ANATOMICAL SUPPORT FOR THE TAXONOMY OF *CALOPHYLLUM* (GUTTIFERAE) IN PANAMA[1]

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Abstract

Leaves of eight species of *Calophyllum* growing in Panama were sectioned and characterized anatomically. A number of characters seemed useful in clarifying the taxonomy and evolution of the genus. Apparently useful characters at the species level include midrib shape, primary vein "V" angle, presence or absence of transcurrent ducts, and presence or absence of a hypodermis. The species *C. nubicola* D'Arcy & Keating is described. Taxonomic relationships among the Panamanian species have been clarified. Old World species introduced into Panama can be distinguished anatomically, but they are not separable statistically as a group from New World species examined.

The genus *Calophyllum* comprises well over 100 species of New and Old World flowering plants. In preparing the treatment of the genus for the *Flora of Panama*, species were difficult to separate on herbarium morphological evidence, so anatomical studies of leaves were undertaken. Such studies had been used to good advantage in the Guttiferae by Vesque (1889, 1893), Schofield (1968), and Stevens (1974, 1976). Smith & Darwin (1974) discussed anatomy of the fruit, but only Vesque had touched on plants of the New World. Although Vesque established three sections for Old World species of *Calophyllum*, he did not classify the New World species into sections. Our anatomical studies summarized in Table 1 tended to confirm tenuous conclusions drawn from study of herbarium sheets, and they suggested changes that might not otherwise have been made, namely, recognition of a new species and separation of *Calophyllum rekoi* and Panamanian plants formerly known as *C. longifolium* from *C. brasiliense*. They also showed the anatomical basis for the leaf ribs which are a useful taxonomic character. A less satisfactory conclusion is that two specimens studied in detail cannot be assigned to any of the known species from Panama, and the herbarium material is insufficient for description of new species. While it is possible that these species have already been described based on material from other countries, our investigations suggested no likely candidates among the relatively few species that have been described from the New World. These results suggest that further realignments will be required, but these possibilities could not be pursued within the limitations of this study.

Materials and Methods

Following careful study of material available from several herbaria (A, F, GH, MO, DUKE, PMA, SCZ, US) a number of elements were selected for

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anatomical study. This selection included specimens of plants believed to be native to the neotropics, and two species known to have been introduced into cultivation from the Old World. One of these Old World species, *C. inophyllum*, has been studied anatomically by several other workers so this species provides a reference of our work to that of others.

Specimens of leaves of eight species of *Calophyllum* were removed from nine herbarium collections (see Table 1) and restored using Aerosol OT (Ayensu, 1967) at 45°C for one week. Two pieces from approximately the center of each leaf were selected and oriented to produce transverse sections of the midrib and transverse sections of the parallel secondary veins. Following restoration, the specimens were fixed in 50% formalin-propiono-alcohol, dehydrated in a tertiary butyl alcohol series (Johansen, 1940), embedded in Paraplast and sectioned at 10 μm. The sections were stained in Safranin O-Fast Green FCF and photographed at 100× magnification. Drawings of the midrib areas were made using a microprojector.

Hand sections were made of leaves of many other available specimens to confirm results drawn from paraffin sections. In addition counts of vein frequency were made of all specimens available.

Gross Morphology

Calophyllum embraces species of trees and shrubs, some of them large and dominant in tropical forests. They sometimes have buttressed bases and the sap is usually milky or yellow. The leaves are entire, usually coriaceous and petiolate. Most treatments in the past have relied heavily on leaf characters, often involving internal anatomical details which are not readily observable. Some species are sparingly pubescent but little taxonomic use has been made of hairs, nor does the inflorescence appear to be a useful character. Interpretation of the perianth is subject to argument, but flowers of most Old World species are usually considered to have petals, while New World species all lack petals. Otherwise there is remarkable similarity in the flowers. The fruits offer a number of taxonomic characters: size, shape, surface appearance, and the size of the seed relative to the locule are all useful, but as fruits are lacking on most herbarium specimens, other characters must be sought for routine determination of specimens.

Anatomical Characters

Histological features of leaves of *Calophyllum* and most other Guttiferae are more regular and highly organized than those found in the majority of mesic dicotyledons. As Stevens (1974) found, midrib type and venation prominence are associated with histological features, and most species can be recognized from leaf sections. The following features appear to be of systematic importance in *Calophyllum* leaves.

Leaf transverse sections, general histology

The leaves are bifacial. The midrib shape is systematically variable and the transectional aspect of the included tissues varies with the midrib shape. The

TABLE 1. Anatomical characteristics of *Calophyllum* leaves

Taxa	Specimen	Midrib Sclerenchyma an Inverted "Y"	Hypodermis	Vein frequency/height ratio	Duct Position[a]	Ducts Transcurrent	Duct Diameter (μm)	Sclereids below Ducts[b]	Ribs per cm	Lamina
NEW WORLD										
1. *C. angulare*	*Krukoff 1442*	+	—	0.75–1.0	M	—	20–30	(2)	44	310
2. *C. brasiliense*	*Claussen 237*	—	+	1.7	M	—	80–90	—	15–18	320
3. *C. longifolium*	*Croat 8532*	+	+	2.0	M	+	50–70	(3)	22–27	320
	Lao 571	+	+	2.0	M	+	50–70	(3)	28	280
	Foster 1480	+	+	1.0–1.5	M	+	50–70	(3)	25	425
	Croat 6451	+	+	1.5	M	+	50–90	(3)	25	470
	Pittier 6597	+	+	1.0	M	+	50–70	(3)	35	500
	Stimson 5132	+	+	1.0	M	+	60–70	(3)	30	500
4. *C. nubicola*	*Dwyer et al. 7301*	+	—	0.7–0.9	H	—	40	+	34–36	400
	Mori & Kallunki 6508	+	—	0.8–1.0	H	—	60–80	+	30	390
5. *C. rekoi*	*Calderón 147*	—	—	2.0–2.5	M	—	40–50	—	24–27	230
	Reko 3557	—	—	2.0–2.5	M	—	40–50	—	17–20	225
	Dwyer 1670	+	—	1.5–2.0	M	—	50–60	—	20–25	290
	Holdridge 6232	—	—	2.5–3.0	M	—	40–50	—	30	200
6. *C. calaba*	*Wagner 1611*	—	—	3.0–4.0	M	—	60–90	—	12–15	300
	Liesner 4881	—	—	3.0–4.0	M	—	50–60	—	20	230
OLD WORLD										
7. *C. soulattri*	*Pittier 6678*	+	+	2.5–3.0	M	+	40	(3)	39–40	270
8. *C. inophyllum*	*Nee 11420*	—	—	1.7–2.0	M	—	70	—	10–14	350
	Standley 30868	—	—	2.0–2.5	M	—	80–90	—	18–20	270

[a] M = medial, H = high.
[b] (2) = nests of fibers where sclereids may occur in other species; (3) = fibers around transcurrent ducts.

midrib is prominent abaxially, either rounded or 1–3-angled. An adaxial ridge or depression may also be present. No palisade layer or other mesophyll is present above or below the midrib. Vascular bundles are collateral. Xylem of the midrib forms a sharp V-shape or transitions to a wide rounded arc or crescent. The angle of the "V" varies from 45° to 90°. On the adaxial side, two patches of fibrous sclerenchyma are attached to the ends of the xylem and adaxial to it (Figs. 2, 7–9), or the fibers may form a continuous inverted "V" running into the adaxial midrib ridge (Figs. 1, 3–5). Secretory ducts may be present above or below the vascular bundle within the ground tissue.

Cross-sections of the lamina cut perpendicular to the secondary veins are bounded by well-cuticularized epidermal layers on both surfaces. Outer walls are well cuticularized with cuticular ridges (flanges) often running between epidermal cells. Stomata are common and level with the abaxial epidermis. An adaxial hypodermis of one layer is present in some species.

Secondary veins are regularly spaced and parallel to each other from the base to the apex of the leaf. They leave the midrib at a uniform acute angle and are always transcurrent with ridges of fibers, 1–3-seriate, running to both epidermal layers. Spacing of secondary veins appears to be species specific (Smith & Darwin, 1974; Stevens, 1974, 1976) and has been expressed here as veins per cm and as the frequency/height ratio. The veins vary from closely spaced (the distance between them being less than the measure of their height, 0.7–1.0:1) or widely spaced, the distance between veins being 1–4 times their height (1–4:1).

The palisade may be 1–3 layers deep over a loosely arranged spongy layer which occupies more than half of the total mesophyll. Secretory ducts are present in all specimens, equidistant between and parallel to the secondary veins. On surface view, transcurrent veins and ducts often look alike and may be indistinguishable. They appear as ribs whose frequency can be counted. This measure, expressed as ribs per cm, may include veins alone or equal proportions of veins and ducts. Without transcurrent sclerenchyma, the ducts are referred to as embedded (Stevens, 1974). The duct lumen may be medially placed between the epidermal surfaces or it may be high (closer to the adaxial epidermis). Duct size and placement appear to be systematically valuable characters.

Sclereids may be present in the spongy layer abaxial to the embedded ducts. Druse crystals and occasionally prisms range from absent to common in ground tissue of the midrib or in the lamina. When present, they are usually within the palisade layer.

Previous writers have commented on the constancy of anatomical characters. Thus Vesque (1893: 533) insisted that the presence or absence of hypoderm is constant for a given species, but he believed that other characters must be considered more of a quantitative than a qualitative nature. Nevertheless, he did not hesitate to rely on other anatomical characters to distinguish species in his key and descriptions. Stevens (1974: 350) stated: "Nearly all species of *Calophyllum* can be recognized by their leaves alone in both the field and the herbarium. Apart from shape, toughness, size, the prominence of the margin and to a certain extent the colour of the leaf on drying, the characters which give the leaf its characteristic look are the midrib and venation." Then he noted that

while these characters are usually fairly constant, they are subject to variation, sometimes even on a single specimen. "Anatomical differences on the whole correlate well with morphological differences and most species can be recognized from sections of the leaves" (p. 352). He was able to prepare a key to *Calophyllum* in Papuasia without relying on anatomical characters, but he did include measurement of vein frequency in his species descriptions, and he sometimes presented other details including, for example, thickness of epidermal cells, angle of midrib bundle divergence, number of veins or latex canals enclosed by the marginal thickenings, etc. Our study does not include a large enough sample to warrant firm statements as to the qualitative nature or specific constancy of any characters, and in the absence of such statements by previous workers such as that of Vesque regarding hypoderm, we must regard all statements regarding individual characters as generalizations to be subjectively judged. Many of the characters we have observed appear to be species specific, but a wider range of material must be examined before their variation can be assessed.

1. *Calophyllum angulare* A. C. Smith, Bull. Torrey Bot. Club 60: 379. 1933. TYPE: Brasil, Matto Grosso, *Krukoff 1442* (MO). FIG. 1.

MIDRIB prominent abaxially, 3-angled with a V-shaped median ridge, raised adaxially in a truncated ridge. *Xylem* forms a prominent V shape. Bundle angle 45–50°. *Sclerenchyma* of fibers forming an inverted V extending into the adaxial ridge. *Secretory ducts* small in the ground tissue.

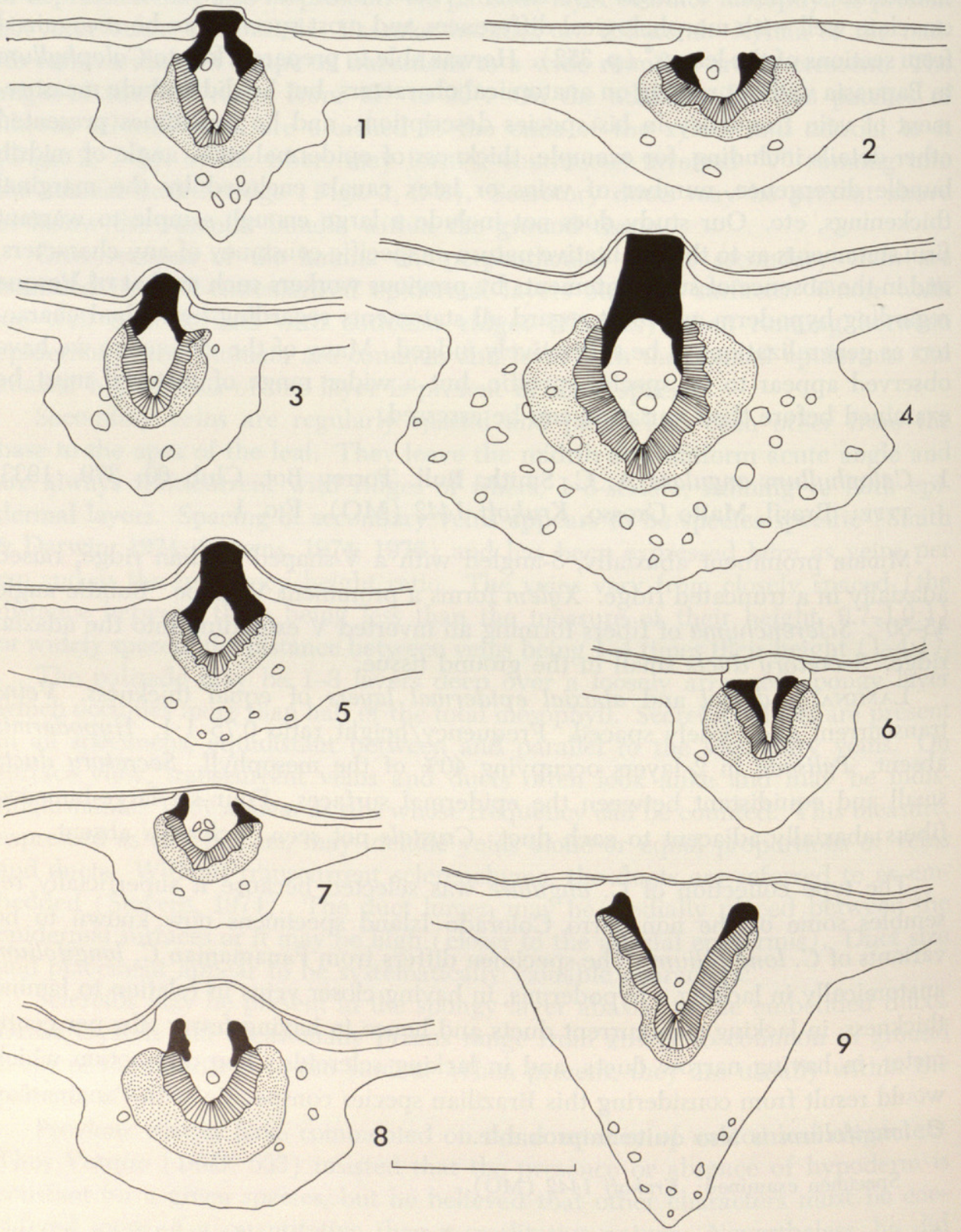
LAMINA. *Adaxial* and *abaxial epidermal layers* of equal thickness. *Veins* transcurrent and closely spaced. Frequency/height ratio 0.75–1:1. *Hypodermis* absent. *Palisade* in 2 layers occupying 40% of the mesophyll. *Secretory ducts* small and equidistant between the epidermal surfaces. Four–six large-lumened fibers abaxially adjacent to each duct. *Crystals* not seen. *Sclereids* absent.

The type collection of *C. angulare* was selected because it superficially resembles some of the non-Barro Colorado Island specimens now known to be variants of *C. longifolium*. The specimen differs from Panamanian *C. longifolium* anatomically in lacking a hypodermis, in having closer veins in relation to lamina thickness, in lacking transcurrent ducts and hence in having fewer ribs per centimeter, in having narrow ducts, and in lacking sclereids. The disjunction which would result from considering this Brazilian species conspecific with Panamanian *C. longifolium* is also quite improbable.

Specimen examined: *Krukoff 1442* (MO).

2. *Calophyllum brasiliense* Camb. in St. Hil. et al., Fl. Brasil Mérid. 1: (ed. folio, MO copy) 247, *tab. 67*. 1827. TYPE: Brazil, Espiritu Santo, *St.-Hilaire 330* (P, not seen, photo MO). FIGS. 2, 14.

MIDRIB rounded abaxially and flat or slightly concave adaxially. The midrib of this specimen agrees with *C. brasiliense* as figured by Schofield (1968). *Xylem* forms a low crescent-shaped arc. Bundle angle 85–90°. *Sclerenchyma* of fibers



FIGURES 1–9. *Calophyllum* leaf midrib cross-sections.—1. *C. angulare, Krukoff 1442.*—2. *C. brasiliense, Claussen 237.*—3. *C. longifolium, Lao 571.*—4. *C. longifolium, Croat 8532.*—5. *C. nubicola, Dwyer et al. 7301.*—6. *C. rekoi, Reko 3557.*—7. *C. rekoi, Calderón 147.*—8. *C. soulattri, Pittier 6678.*—9. *C. inophyllum, Nee 11420.* Scale line equals 1.0 mm. Hatching = xylem; stippling = phloem; solid black = sclerenchyma.

forming 2 patches adaxial to the ends of the xylem. *Secretory ducts* present in the ground tissue.

LAMINA. *Adaxial epidermis* thicker than the abaxial epidermis. *Veins* transcurrent, widely spaced. Frequency/height ratio 1.7:1. *Hypodermis* present. *Palisade* parenchyma in 2 rows occupying 40% of the mesophyll. *Secretory ducts* very large, equidistant between the epidermal surfaces. No sclerenchyma near the ducts. *Druses* present but uncommon. *Sclereids* absent.

The specimen examined was the only one available which had been annotated by Vesque as *Calophyllum brasiliense* subsp. *verum* var. *α genuina.* It was examined to confirm whether *C. longifolium* and *C. rekoi* could reasonably be considered conspecific with *C. brasiliense* as was affirmed by Vesque (1893) and by Standley (1932) and followed by subsequent workers. The specimen was collected in Minas Gerais but no other collection data are available. This same specimen was examined and figured by Vesque (1889, 1893).

The material examined of this species differs from *C. rekoi* in having a hypodermis and in having very large ducts in the lamina. It differs from Panamanian *C. longifolium* in details discussed under that species.

Specimen examined: Brazil, Minas Gerais, *Claussen 237* (MO).

3. *Calophyllum calaba* [Plum.] L., Sp. Pl. 514. 1753. LECTOTYPE: *Calaba* Plum., Pl. Nouv. Amér. Gen. 39, *tab. 18.* 1703.

MIDRIB prominent abaxially, rounded to slightly 3-angled, adaxially with a slight groove. *Xylem* forms a V shape. Bundle angle of 70–80°. *Sclerenchyma* of fibers forming 2 small patches of thick-walled cells attached at the ends of the xylem strands; in addition, much of the tissue of larger-lumened cells beneath the adaxial surface of the midrib are lignified. *Secretory ducts* are few and large in the ground tissue.

LAMINA. *Adaxial* and *abaxial epidermal layers* of equal thickness. *Veins* transcurrent and widely spaced. Frequency/height ratio 3–5:1. *Hypodermis* absent. *Palisade* of 1 layer occupying 25–40% of the mesophyll. *Secretory ducts* large and equidistant between epidermal surfaces; ducts may be partially encircled by a single sheath of fibers with the sheath having no consistent orientation. *Druses* common in the palisade layer. *Sclereids* not seen.

Typification of this name has been subject to controversy and confusion in the past, but most writers, both ancient and recent, have applied the name to plants of the Caribbean based on either Plumier or Jacquin descriptions. Furtado (1941) and Howard (1962) have discussed the merits of this choice of name, and it is in this Antillean context that we use the name here. The species is recognizable by its widely spaced ribs, small rounded leaves, and short petioles, and anatomically by its widely spaced ribs and lack of hypodermis. No specimens of this species have been seen from Panama. *Liesner 4881* from neighboring Costa Rica appears to be a good representative of *C. calaba.* It is from Guanacaste Province which is subject to seasonal drought, a condition also prevailing in much of the Antillean region. Anatomically it compares well with *Wagner 1611* from Puerto Rico.

Specimen examined: Costa Rica, *Liesner 4881* (MO). Puerto Rico, *Wagner 1611* (MO).

4. *Calophyllum inophyllum* L., Sp. Pl. 513. 1753. TYPE: East Indies, Herb Linn (LINN 676.1, not seen; microfiche MO). FIGS. 9, 12–13.

MIDRIB prominent, strongly V-shaped abaxially, no protrusion or concavity adaxially. *Xylem* forms a V-shaped strand. Bundle angle 60–62°; Stevens (1974) records a bundle angle of 70–90°. *Sclerenchyma* of fibers forming 2 small patches attached to the ends of and adaxial to the xylem. *Secretory ducts* small, frequent in the ground tissue of the midrib.

LAMINA. *Adaxial* and *abaxial epidermal* layers of equal thickness. *Veins* transcurrent, widely spaced. Frequency/height ratio 1.7–2:1. *Hypodermis* absent. *Palisade* in 2 layers occupying 30% of the mesophyll. *Secretory ducts* large, between the secondary veins and equidistant between the epidermal surfaces. No sclerenchyma associated with the ducts. *Druses* and *prismatic crystals* common in the ground tissue of the midrib. *Sclereids* absent.

This species is widespread on coastal sites in the Indian Ocean and South Pacific, and it has been introduced into the New World as a cultivated street tree. It resembles some collections of *C. longifolium* but is easily distinguished by having petals and fewer leaf ribs than any other Panamanian element. This species was studied by Kienholz (1926), Stevens (1974, 1976), and Vesque (1889, 1893), and our inclusion of it was in part to relate this study to previous work. Our observations agree with those of previous descriptions. *Calophyllum inophyllum* has a strikingly different midvein shape from other species studied. Anatomically, the combination of having no hypodermis, no inverted V sclerenchyma, no transcurrent ducts, and no sclereids is unique to this species.

Specimens examined: Panama, Canal Zone, cultivated, Ancón, *Nee 11420* (MO).

5. *Calophyllum longifolium* Willd., Ges. Naturf. Freunde Berlin Mag. Neusten Entdeck. Gesammten Naturk. 5: 80. 1811. TYPE: America meridionalis, Herb. Willd. 10117 (B-Willd., not seen; microfiche MO). *C. brasiliense* subsp. *longifolium* (Willd.) Vesque, in D.C., Monogr. Phan. 8: 592. 1893. FIGS. 3–4, 11.

MIDRIB prominent abaxially, 3-angled with a V-shaped median ridge, raised adaxially in a smaller truncated or rounded ridge. *Xylem* forms a V-shaped strand. Bundle angle 45–55°. *Sclerenchyma* of fibers forming an inverted V extending into the adaxial ridge. *Secretory ducts* few or numerous in the midrib ground tissue.

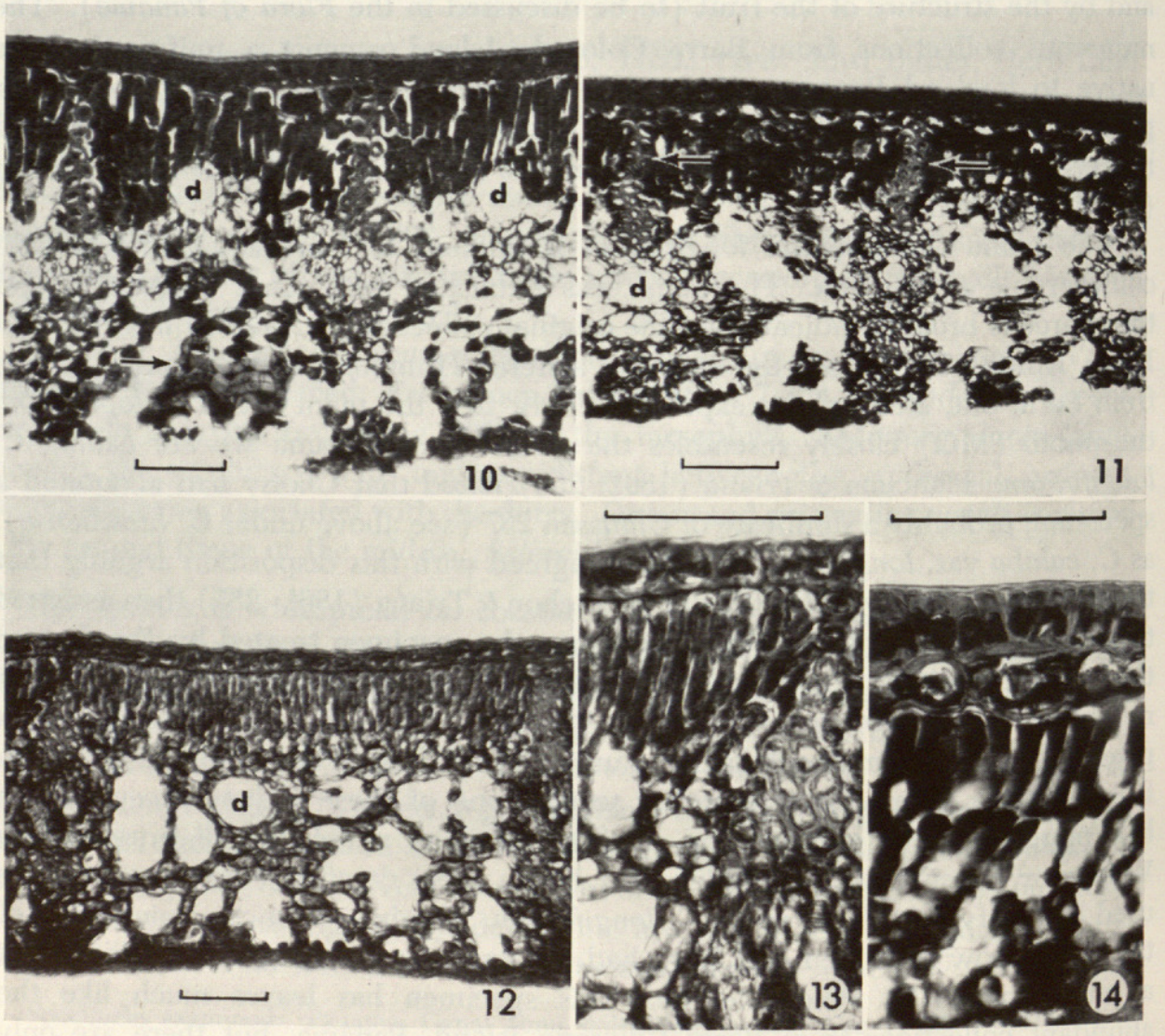
LAMINA. *Adaxial epidermis* about twice as thick as the abaxial. *Veins* transcurrent, widely spaced. Frequency/height ratio 2:1. *Hypodermis* present. *Palisade* in 1–2 layers occupying 30–50% of the mesophyll. *Secretory ducts* large, between the secondary veins and weakly or strongly transcurrent. *Druses* uncommon or common in the mesophyll or in the midrib ground tissue. *Sclereids* absent or present as 1–2 cells in the spongy layer near the abaxial epidermis.

Calophyllum longifolium can be distinguished by the number of leaf ribs

and by the structure of the fruit (to be discussed in the *Flora of Panama*). The numerous collections from Barro Colorado Island suggest a uniform species native to lowland Panamanian forests. However, collections taken from other parts of the country are not all superficially similar, and some have been annotated by Bassett Maguire, longtime specialist in the group, as *C. brasiliense* var. *rekoi* (Standl.) Standl.

The name *Calophyllum longifolium* is applied to Panamanian plants with considerable reservation. The name was published with scant description and the source is broadly indicated on the original collection as *America meridionalis*. Later, Kunth (1821) used the name for a sterile Humboldt & Bonpland specimen from Peru, but, although he expressed doubt that the plant was a *Calophyllum*, the photo (MO) closely resembles the plants from Panama we are calling *C. longifolium*. Planchon & Triana (1861: 253) noted that Choisy had annotated a specimen, probably a duplicate of *Claussen 237* (see above under *C. brasiliense*), as *C. calaba* var. *longifolia*, but they disagreed with this disposition arguing that the specimen is really *C. brasiliense*. Planchon & Triana (1861: 255) then assigned two other specimens to *C. longifolium*, one the specimen treated by Kunth, and the other a specimen at MPU from Panama. Somewhat later Vesque (1893: 592) reexamined both specimens cited by Planchon and Triana and on this basis placed *C. longifolium* at subspecific rank under *C. brasiliense*, adding a specimen from Jamaica to his list of material seen. None of these writers except Kunth had opportunity to examine the original specimen which is still preserved in Willdenow's herbarium at Berlin, and hence their detailed analysis cannot be used to justify use of the name *C. longifolium*. We have seen the microfiche of the Willdenow specimen and have had the use of a pencil rubbing kindly put at our disposal by Peter Stevens. This specimen has leaves much like the abundant collections from Barro Colorado Island, Panama, but there are only 17–20 ribs per cm, fewer than is usual in the Panamanian plants, and in the same range as *C. rekoi*. The petioles are shorter, and Stevens noted that the twig is glabrous, while in the Panamanian plants the twigs and petiole undersides are minutely puberulent. However, being unable to examine the type collection personally, we are unwilling to affirm that it is not the same as the Panamanian plants. Such an affirmation would require a new name for the Panamanian element, and we would have to find another Central American species to which the name could confidently apply.

Calophyllum longifolium differs from both *C. brasiliense* and *C. rekoi* in several respects, which we believe to be significant at the species level. From *C. brasiliense* it differs in having transcurrent ducts, in having more frequent veins, in the midrib sclerenchyma forming an inverted V, and in having sclereids. It also has smaller ducts, slightly thicker lamina, cuticle, mesophyll, and thinner hypodermis. From *C. rekoi*, *C. longifolium* differs in having a hypodermis, in having the midvein sclerenchyma in a V, in having transcurrent ducts, in having sclereids, and it also has slightly larger ducts, and much thinner lamina, cuticle, mesophyll, spongy mesophyll, and slightly thinner palisade tissue. We can see no justification for considering these three elements as conspecific as Vesque and Standley have done in the past.



FIGURES 10–14. Cross-sections of *Calophyllum* leaves perpendicular to the secondary veins. —10. *C. nubicola, Dwyer et al. 7301.* Note small ducts (d) between transcurrent veins and above the center of the leaf. Note sclereids (arrow) below the ducts.—11. *C. longifolium, Croat 8532.* Both veins and ducts (d) are transcurrent (arrows).—12. *C. inophyllum, Nee 11420.* Duct (d) is not transcurrent and is centered between the epidermal layers.—13. *C. inophyllum, Nee 11420.* Note absence of a hypoderm over the palisade layer.—14. *C. brasiliense, Claussen 237.* Note hypoderm over the palisade layer. Scale lines equal 100 μm.

Specimens examined: Panama, Canal Zone, Barro Colorado Island, *Croat 8532* (MO); *Foster 1480* (SCZ); *Croat 6451* (MO, SCZ). Darién, *Pittier 6597* (US); *Stimson 5132* (GH). Herrera, El Monteroso–Las Minas, *Lao 571* (MO).

6. *Calophyllum nubicola* D'Arcy & Keating. TYPE: Panama, *Mori & Kallunki 7301* (MO). FIGS. 5, 10.

Arbor ve arbuscula ad 12 m altus. Folia coriacea, parva, plana, rugis lateralis obscuris, 34–36 per cm. Fructus globusus, apiculatus, semine loculum ⅕ inplenti.

Tree or *shrub* to 12 m tall, much branched, the twigs slender, stiff, ascending, drying yellowish or reddish brown but soon greyish, scruffy. *Leaves* elliptical, sometimes broadly so, small, to 6 cm long, 4.5 cm wide, stiffly coriaceous, plane but the margins minutely revolute, the costa conspicuous, subplane or elevated above, rounded to sharply raised beneath, evanescent distally, drying reddish

brown, the lateral ribs somewhat obscure and appearing very numerous, actually 34–36 per cm, usually more distinct above; petiole 5–10 mm long, drying ca. 2 mm thick, with a narrow groove above. *Inflorescence* not seen. *Fruits* globose or slightly ellipsoidal, 20–25 mm long, conspicuously apiculate, the apicule 2–4 mm long, longer than broad, the basal stipe resembling the apicule, the surface drying glaucous, reddish brown, wrinkled; fruiting pedicels 10–12 mm long, resembling the twigs; interior wall of the fruit papery, thin, dark brown, the endocarp stony; seeds globose, brown, occupying ⅘ of the locule.

This species is known only from Cerro Jefe, a lowland cloud forest reaching ca. 900 m elevation just northeast of Panama City. Woody plants in this habitat have notably coriaceous, small, entire leaves and much-branched twigs.

Midrib rounded abaxially and raised adaxially into a narrow, rounded ridge. *Xylem* forms a broad V. Bundle angle 70–75°. *Sclerenchyma* of fibers forming an inverted V extending upward into the adaxial ridge. *Secretory ducts* common in the ground tissue of the midrib.

Lamina. *Adaxial epidermis* slightly thicker than the abaxial. *Veins* transcurrent, closely spaced. Frequency/height ratio 0.9:1. *Hypodermis* absent. *Palisade* cells in 2(–1) layers occupying 30% of the mesophyll. *Secretory ducts* small, between the transcurrent veins and closer to the adaxial epidermis at the boundary of the palisade and spongy layers, not transcurrent or associated with sclerenchyma. *Druses* and prismatic crystals common in the ground tissue of the midrib, uncommon in the mesophyll. *Brachy-* and *macrosclereids* forming prominent groups in the spongy mesophyll beneath the ducts.

This species is distinctive in its small flat leaves and in the much-branched appearance of the specimens. The lateral venation is obscure and the ribs are difficult to count. The seed is loose and rattles inside the fruit, but it fills over ⅘ of the locule. There is no spongy tissue in the unfilled portion of the locule. *Calophyllum nubicola* superficially resembles *C. angulare*, but differs in its smaller, more coriaceous, more rotund leaves with numerous, obscure ribs. The twigs are narrower and the petioles are shorter. Anatomically, the laminar secretory ducts of *C. nubicola* are situated closer to the adaxial epidermis than in any other species. Sclereids in *C. nubicola* are strongly developed between the veins, near the abaxial epidermis. In *C. angulare*, fibers are found between the veins, closer to the abaxial side of the duct.

Specimens seen: Panama, Cerro Jefe, *Dwyer et al. 7301*; *Mori & Kallunki 6503, 7301*; *Mori 7128* (all MO).

7. *Calophyllum rekoi* Standl., Contr. U.S. Natl. Herb. 20: 192. 1919. TYPE: Mexico, Oaxaca, *Reko 3557* (US). *Calophyllum brasiliense* var. *rekoi* (Standl.) Standl., Trop. Woods 30: 7. 1932. Figs. 6–7.

Midrib prominent, rounded or slightly 3-angled abaxially with a low rounded adaxial ridge. *Xylem* forms a narrow or broad V. Bundle angle 57–62° or 80–85°. *Sclerenchyma* forming 2 strands of fibers adaxial to the ends of the xylem trace,

the tissue between the two strands weakly lignified and in the Reko specimen lying against the adaxial ridge. *Secretory ducts* 1–5 in the ground tissue of the midrib.

LAMINA. *Adaxial epidermis* slightly thicker than the abaxial. *Veins* transcurrent, widely spaced. Frequency/height ratio 2–2.5:1. *Hypodermis* absent. *Palisade* cells in 2 layers occupying 40% of the mesophyll. *Secretory ducts* medium-sized, between the secondary veins and equidistant between the epidermal surfaces, not transcurrent or associated with sclerenchyma. *Druses* common in midrib ground tissue and in palisade and spongy mesophyll. *Sclereids* absent.

Much material collected in Central America has been determined as *C. rekoi* or as *C. brasiliense* var. *rekoi*, and these names have covered plants with small leaves as well as many with large leaves. The type collection has relatively large leaves, 12–14 cm long, 4–5 cm wide, superficially resembling those of *C. longifolium*, while the Calderón specimen has small leaves, 3.5–9 cm long, 2.5–3.5 cm wide, and seems representative of the relatively uniform small-leaved forms of Belize, Nicaragua, and El Salvador. It does not occur in Panama.

The two specimens examined differ very little. There is a slightly lower vein count in the Reko specimen. The shape of the midvein varies somewhat. There is more sclerenchyma beneath the adaxial surface of the midvein in the Reko specimen. It remains reasonable to associate them in a single variable species which differs notably from the other taxa studied in having a thin lamina and in having the lowest vein frequency/height ratio of the American species.

It is quite unreasonable to consider the Central American *C. rekoi* to be conspecific with the Brasilian *C. brasiliense*. Superficially, the specimens are different, *C. brasiliense* having subterete twigs while *C. rekoi* has notably angled twigs. *Calophyllum rekoi* has a hypodermis and the laminar ducts are only about half the size of those in *C. brasiliense*. It is unlikely that this species is conspecific with the type of *C. longifolium* and that *C. rekoi* should be placed into synonomy with the earlier *C. longifolium* (see discussion under *C. longifolium* above).

Specimens examined: Mexico, *Reko 3557* (US). El Salvador, *Calderón 147* (MO). Panama, *Dwyer 1670* (MO); *Holdridge 6232* (MO).

8. *Calophyllum soulattri* Burm. f., Fl. Ind. 2: 121. 1768. TYPE: Java, ?*Burman* (?G, not seen). *C. kunstleri* sensu auct., non King. FIG. 8.

MIDRIB prominent, rounded, slightly 2-angled abaxially with a low, rounded adaxial ridge. *Xylem* forms a wide rounded V. Bundle angle 70–75°. *Sclerenchyma* of fibers forming 2 strands adaxial to the ends of the xylem strand. There is weak lignification in the form of an inverted V of cells adjacent to the adaxial surface of the midrib. *Secretory ducts* small and frequent in the ground tissue of the midrib.

LAMINA. *Adaxial* and *abaxial epidermal* layers of equal thickness. *Veins* transcurrent, widely spaced. Frequency/height ratio 2.5–3:1. *Hypodermis* present. *Palisade* in 1(–2) layers occupying 25% of the mesophyll. *Secretory ducts* large, between the secondary veins and equidistant between the epidermal surfaces, surrounded by epithelium and by fibers with biseriate extensions, transcurrent and

strongly resembling secondary veins. *Druses* and *prismatic crystals* common in the ground tissue of the midrib, uncommon in the palisade layer. *Sclereids* absent.

Calophyllum soulattri has a high frequency/height ratio, but the hypodermis and transcurrent ducts in the lamina are reminiscent of *C. longifolium*. The duct diameter is smaller in *C. soulattri*, and its midrib is less strongly angular than *C. longifolium*. Otherwise, the two species are closer structurally than would be anticipated by their native ranges and floral characters. Our analysis reveals a species quite different from what Vesque (1889, 1893) described as *C. kunstleri* King.

This species is represented in Panama by several specimens collected early in this century from cultivation, and was listed, as *C. kunstleri*, in *Annual Reports of the Canal Zone Plant Introduction Gardens* (e.g., Johansen, 1926). The species is widespread in the Malaysian region. It is sometimes cultivated elsewhere (Stevens, pers. comm.) and it may be naturalized on Mauritius (Stevens, 1976). *Calophyllum soulattri* is distinct from all New World species of the genus in its small, glaucous fruits, only 11 mm across when mature (Stevens, 1976) and in its compound inflorescence which may appear subumbellate or fan shaped. Assistance from P. F. Stevens enabled us to determine our material of this species with confidence.

Unassignable Specimens

1. *Cooper & Slater 204* (MO, US). This sterile specimen has ovate leaves to 10 cm long and 4.5 cm wide, the apex is conspicuously acuminate and the base is obtuse. The veins are well spaced, ca. 16 per cm.

The label reads: Large forest tree over 75 ft × 24 inches; bark is rough and plated and when cut a sticky yellow sap exudes; wood is coarse, hard and used for general construction but not durable in contact with the ground. "Maria." Progreso, Chiriquí Province, 1927.

2. *Stern et al. 60* (MO, US). This sterile collection has ellipitical leaves to 13 cm long, 6 cm wide with blunt, obtuse and short acuminate tips and obtuse bases. They have dried partly yellowish and partly blackish and appear quite different from other Panamanian collections. The costa is contrasting. Except for the unusual coloration they appear much like *C. longifolium*.

The label reads: Tree 18 inches in diameter; bark with sticky yellow sap. "Maria." Foothills of Volcán Baru NW of El Hato, 4500 ft, Chiriquí Province, 6 June 1957.

Discussion

This study satisfied its basic goal of clarifying taxonomic relations between the main species of *Calophyllum* in Panama. From this work is became clear that neither *C. longifolium* nor *C. rekoi* can be justified as infraspecific taxa of *C. brasiliense*. Confidence could be attached to the placement of plants from other localities than Barro Colorado Island under *C. longifolium*, and recognition of the new species *C. nubicola* is now based on less subjective, superficial criteria than could otherwise have been noted. Of course, this is far short of what should

be done in preparing a revision of the New World or even Central American species of *Calophyllum*, for little extra-Panamanian material was examined. The full range of variability within a given taxon was only partly established. Extension of this study should produce useful results of application in other countries.

Our findings show that three native species of mainland America normally lack a hypodermis, while Vesque found hypodermis lacking in *C. calaba*. Clearly, a detailed study of New World *Calophyllum* is likely to produce an extended picture of the variability within the genus as a whole. For studies based on leaf histology, herbarium specimens are quite adequate since restoration is not a problem due to rigid support of the tissues by transcurrent veins and ducts. But while anatomical studies can provide valuable insights into systematic relationships and provide a check on variations in gross morphology, only study of many parts of the plants can support confident disposition of specimens into taxonomic groupings. Studies of living plants and of other features of the species will undoubtedly reveal many other characters of systematic importance in this taxonomically difficult genus.

A number of scatter diagrams were made in an attempt to detect clustering of characters or suggestions of trends which might be tested in future work. With one exception, almost no conclusions are possible with these data. The characters occur in almost any conceivable combination and appear to have been independently selected in each species. One would expect some correlation among functionally related features of leaf histology. The one trend noted is that vein frequency increases with lamina thickness.

The Old World species *C. inophyllum* and *C. soulattri* are not separable as a group from the New World species. *Calophyllum inophyllum* has an unusual midrib profile but its histological features are quite within the range of the other species examined.

An interesting phytogeographical comment concerns the close anatomical similarity between *C. longifolium* and the Old World introduction *C. soulattri*. One might have expected greater structural divergence based on geography, but perhaps various elements in the New World are more closely related to specific Old World taxa than others, and in some cases, the relationship might be quite close. We have not been able to explore this possibility and must advance it as speculation.

LITERATURE CITED

AYENSU, E. S. 1967. Aerosol OT Solution—An effective softener of herbarium specimens for anatomical study. Stain Tech. 42: 155–156.

FURTADO, C. X. 1941. The typification of *Calophyllum calaba* L. Gard. Bull. Straits Settlem. 11: 258–260.

HOWARD, R. A. 1962. Some Guttiferae of the Lesser Antilles. J. Arnold Arbor. 43: 389–399.

JOHANSEN, D. A. 1940. Plant Microtechnique. McGraw-Hill, New York.

JOHANSEN, H. 1926. Second Annual Report of the Canal Zone Plant Introduction Gardens. Mount Hope, Canal Zone.

KIENHOLZ, R. 1926. An ecological-anatomical study of beach vegetation in the Philippines. Proc. Amer. Philos. Soc. Suppl. 65: 58–100.

KUNTH, C. S. 1821. Guttiferae. *In*: A. B. Bonpland & A. de Humboldt. Nova Genera et Species Plantarum. Vol. 5: 198–204.