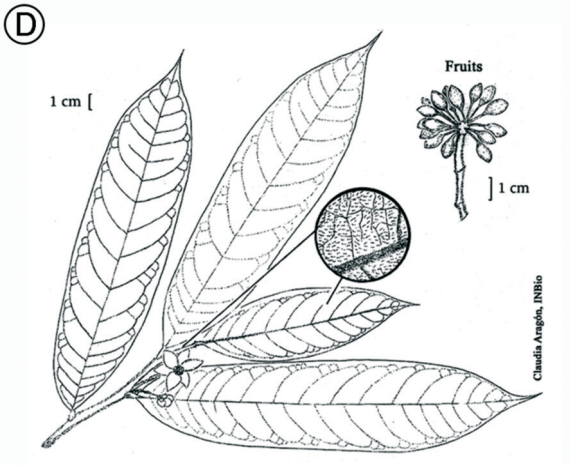
Plate 4:
Guatteria chiriquiensis



1 mid vein
2 leaf blade
3 phloem
4 xylem
5 sclerenchyma
6 kollenchyma
7 parenchyma
8 epidermis
9 hair
10 palisade mesophyll
11 spongy mesophyll
12 crystal
13 cell with lipid store

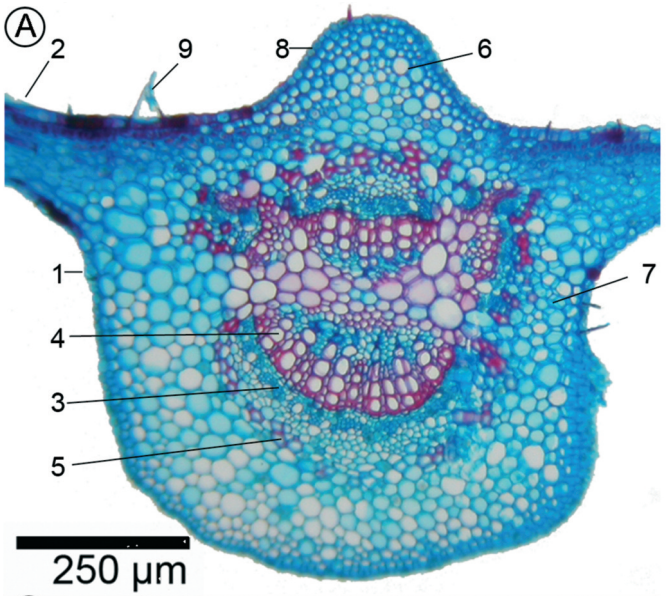


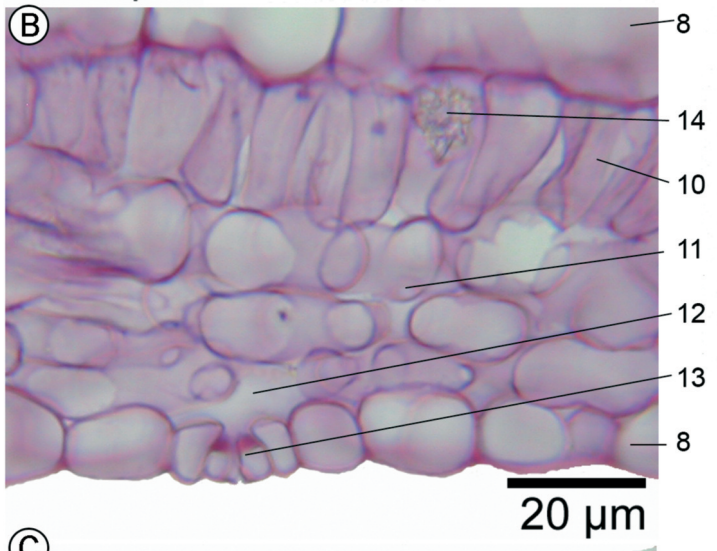


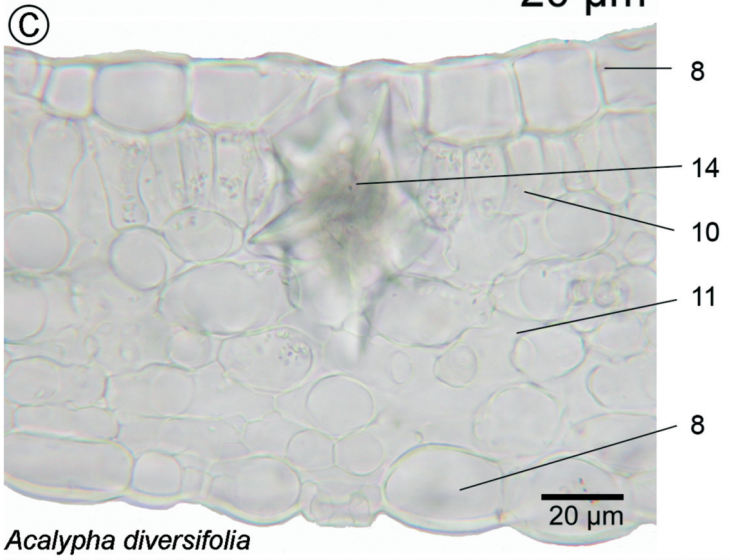


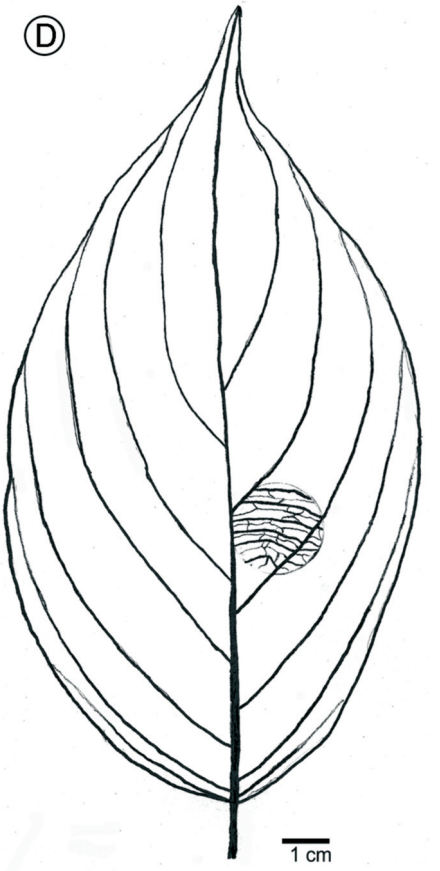
Guatteria chiriquiensis
A mid vein (bleached, safranin - astrablue)
B leaf blade (bleached, unstainded)
C leaf blade (bleached, gentian violet)
D draft of the leaf (WEBER et al. 2001)

Plate 5: *Acalypha diversifolia*





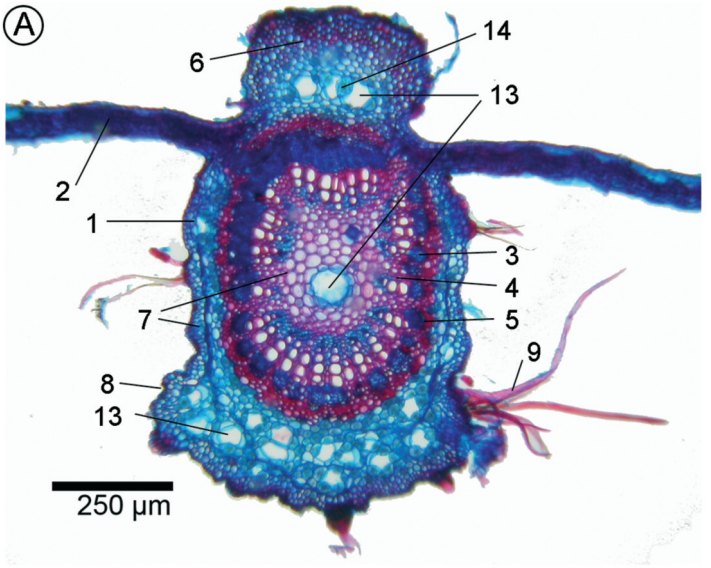


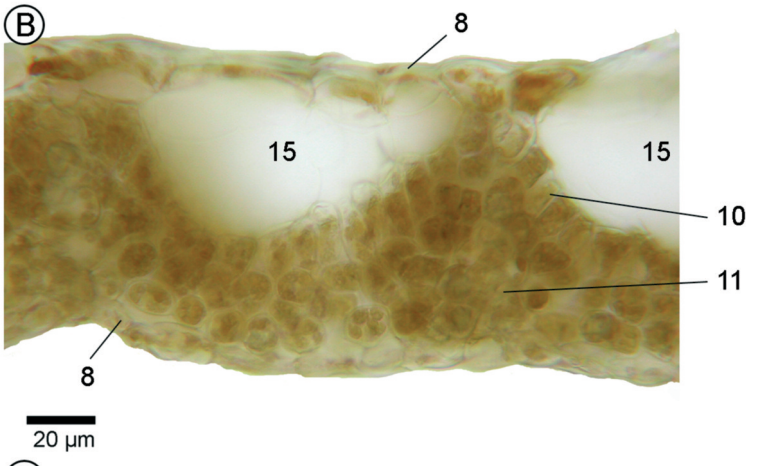


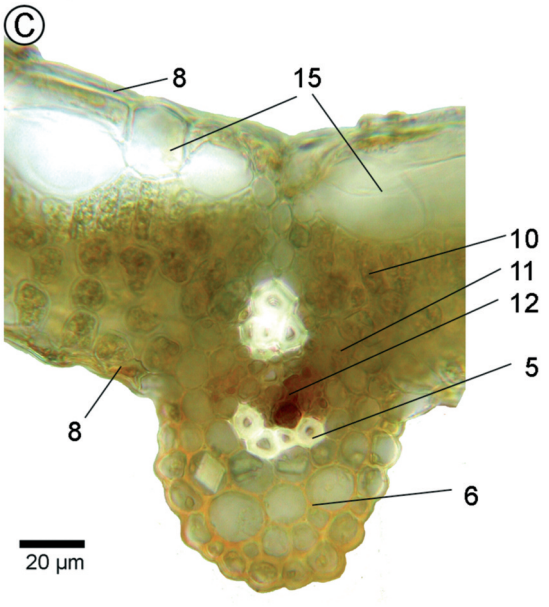
1 mid vein
2 leaf blade
3 phloem
4 xylem
5 sclerenchyma
6 kollenchyma
7 parenchyma
8 epidermis
9 hair
10 palisade mesophyll
11 spongy mesophyll
12 stomatal space
13 stoma
14 crystal

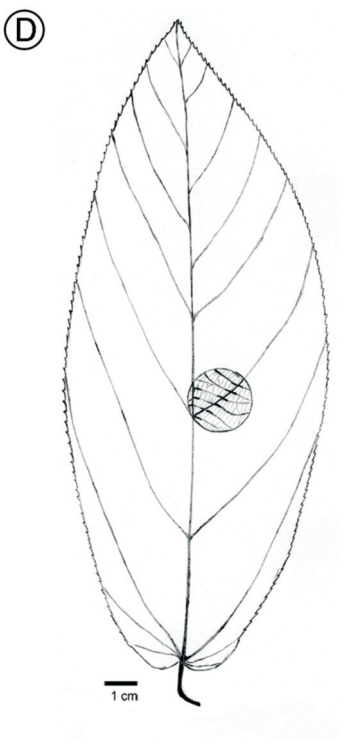
Acalypha diversifolia
A mid vein (bleached, safranin - astrablue)
B leaf blade (bleached, auramin - mucicarmin)
C leaf blade (bleached, unstained)
D draft of the leaf

Plate 6:
Apeiba tibourbou





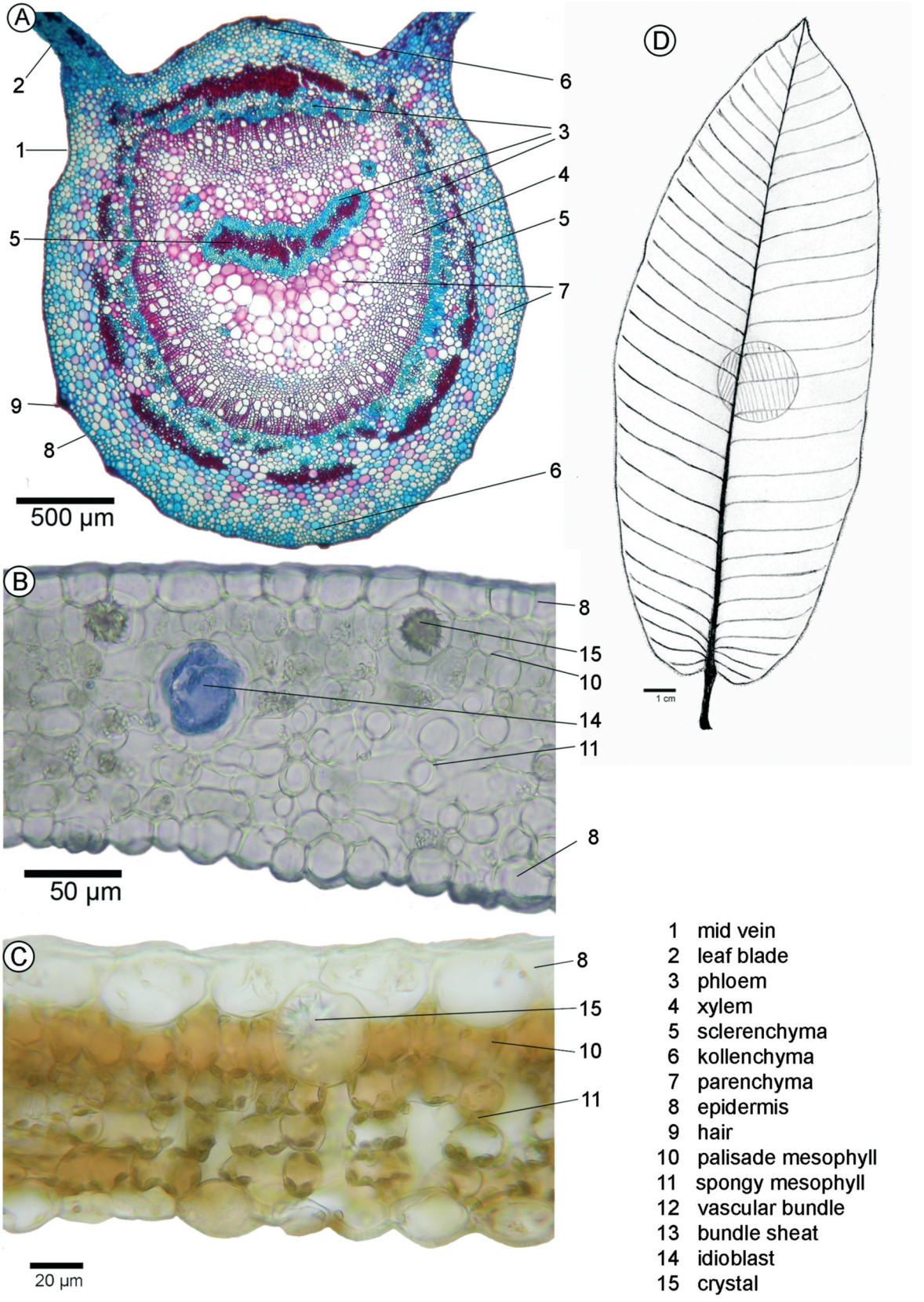




1 mid vein
2 leaf blade
3 phloem
4 xylem
5 sclerenchyma
6 kollenchyma
7 parenchyma
8 epidermis
9 hair
10 palisade mesophyll
11 spongy mesophyll
12 vascular bundle
13 secretion canal
14 crystal
15 idioblast

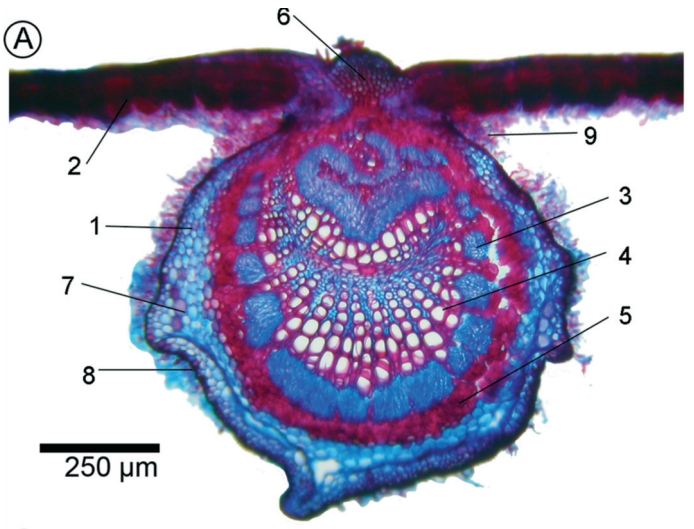
Apeiba tibourbou
A mid vein (bleached, safranin - astrablue)
B leaf blade (bleached, unstained)
C leaf blade (unbleached, unstained)
D draft of the leaf

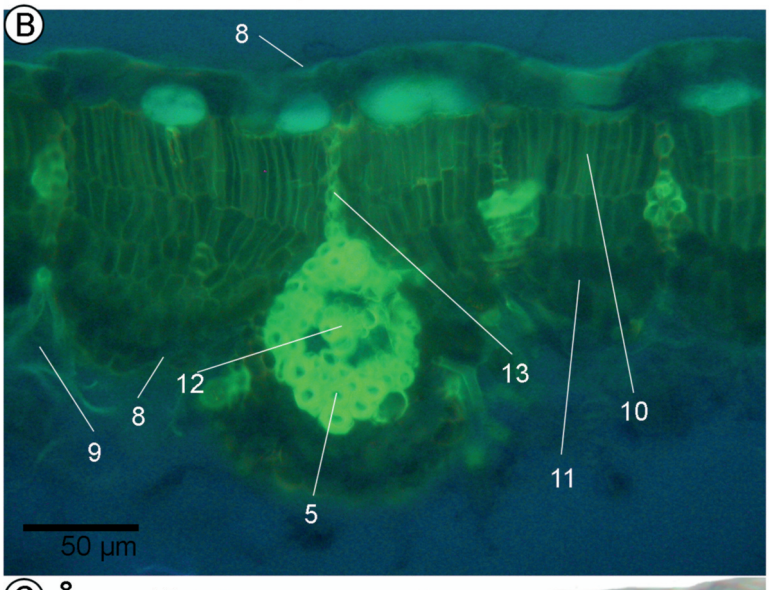
Plate 7:
Virola koschnyi

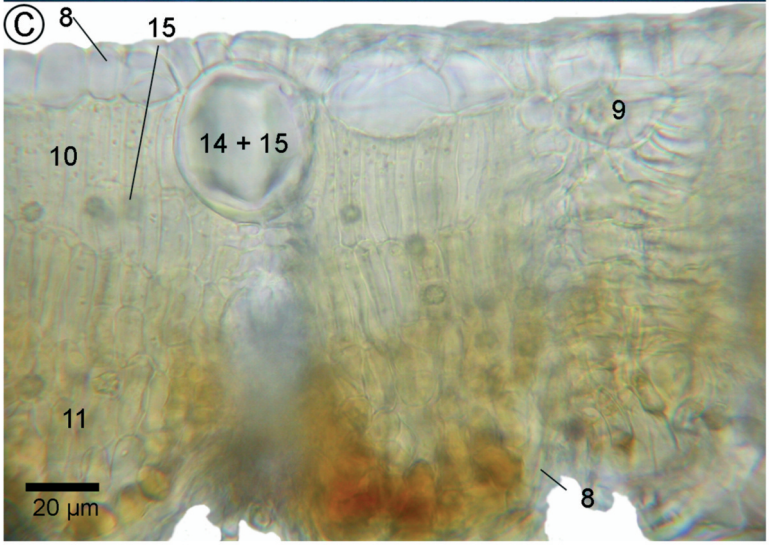


1 mid vein
2 leaf blade
3 phloem
4 xylem
5 sclerenchyma
6 kollenchyma
7 parenchyma
8 epidermis
9 hair
10 palisade mesophyll
11 spongy mesophyll
12 vascular bundle
13 bundle sheat
14 idioblast
15 crystal

Virola koschnyi
A mid vein (bleached, safranin - astrablue)
B leaf blade (unbleached, gentian violet)
C leaf blade (unbleached, unstained)
D draft of the leaf







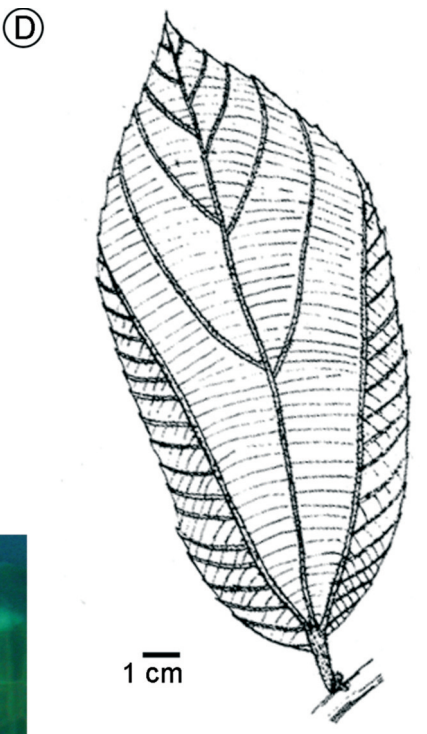
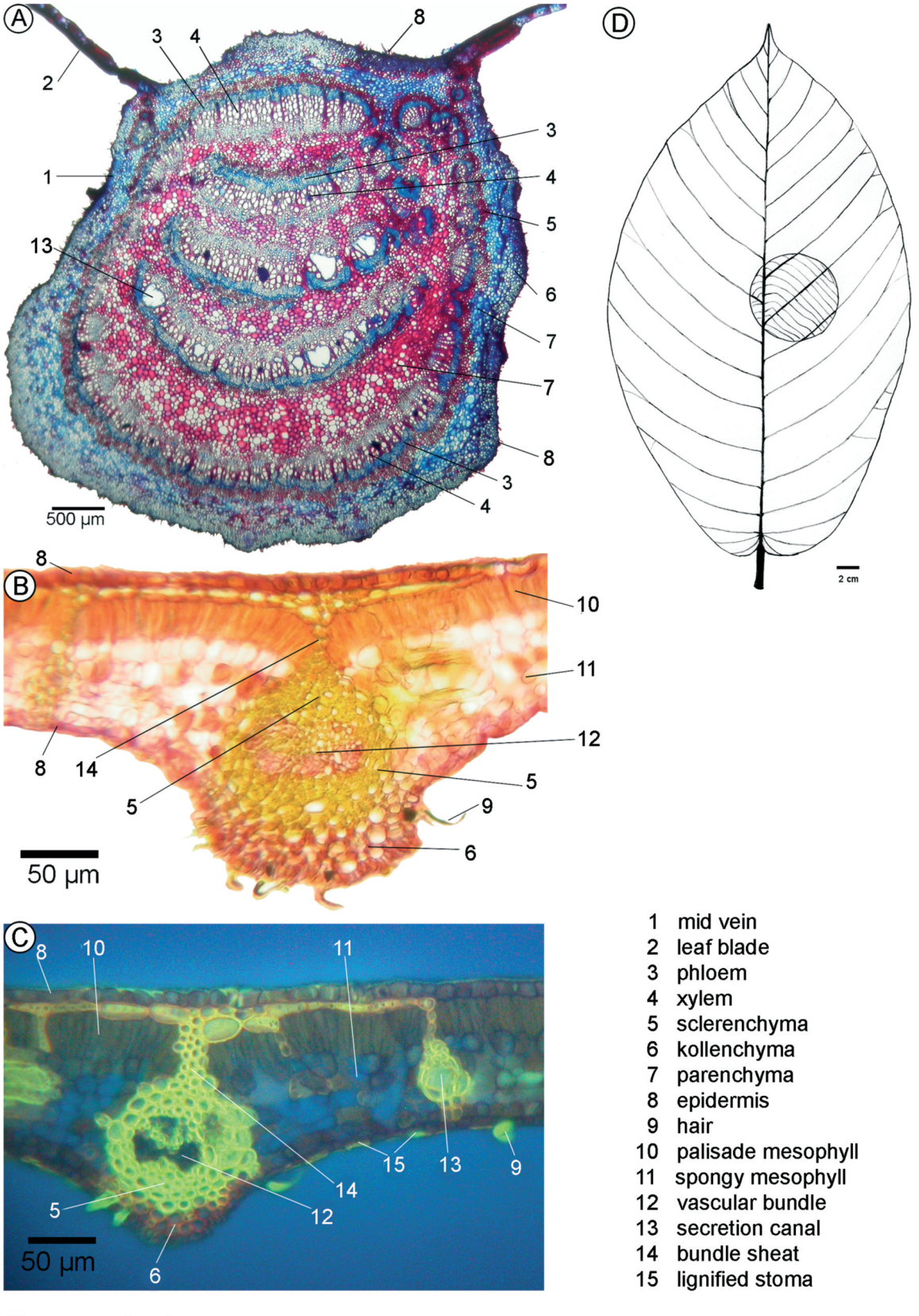


Plate 8:
Luehea seemannii

1 mid vein
2 leaf blade
3 phloem
4 xylem
5 sclerenchyma
6 kollenchyma
7 parenchyma
8 epidermis
9 hair
10 palisade mesophyll
11 spongy mesophyll
12 vascular bundle
13 bundle sheat
14 idioblast
15 crystal

Luehea seemannii
A mid vein (bleached, safranin - astrablue)
B leaf blade (bleached, auramin - mucicarmin, fluorescence microscopy)
C leaf blade (bleached, unstained)
D draft of the leaf

Plate 9:
Sloanea medusula



1 mid vein
2 leaf blade
3 phloem
4 xylem
5 sclerenchyma
6 kollenchyma
7 parenchyma
8 epidermis
9 hair
10 palisade mesophyll
11 spongy mesophyll
12 vascular bundle
13 secretion canal
14 bundle sheat
15 lignified stoma

Sloanea medusula
A mid vein (bleached, safranin - astrablue)
B leaf blade (bleached, auramin - mucicarmin)
C leaf blade (bleached, auramin - mucicarmin, fluorescence microscopy)
D draft of the leaf