VASCULAR ANATOMY OF THE SAPODILLA FLOWER (MANILKARA ZAPOTILLA (JACQ.) GILLY)

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Marie Leonore Ehrmann

1950

This is to certify that the

thesis entitled

"VASCULAR ANATOMY OF
THE SAPODILLA FLOWER
(Manilkara zapotilla (Jacq.) Gilly)"

presented by

Marie Leonore Ehrmann

has been accepted towards fulfillment
of the requirements for

M. S. degree in Botany

E. F. Woodcock
Major professor

Date May 25, 1950

O-169

VASCULAR ANATOMY OF THE SAPODILLA FLOWER

(*Manilkara zapotilla* (Jacq.) Gilly)

by

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A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

1950

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ACKNOWLEDGMENTS

For assistance, as specified below, the author would like to express her appreciation to the following members of the staff of the Department of Botany and Plant Pathology of Michigan State College: Dr. E. F. Woodcock, for his supervision and constant aid in histological and anatomical technique; Mr. C. L. Gilly, for his supervision of the systematic (taxonomic) phase of this work, for his continuous encouragement, and for supplying the materials studied; Dr. G. B. Wilson, for advice on histological technique and for supplying reagents used in some of the experimental histological work involved in this study; and Drs. W. B. Drew and C. A. Alexopoulos for general helpful suggestions during the course of this project.

INTRODUCTION

The family Sapotaceae includes a group of plants widely distributed in the tropics and subtropics. This family is characterized by a considerable variation and complexity of floral structure; this diversity, resulting from the existence of several evolutionary trends of reduction and fusion of various parts and of various whorls in the flower, has led to conflicting interpretations of the floral structure of some of the members of this family. To date, these interpretations have been based solely upon observations of the gross morphology of the flowers. So far as can be determined, no previous study of vascular anatomy has been made in flowers of the Sapotaceae.

The principal purpose of this study is an attempt to prove, through investigation of the vascular system, the proper designation of each whorl of parts in the flower of the Sapodilla, Manilkara zapotilla (Jacq.) Gilly, one of the economically important members of the family Sapotaceae. A secondary objective of the study is to provide additional data of use in determining the systematic position of the Sapodilla complex of species within the family.

Taxonomic and anatomical workers sometimes differ in their opinions regarding the importance or the validity of basing an interpretation of floral structure upon the vascular system of the flower. However, it is believed that a study of the vascularization is, at the very least, a valuable supplementary

tool in cases where morphological features alone fail to give a complete or correct picture of floral structure. With this idea in mind, it is hoped that this investigation will throw light on the flower structure of the Sapodilla and related plants and thus prove of assistance in the interpretation and classification of the Sapotaceae.

REVIEW OF LITERATURE

Relation of Floral Anatomy to Morphology and Taxonomy

Work has been done by numerous authors to demonstrate relationships between plant families, and subordinate groups within families, on the basis of anatomical studies of the vascular system in the flower. It has been shown that the old classification of Dicotyledons along two specific lines, Polypetalae and Gamopetalae, is invalid, since some members of the two groups can be shown to be closely related on basis of floral anatomy, though they are morphologically different with respect to the extent of petal fusion (Douglas, 1936). The families Primulaceae and Caryophyllaceae may serve as examples of families in which both apopetalous and sympetalous members occur.

The phylogenetic origin of a floral organ may be indicated by tracing its vascular tissue to its level or point of origin in the main vascular cylinder of the floral axis. For instance, according to Tillson (1940), it is indicated that the scales found between the stamens and carpels in the flower

of Crassula sp. are derived from the gynoecium, since they receive branch traces from the dorsal bundle traces and often from the median lateral bundle traces of the carpels.

There is, however, disagreement as to the validity of using the vascular system as a guide to phylogenetic history. Grélot (quoted by Arber, 1933) states: "When there is partial abortion of an organ, one finds ordinarily one or more rudimentary bundles, which lead to it; when there is total abortion there are never any bundles."

Hunt (1937), on the other hand, makes this statement: "It has been found that ancestral conditions of a structure, long since lost to external view, may often be represented by a lingering vascular supply. Plant vascular tissue, like the skeleton of animals, is slow to change."

Van Tieghem and Henslow (quoted by Arber, 1933) described bundles branching off the calyx midribs in some members of the Primulaceae, as representing an absent series of stamens. Arber, in disagreement, states: "Evidence is absent for the survival of vascular tissue after the organ which it supplied ceased to exist. [Furthermore] an organ, which retains some trace of its normal external form, may yet show complete lack of vascular tissue."* On the other hand, she states that the androecium is particularly favorable to anato-morphological

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* The author of the present paper found this to be the case in the glands at the base of the gynoecium in Crassula corymbulosa.

study, since abortive stamens occur more frequently than rudiments of other floral parts.

In general, sepals are essentially leaf-like in their venation, whereas the vascular pattern of petals resembles that of sterile stamens; i.e. there are usually one to three (sometimes more) prominent veins in a petal. According to Eames (1931), however, petals in their vascular supply may often be like leaves. He states "Petals appear to be sometimes modified leaves, like the sepals.....".

Swamy (1949) reports a striking similarity in the method of vascular supply of sepals and outer petals in members of the family Degeneriaceae. Each petal and each sepal of Degeneria sp. is supplied by one median, and two marginal traces. The inner petals of the flower receive one trace, which forks into a median, and two marginal traces.

Systematic Position of the Sapodilla

The Sapodilla (Manilkara zapotilla (Jacq.) Gilly; the Achras zapota of most authors) is a member of the family Sapotaceae; this family is generally included in the order Ebenales. The Sapotaceae is a family of tropical and subtropical woody plants (mostly trees); some members of this family produce latex. Some types of this latex are used commercially in the production of various gums, such as balata rubber, chicle, and gutta percha. The leaves are usually simple, entire, and of coriaceous texture. Arrangement is alternate or rarely opposite. The perfect (or rarely

polygamous) flowers are solitary in the leaf axils, or borne in axillary clusters. They are generally described as being sympetalous, with epipetalous stamens in one to three whorls. The anthers are extrorse, and petaloid staminodes are frequently present in the flowers.

The ovary may consist of as many as fourteen or as few as one, uni-ovulate carpels. Placentation is essentially axile. The style is sometimes obscurely lobed at the summit. The fruit is a large, generally edible, berry; the seeds are variable in shape and structure.

The genera of the family Sapotaceae have been variously grouped by taxonomists (Baillon, 1892; Dubard, 1915; Lam, 1925, 1927, 1939, 1941). One of the more recent classifications is that of Lam (1939), in which the family is subdivided as follows:

Subfamily I. Sideroxyloideae, divided into three tribes: Sideroxyleae, Bumelieae, and Pouterieae;

Subfamily II. Mimusopoideae, divided into two tribes: Mimusopeae and Manilkareae;

Subfamily III. Madhucoideae, divided into two tribes: Madhuceae and Palaquieae.

This classification is based primarily upon presence or absence of appendages on the corolla, the number of members in the perianth, the general type of seed scar, and the presence or absence of staminodes. In this classification, the Sapodilla is placed in a group "Acyclicae" of the subtribe

Pouterilineae under Tribe Pouterieae of the Sideroxyloideae. The rather obviously related balata producing members (Manilkara spp.) of the family are placed in the subfamily Mimusopoideae, while the gutta-percha producing plants are placed in subtribe Palaquieae of the Madhucoideae.

More recently, Lam (1941) has included the Sapodilla in the subtribe Manilkareae of the Mimusopoideae, under the generic name Achras L.

Cronquist (1946), in a consideration of the North American members of the Sapotaceae, seems to be in agreement with Lam as to characters used in recognizing subdivisions of the family. The presence or absence of staminodes, presence and position of appendages on the corolla lobes, type of seed scar and of ovule attachment, and the presence or absence of endosperm, are generally important features. He states, however, that number and arrangement of parts per whorl often fail in this respect. Like Lam (1941), Cronquist places the Sapodilla in close relationship with the balata producing members of the Mimusopoideae. Unlike Lam, however, and following the treatment of Gilly (1942, 1943), Cronquist places the Sapodilla in the genus Manilkara Adans.

The subfamily Mimusopoideae is generally characterized by cyclic flowers and by possession of paired dorsal appendages subtending the corolla lobes. The genera in this subfamily are grouped (Lam, 1939, 1941) into two tribes, the Mimusopeae and the Manilkareae; the former are characterized by having small,

basilateral seed scars, and eight members (in two groups of four) in their floral whorls. Mimusops L. (which is frequently divided into several sections), Baillonella Pierre, and Butyrospermum Kotschy are the more important genera of this tribe.

The Manilkareae is differentiated from the Mimusopeae by the elongated, generally lateral seed scars, and six members (in two groups of three) in the floral whorls. The principal genera in the Manilkareae are Manilkara Adans. (which includes the sapodilla and the balata), Muriea Hartog, Northia Hook.f., Shaferodendron Gilly, and several other small genera of the eastern hemisphere. The most striking feature of this tribe is a tendency towards reduction of dorsal appendages and staminodes. According to Lam (1941), these reductions occur independently of each other. In addition, Gilly (1942) has shown that the paired dorsal appendages may be partially or entirely fused with the petals.

The Sapodilla was originally placed in Achras by Linnaeus (1753). This genus was separated by subsequent workers (Eichler, quoted by Lam, 1938; Engler, 1903; Dubard, 1915; Lecomte, 1920; Lam, 1925, 1927, 1938, 1939, 1941) from Manilkara only because, in the former, the dorsal appendages were completely obsolete, perhaps through fusion with the petals. In Manilkara the appendages were always present. Gilly (1942) has shown, however, that a large number of intermediate forms, exhibiting various degrees of union between the "dorsal (or

stipular) appendages" and the "petals", exist; these range from complete separation (as in the balata flower) to complete fusion (as in the Sapodilla flower). For this reason he incorporated the Sapodilla complex into Manilkara; the nomenclaturally technical reasons for the choice of generic name were discussed in detail by Gilly (1943).

Histological and Anatomical Techniques

The two most common methods used in the initial process of preparing dried flowers from herbarium specimens are (a) soaking in water and (b) boiling in water. One author working with buds from herbarium sheets (Tillson, 1940) reported soaking specimens in water at 80 degrees C. for two hours prior to bleaching in 5% NH_4OH to be satisfactory for softening the buds of Kalanchoe and Bryophyllum. Boiling a few minutes in tap or distilled water has been recommended by others.

To dehydrate specimens in preparation for clearing and embedding, a series of ethyl alcohol solutions of increasing concentrations is generally used. This process, in addition, necessitates gradual transfer of the material from absolute alcohol into pure xylol, since paraffin, the commonly used embedding matrix, is soluble only in the latter.

The use of xylol as intermediate agent between dehydration in ethyl alcohol and paraffin embedding may be eliminated by applying Zirkle's Butyl Alcohol Method (Rawlins, 1933), since paraffin is soluble in this reagent. Since butyl alcohol is

slower in penetrating the tissues, it is preceded by ethyl alcohol, then gradually mixed in. (This method is described later in this paper.)

A modification of this method consists in setting the material into a 10% glycerin solution, allowing the water to evaporate, and then transferring to a 50% glycerin-50% normal butyl alcohol solution for 36 hours. This is followed by three changes of pure normal butyl alcohol, 36 hours each, then by infiltration with paraffin (cited in Rawlins, 1933).

Ethyl alcohol may be substituted for butyl. Three changes of 95% ethyl alcohol, three hours each, are followed by two changes, one to two hours each, of absolute alcohol. This is much less time consuming than the use of butyl alcohol, but necessitates transfer into xylol, benzol, or a similar clearing agent.

Numerous materials are used for the purpose of clearing plant tissues after dehydration. Generally they belong in either of two groups: the hydrocarbons, such as xylol, benzol, toluol, phenol, chloroform, etc., and the essential oils, including clove oil, cedar oil, bergamot oil, aniline oil, oil of wintergreen, and others. Vegetable oils cause the least amount of shrinkage (Lee, 1928).

The above described clearing agents, however, will not remove brown pigments of dried plants, and thus, for treating herbarium specimens, definite bleaching agents are required.

NH_4OH, NaOH, lactic acid, peroxide, dioxan, chlorine, and sulfurous acid, and others, are listed as bleaching agents by various authors. (Methods utilizing the first four bleaching agents mentioned are described later in this paper.) It must be remembered that plant tissues vary in chemical composition and physical properties, and that this, as well as the treatment the tissue has previously undergone, will necessitate the use of different bleaching agents for different plant materials.

Several stains were recommended by various authors to differentiate vascular tissue. According to Johansen (1940), a saturated solution of light green, aqueous or alcoholic, acidulated with hydrochloric or acetic acid, will stain lignified tissue, and wash out from the remaining tissue. The same author recommends a one percent solution, in 70% alcohol, of Iodine Green. Other stains in use for this purpose are Methyl Green, Safranin O, Crystal Violet, and Eosin. McClung (1937) suggests a one percent solution of Safranin O in 50% alcohol, the alcohol solution being prepared with "aniline water". The latter reagent consists of two parts aniline oil per 100 parts water. Combinations of a) Methyl Green and Bismarck Brown, b) Cyanin and Erythrosin, and c) Safranin and Light Green are suggested by Woodcock (1945).

INVESTIGATION

Plant Materials Utilized

Flowers from the following herbarium specimens were used in this study of the vascular anatomy of the Sapodilla, and related plants:

Species	Specimen	Locality
Manilkara zapotilla (Jacq.) Gilly	Alexander s.n.	Jamaica
	Box 1210	Antigua
	Broadway s.n.	Grenada
	Duque 1747	Colombia
	Eyerdam 212	Haiti
	Harris 8634	Jamaica
	Jack 7820	Cuba
	Lundell 500	British Honduras
	Matuda 3254	Mexico
	Pittier 779	Colombia
	Ricksecker 316	Virgin Islands
	Rose and Rose 21609	Curacao
Manilkara bidentata (A. DC.) Chev.	Duss 3263	Guadalupe
Manilkara bahamensis (Baker) Lam & Meeuse	Curtiss 5437	United States
	Hitchcock s.n.	Bahama Islands
	Seibert 1317	United States
Manilkara tabogaensis Gilly	Woodson, Allen, and Seibert 1455	Panama

Methods Used in Anatomical Studies

To facilitate explanation and discussion of techniques used in this study, all methods are outlined in the following pages, and numbered or lettered. The time factor was important

only in certain steps of each method, and therefore, is not given in all instances.

Dehydration

I. Ethyl Alcohol Method

1. 35% alcohol.....2 - 4 hours minimum
2. 50% alcohol..... " " "
3. 70% alcohol..... " " "
4. 85% alcohol..... " " "
5. 95% alcohol..... " " "
6. 100% alcohol..... 2 hours minimum
7. Change of 100% alcohol.... " " "
8. Change of 100% alcohol.... " " "

II. Zirkle's Butyl Alcohol Method, using Normal Butyl Alcohol

1.	95%	water,	5%	ethyl alcohol	---	...	one hour		
2.	89%	"	11%	"	"	---	...	"	"
3.	82%	"	18%	"	"	---	...	"	"
4.	70%	"	30%	"	"	---	...	"	"
5.	50%	"	40%	"	"	10%	butyl alcohol	"	"
6.	30%	"	50%	"	"	20%	"	"	"
7.	15%	"	50%	"	"	35%	"	"	1 hr. minimum
8.	5%	"	40%	"	"	55%	"	"	"
9.	---		25%	"	"	75%	"	"	"
10.	---		---			100%	"	"	"
11.	Change of 100% butyl alcohol							"	"
12.	Change of 100% butyl alcohol							"	"

III. Same technique as II, but combining steps 2 and 3, and substituting 85% water and 15% ethyl alcohol.

IV. Same sequence as II, using Tertiary Butyl Alcohol instead of Normal Butyl Alcohol.

V. Glycerin Method

1. Immerse in 10% glycerin solution, allowing water to evaporate .....(approx. 31 hours at 60° C)
2. 95% ethyl alcohol.....3 hours
3. Change of 95% ethyl alcohol....3 hours
4. Change of 95% ethyl alcohol....3 hours

Clearing and Bleaching

A. Xylol Method

1. 100% ethyl alcohol.....2 to 6 hours
2. 3 parts ethyl alcohol, 1 part xylol
3. 2 " " " 2 parts "
4. 1 part " " 3 " "
5. 100% xylol
6. Change of 100% xylol
7. Change of 100% xylol

B. Benzol Method

Same sequence as in Method A, substituting benzol for xylol.

C. Ammonium Hydroxide Method

1. Water at 60° C.....6 hours
2. 5% solution of NH_4OH at 60° C.....overnight
3. Rinse in running water bath.....12 hours minimum

D. Same sequence as C, using 10% strength of NH_4OH

E. Sodium Hydroxide Method

Same sequence as method C, substituting 5% NaOH for NH_4OH

F. Same sequence as method C, using 10% NaOH instead of NH_4OH

G. Peroxide Method

1. 95% ethyl alcohol
2. 95% ethyl alcohol and 3% peroxide at 60° C...24 hrs. minimum
3. As many changes of above step as required for complete bleaching of tissue (variable).....24 hrs. each
4. 95% ethyl alcohol
5. One or two changes of 95% ethyl alcohol

H. Lactic Acid Method

1. Water (distilled)
2. 75% lactic acid.....several days
3. Wash in running water bath.....24 hours

J. Clove Oil Method

1. 100% ethyl alcohol
2. Clove oil, at 60° C.....2 days minimum
3. 100% ethyl alcohol

K. Cedar Oil Method

1. 100% ethyl alcohol
2. 2 parts ethyl alcohol, 1 part cedar oil...26 hours
3. 1 part " " 2 parts " " ...2 days
4. 100% ethyl alcohol.....24 hours
5. One change of 100% ethyl alcohol..... " "

L. Chloroform Method

1. 100% ethyl alcohol
2. 100% ethyl alcohol and chloroform, equal parts, at 60° C.....2 days
3. 100% ethyl alcohol

Staining of Sectioned Material

1) Methyl Green- Bismarck Brown

1. Methyl Green (aqueous).....overnight
2. Water.....2 minutes
3. Bismarck Brown, 2% (70% alcoholic)1/2 minute
4. 95% ethyl alcohol..... " "
5. 100% " " .....2 minutes
6. Xylol

Staining of Bulk Material

1) 25% Alcoholic Eosin

1. Stain.....approx. 2 days
2. Destain in 35% alcohol (ethyl) or 35% acid alcohol
3. 70% ethyl alcohol.....few minutes
4. 95% " " ..... " "

2) 1% Aqueous Eosin

1. Stain.....approx. 10 hours
2. Water.....few minutes
3. 70% ethyl alcohol..... " "
4. 95% " " ..... " "
5. 100% " "

3a) 1% Aqueous Saffranin O

1. Stain.....approx. 10 hours
2. Water.....few minutes
3. 70% ethyl alcohol..... " "
4. 70% acid ethyl alcohol.....up to 5 minutes
5. Change of acid alcohol..... " " " "
6. 70% ethyl alcohol.....15 minutes
7. 95% ethyl alcohol..... " "
8. 100% " "

3b) 50% Alcoholic Saffranin O, the alcohol solution consisting of "anailine water" (approx. 1/3 aniline oil and 2/3 water) and 95% ethyl alcohol, equal parts.....approx. 2 days
2. 50% acid ethyl alcohol..... " minutes
3. 70% " " " .....10 to 15 minutes
4. 95% ethyl alcohol.....few minutes
5. 100% " "

4a) 1/2% Aqueous Iodine Green

1. Stain.....approx. 24 hours
2. Water..... " 2 hours
3. 50% ethyl alcohol.....few minutes
4. 70% acid ethyl alcohol.....few hours
5. 70% ethyl alcohol.....10 minutes minimum
6. 95% " " ..... " " "
7. 100% " " ..... " " "
8. 100% " " and xylol, equal parts

4b) 70% Alcoholic Iodine Green

1. Stain.....approx. 2 days
2. 70% ethyl alcohol.....10 minutes
3. 70% acid ethyl alcohol.....10 to 15 minutes
4. 95% ethyl alcohol..... " " " "
5. 100% " "

5) 1% Aqueous Crystal Violet

1. Stain.....approx. 10 hours
2. Water.....10 minutes minimum
3. 50% ethyl alcohol..... " " "
4. 70% " " .....15 minutes minimum
5. 70% acid ethyl alcohol..... " " "
6. 70% ethyl alcohol..... " " "
7. 95% " " .....10 minutes minimum
8. 100% " " .....3 minutes
9. 100% ethyl alcohol and xylol, equal parts.....few minutes
10. Xylol

6) 1% Aqueous Methyl Green

1. Stain.....1 to 10 hours
2. Water.....10 minutes
3. 70% ethyl alcohol..... " "
4. 70% acid ethyl alcohol.....1 hour minimum
5. 95% ethyl alcohol.....few minutes
6. 100% " " ..... " "
7. 100% ethyl alcohol and xylol, equal parts..... " "
8. Xylol

Discussion of Methods

All herbarium flowers were softened by boiling very gently for ten to twenty minutes in water. Both distilled and tap water were used in order to determine the effect of each upon solubility of the brown pigment of the dried flowers during the boiling process. No difference in effect was noticed, neither type of water removing more than a negligible amount of the color. In some specimens single parts of the perianth, and in one or two cases, the so-called corolla tube became detached in the boiling process.

No difference in efficiency of dehydration was noted in comparing the ethyl alcohol, butyl alcohol, and glycerin methods. Experimentation with these methods was carried out on freshly cut Crassula, Saintpaulia, Verbena, and Solanum flowers. It was observed that the butyl alcohol methods (numbers II, III, and IV) seemed to render these flowers more brittle than ethyl alcohol; thus these methods were not employed in the work with sapodilla flowers. No difference in effect was evident between Normal and Tertiary butyl alcohols. Because of availability of materials, and closer familiarity

with the method, the ethyl alcohol method (number I) was used, in preference to the glycerin (number V) method, in dehydrating the Sapodilla flowers used for study.

By experimenting on Sapodilla flowers from herbarium specimens, it was found that none of the ordinary clearing agents (A, B, J, K) removed any amount of the brown pigment (which was, at this point, almost entirely retained in the specimens). Lactic acid (method H), after a few days, removed some, but by far not enough, color to clear the flowers. Chloroform (method L), NaOH, and NH_4OH (methods C through F) were superior to lactic acid, but still not strong enough. The only reagent which completely bleached the flowers to a transparent white color, and thus rendered them fit for staining, was peroxide (H_2O_2), applied as described in method G. Rawlins (1933) suggests only one application of peroxide in 95% alcohol, but it was found that, to clear adequately the dried Sapodilla flowers, at least three changes of H_2O_2 were necessary. Two undesirable effects of this method were (a) undue softening of the flower to the point where it practically disintegrated upon attempt to dissect it or cut it into longitudinal halves, and (b) release of oxygen, from the peroxide solution within the tissues, which caused the flower to float. The latter condition necessitated aspiration of the specimens before paraffin infiltration. Two or three changes of 95% ethyl alcohol subsequent to bleaching served to remove the peroxide and harden the tissue to a workable texture. The specimens to be sectioned were then transferred through 100%

ethyl alcohol, xylol (method A), and gradually infiltrated with paraffin.

Apparently there is some air trapped within the flower, which cannot be removed by aspiration, because the paraffin blocks with the embedded Sapodilla material always contained numerous air bubbles, especially in and around the gynoecium; this often resulted in greater or lesser failure of ribbon formation. (This difficulty was never encountered in working with fresh Crassula, Saintpaulia, Verbena, and Solanum flowers.)

The sections were attached to slides by egg albumen fixative, the latter being made up in proportion of 50 cc. egg albumen, 50 cc. glycerine, and one gram sodium salicylate (Woodcock, 1945), then floated in two percent formalin. Upon drying, the sections were stained by the method listed. They were then mounted in Canada Balsam.

Specimens which were studied in bulk were run back from 95% alcohol to the medium in which a particular stain was soluble. At first, pieces of tissue, such as a sepal or petal, and later, longitudinal quarters of flowers, were used to test the six stains listed.

The Eosin stains, both aqueous and 25% alcoholic, were the least usable, and thus were discarded after a few trials. There was an unequal distribution of the stain throughout the tissue, with scarcely any differentiation of the vascular bundles. Destaining in acid alcohol resulted in precipitation of the stain.

The Saffranin solutions showed more promise than the eosin, but were surpassed by Iodine Green, which displayed a better differentiation of the veins. In these preliminary staining trials, stains 4a and b were best; stains 5 and 6 were next best. The green and purple stains seemed in all cases superior to the red ones.

The four stains mentioned in the preceding paragraph were repeated, each on one one-fourth of a sapodilla flower (bleached in peroxide). In the final analysis, stains 5 and 6 were found most efficient; timing experiments showed the optimum amount of time for each step to be as described in the preceding section of the paper. Methyl Green was slightly superior to Crystal Violet, and was finally used in the bulk staining of all other flowers studied. One difficulty was encountered in all types of stains (though least in numbers 5 and 6); a much heavier stain would be retained in the lower portion of the gynoecium, and in the receptacle, than in the other floral parts, so that it was impossible in case of the red stains, and difficult with the most workable stains, to strike a favorable balance between understaining the latter, and overstaining the former.

The specimens stained and destained in bulk were carefully laid open by making a shallow cut along the length of the peduncle and the receptacle. Through careful manipulation it was possible in most cases to determine the relative levels at which the various traces diverged from the main cylinder of

the peduncle into the floral parts, and to follow the entire course of each branch. Two of the flowers were badly disintegrated by the peroxide, and two or three others did not take the stain well enough for conclusive study, but the partial results that could be obtained from them appeared to be in accordance with the vascularization picture presented by the others.

Longitudinal sections, 14 microns in thickness, were cut from one flower (Alexander s.n.) and transverse sections of the same thickness were cut from another flower (Broadway s.n.). Since the flowers were at a stage of maturity where the sub-epidermal cell layers of the ovary had already become lignified, extreme difficulty was encountered in cutting paraffin ribbons. Sufficient sections were obtained from each flower, however, to include the area where differential vascularization into the floral whorls occurred.

OBSERVATIONS

Because of disagreement in the interpretation of the floral structure of the subfamily Mimusopoideae, the terminology of the different floral whorls varies with different authors. To avoid possible confusion in reporting observations, each whorl will be designated by a letter, corresponding to the floral diagram, Figures 1 and 2. The following tabular arrangement shows a comparison of the nomenclature used for each whorl by previous workers (see Review of Literature) and by Gilly (1942).

Whorl	Previous Workers		Gilly	
A	Sepals, outer series	perianth	perianth	Outer perianth, valvate (sepals)
B	Sepals, inner series			Inner perianth, imbricate (petals)
C	Dorsal (stipular) appendages to petals (in Balata complex)	perianth	androecium	Exterior staminodes (C and D are fused in the Sapodilla, separated in the Balata flowers)
D	Petals			
E	Staminodes	androecium		Interior staminodes
F	Stamens			Stamens
G	Carpels (ovary)	gynoecium		Carpels (ovary)

The vascular skeleton of the flowers that were laid open as shown diagrammatically in Figure 3. The first three traces which separate from the vascular cylinder, at the lowest level, form the midribs of the three segments of whorl A; two of these three traces are indicated in black in Figure 3. These traces closely parallel the cylinder for some distance and, after diverging into the base of the segments, each gives rise to two branches which parallel the main trace toward the apex of the segment.

At the next level of divergence, three strands separate. Two of these (stippled in Figure 3) make their way also into the sepal, one above the other, and there give rise to several veins, each of which branches again to form the conducting system of this segment.*

*Another case, where the lateral sepal traces arise independently of the midrib is described by Tillson (1940) in some members of the Kalanchoideae (subfamily of the family Ericaceae); there the laterals separate at a lower level than the midrib. The family is close in taxonomic position to the Sapotaceae.

The third strand (shown by the horizontal lining in Figure 3), passes into a segment of whorl B as a midrib. At the base of this strand two branches arise; each dividing several times to form the vascular supply of this segment.

The veins entering the combined whorls C-D branch off independently and at a much higher level, as shown by the diagonally striped lines in Figure 3. In making this diagram, a petal was removed to expose the vascular system, and only a part of the structure formed by whorls C, D, E, and F, is shown.

The venation pattern of the lobe (whorl C-D) to which the stamen is attached, and that of the staminode (whorl E) are essentially the same, both structures generally containing one strong vein that branches into two parts. The midribs of each member of whorl C-D and the adjacent member of whorl E originate from the same strand at the base of the tube. The single trace of the filament (whorl F) diverges as a third branch from the midrib of each segment of whorl C-D. The exact point of divergence varies somewhat from flower to flower and to a greater degree from species to species, but is generally close to the base of the tube.

The last two groups of traces to separate from the central cylinder are those of the gynoecium (whorl G). The dorsal carpel traces separate first and continue through each carpel and upwards within the style. The two lateral traces of each carpel are apparently fused into a single ventral bundle and

these ventral bundles terminate below the middle of the ovary. The style is hollow and internally lobed (the number of lobes corresponding to the number of carpels) throughout its length.

The parallel broken lines in Figure 3 correspond to the levels at which cross-sectional diagrams (Figures 4 to 10) were drawn. The lowest cross section (Figure 4) shows three strands diverging from the main central cylinder; these evidently represent the midribs of whorl A. The separation of these traces apparently takes place at a relatively low level in the peduncle; at least it was below the point at which the first sections of this flower were cut.

The next diagram (Figure 5), approximately 550 microns above the first, shows that three more bundles, alternating with the first three, have separated from the main cylinder. In Figure 6, each of these additional three bundles has divided once to form a pair. One member of each pair divides again, so that three groups of three bundles are formed. This is shown in Figure 7. In each of these groups, two traces apparently represent the lateral traces of whorl A (represented by stippling in Figure 3), the third being the midrib of whorl B (represented by horizontal lining in Figure 3).

The fourth cross section (Figure 8) represents a level 550 microns above the previous one, and shows the divergence of the six traces which enter the tube formed by the fusion of whorls C, D, E, and F.

In the next diagram (Figure 9) a cross section through

the lower part of the ovary is shown; in this flower the number of carpels is eleven. One dorsal and one ventral trace (formed of the two united ventral bundles) per carpel are evident. The last cross section (Figure 10) includes a section through the style, showing its internal structure, and the dorsal bundles, which are its sole vascular elements. The large number of traces seen in each of the surrounding floral whorls indicates the leaf-like character of venation displayed by all floral organs. Only one trace is present in the anther. The extrorse anthers are turned at an angle from their natural position, evidently to fit into the lobed shape of the tube resulting from compression of the flower in the embedding process.

The diagram, Figure 11, representing a longitudinal slice through a sapodilla flower near the center, was drawn from ten successive sections, each 14 microns thick. Since the section is not a perfect median one, a small portion of a segment of whorl A is present on the right side. The portion of a vascular trace in the lower part of this tissue is probably a part of the midrib; the trace passing into the upper tissue of the segment is evidently one of the laterals, since it diverges from the central cylinder at the same point as the trace extending into the segment of whorl B.

The trace entering the left-hand segment of whorl B is probably the midrib of that segment, judging from its level of divergence.

At a much higher level (the same level on both sides), a single trace departs from the main cylinder to enter the staminode (whorl E) on the left side, while a similar trace on the right passes into a segment of whorl C-D as a midrib. This trace divides, the left branch extending up through the stamen (whorl F), the right arm continuing upward within segment C-D. Two dorsal carpel traces are shown, extending upward within the hollow style.

The venation of individual members of the floral whorls is shown in Figures 12-14. Figure 12 represents one of the three valvate segments of whorl A; the three segments are joined at the base into a tube, and each segment is very thick in the basal part. The midrib is embedded in the lower part of the tissue, while the two lateral traces are situated near the upper epidermis. As may be noted from the drawing, the two lateral bundles are thicker than the midrib, and, by branching, each gives rise to the vascular supply of one half of the segment.

One of the three segments of whorl B is represented by Figure 13; these three segments are imbricate and independently attached upon the tube formed by whorl A. They are somewhat more pointed than the segments of whorl A, and not so thickened at the base. The vascular supply of each of these segments originates from the single prominent trace which gives rise to the midrib and a branch on each side; several veins diverge from these branches and run almost parallel to the midrib.

The venation pattern of the segments in both whorls A and B is essentially leaf-like, and may be described as flabellate. It is very similar in both series, the only difference being the presence of the two lateral veins in the outer one.

The basic vascularization of segments of the united whorls C, D, E, and F is shown in Figure 14. There is always one prominent midrib in each segment of whorl C-D, and generally one strong trace passing into each segment of whorl E. The midribs of segment C-D and of one of the adjacent members of whorl E are united at, or even below, the base of the tube. As previously mentioned, one strand separates from the midrib of each segment of whorl C-D and extends into the stamen (a segment of whorl F).

Though the basic pattern described above is constant in the flowers of the Sapodilla and related plants, such as the Balata, minor variations occur within a single species; some of these are illustrated in Figures 15-21. Figures 15-18 show portions of the tubes taken from different specimens of Manilkara zapotilla. Figures 19 and 20 represent tubes of related species (the former being from M. emarginata Gilly, and the latter from M. tabogaensis Gilly).

In Figure 15, each branch of the midrib of each broad segment (representing whorls C-D) is almost immediately divided again to form a pair of branches; the inner one of each pair is slender and extends toward the tip of the segment. There is, therefore, no true midrib within the segment.

A somewhat different type of venation in whorl C-D is shown in Figure 16. The midrib forks at or near the base of the tube, and one of the branches divides almost immediately, so that there are three principal veins extending into the segment. The other branch divides too, but at a much higher level. The venation of the staminode (whorl E) in this specimen is quite weak.

An interesting example of accessory venation occurs in the specimen shown in Figure 17. In addition to the midribs of the broad segment and the staminode, there is (in four or five pairs out of the six) on one or both sides of the staminode midrib, a rather prominent trace, which forks near the summit of the tube and sends one branch up into the staminode, the other into the adjacent broad segment. These prominent accessory veins are joined to the staminode midrib at the base of the tube. In one of the six broad segments, one of the branches of the midrib diverges at a wide angle before forking; one arm of the fork extends upward along the edge of this segment, the other enters the adjacent staminode.

This extra venation seems to predominate in those forms of Manilkara zapotilla, where the segments of whorl C-D are very slightly three-lobed; that is, apparently the fusion between segments of whorl C and those of whorl D is not so entirely complete as in the specimens where the broad segments (whorls C and D combined) are entire. Only in one specimen of the latter type was evidence found of prominent venation.

In the specimen shown in Figure 18, no accessory prominent veins are present, but there is a distinct network of small veinlets connecting the broad segments and the staminodes.

In Manilkara emarginata, M. tabogaensis, and M. bidentata, in all of which whorls C and D are distinct (that is, each broad segment has a pair of "dorsal appendages"), the venation pattern is closely similar and quite uniform. Segments of the tube formed by whorls C, D, E, and F of the first two species mentioned are shown in Figures 19 and 20. In both species, the venation of a staminode and of a segment of whorl C are very similar: the midrib of each gives off one branch on each side, which diverges to pass into the adjacent "dorsal appendage"; the central trace of each continues upward in the whorl C segment and forks once. Thus each "dorsal appendage" receives traces from the members adjacent to it on each side, but the greater part of its supply results from the branching of the trace received from whorl C.

A very interesting feature observed in the specimen of M. emarginata studied, was the fact, that there are three distinct traces extending up through the filament. Time did not permit sections to be made through the stamen, but from superficial examination of the transparent anther under the dissecting microscope, the venation of that organ appeared to be as shown in Figure 19. The central one of the three traces continues upward through the connective, but the other two apparently each enter a pollen sac, and then immediately become obsolete.

INTERPRETATION AND DISCUSSION

This section of this paper is concerned with a consideration of floral whorls A to F, inclusive, of the sapodilla flower. Since the interpretation of whorl G (the gynoecium) has never been in dispute, it will not be considered here. An adequate description of the gynoecium has been given in the preceding portion of this paper.

Perianth (Whorls A and B):

Though the venation pattern is similar in both whorls A and B, the inner one is supplied by a different system than the outer; in both there is one trace functioning as midrib (the midrib of the inner series branching off at a higher level than that of the outer), but each part of whorl A is also supplied by two other traces separating from the main cylinder at the same level as the midrib of whorl B. In the segments of both whorls A and B, the midribs branch near the base, giving rise to one vein to the right and one to the left. While in the segment of whorl B the minor venation pattern originates from the branching of the midrib, that of the segments of whorl A originates primarily from branches of the lateral traces entering the sepal at a level above that of the midrib.

Furthermore, close observation of the so-called biseriate calyx (whorls A and B) reveals, that the outer three parts (whorl A) are valvate in position, and have acute apices, while the members of the inner series are imbricate, and have

more rounded, blunt apices. In addition, the three segments of series A are united at the base into a short tube, and the three members of series B are separately attached on the tube formed by the union of the segments of A. The members of whorl B are much less thickened at the base than are the segments of whorl A.

Eames' statement to the effect, that petals may be leaflike in venation, and Swamy's report of the similarity in the venation patterns of the sepals and of the outer petals of Degeneria sp. (see Review of Literature) show, that in some other plant families, the relationship between sepals and petals is comparable to that existing between whorls A and B of the Sapodilla flower. The petals of the flower of the Degeneria sp. receive one trace, which forks into a median and two marginal traces. This situation is the same as that found in members of whorl B in the Sapodilla flower.

The above described results of previous morphological, and present anatomical studies seem to indicate, that whorls A and B (the so-called biseriate calyx) represent two distinct perianth whorls: an outer series of three sepals (whorl A), and an inner series of three petals (whorl B). In the light of this interpretation, members of whorl A shall, in the remainder of this paper, be referred to as sepals, and segments of whorl B, as petals.

Androecium (Whorls C, D, E, and F):

The high level, at which the traces depart into the base of the tube formed by the united whorls C-D (the structures considered originally to be corolla lobes) indicates, that these structures are androecial in nature, rather than corolloid. The venation of each member of series C-D is similar to that of the staminodes (whorl E); it is especially noticeable that, in the members of both whorls, the midrib forks into two branches, each of which again may branch. In general, the main venation pattern of members of whorls C-D and E tends to be dichotomous. The single trace leading through the stamen arises as a third branch which is separated, almost at the base of the tube, from the midrib of the broad petaloid segment (whorl C-D).

Since the basic pattern of venation is the same in all Sapodilla specimens studied, the slight variations in the levels, where the branches from the midribs (in both whorls C-D and E) diverge and branch again, (as shown in Figures 15-18) seem to be of minor importance. These variations are not always constant in different segments of the tube of one flower.

An interesting feature is the extra or accessory venation found mostly in those forms of Manilkara zapotilla where the somewhat three-lobed shape of each broad segment is apparently suggestive of incomplete fusion of the dorsal appendages.

(whorl D in the Balata flower) with the parts of whorl C.

Usually, there is an accessory trace near the zone of fusion between a segment of whorl C-D and one of whorl E. This trace forks, one branch extending up into the broad segment (whorl C-D) near its margin, the other branch passing into the marginal portion of the staminode (whorl E). It should be noted, also, that the accessory traces are joined to the staminode midribs at the base of the tube, as shown in Figure 17.

In an attempt to determine the possible significance of this accessory venation, one should refer to Figures 19 and 20, which show the species of Manilkara, in which the dorsal appendages (whorl D) are distinct from the segments of whorl C. The reader may compare these Figures with Figure 17, and picture the slightly lobed lower part of each margin of a broad segment as representing the vestige of a dorsal appendage. In this case, the accessory trace may be comparable to a branch diverging from the midrib of the staminode into an adjacent dorsal appendage. In some of the broad segments (whorl C) in Figure 17, the branches of the midrib diverge at a wide angle to pass into the small lobes (the vestigial dorsal appendages) on either side; in one broad segment, the angle of divergence of the right branch of the midrib is so wide, that, as this branch forks, the right arm passes into the adjacent staminode, while the left one continues up near the lobed margin of the lower part of

the segment (see Figure 17). This trend of wide divergence of the branches of the midrib toward the slightly lobed margin may possibly be interpreted as a vestige of the vascular connection between a segment of whorl C and its adjacent dorsal appendages, as found in the flowers represented by Figures 19 and 20. As shown in these Figures, one branch on each side diverges at a wide angle toward the margin from the midrib of a segment of whorl C, to pass into the dorsal appendage.

These observations seem to indicate, that when whorls C, D, E, and F are all distinct and present, they are connected by a prominent system of vascularization, and that a reduction of this vascular pattern apparently takes place in proportion to the amount of fusion of whorls C and D.

It is interesting to note, that an interpretation of the vascularization of a segment of whorl C-D as an androecium segment (rather than as a corolla segment) seems to be in accord with the telome theory of the phylogeny of stamens, as expounded and illustrated by Wilson (1937, 1942). This theory describes the plant body as one axis, branching dichotomously, with some branches being sterile, and others containing solitary sporangia at their tips. Sepals and petals of modern flowers, according to this theory, have arisen by the flattening, webbing, and fusing of sterile telomes, while the stamens and carpels are the result of fusion of sporangia-bearing telomes.

According to the telome theory, the typical anther of four

pollen-sacs originated in this way (Wilson, 1942): "The stalks of two pairs of sporangia became reduced, so that the sporangia were almost sessile and were contiguous. Each pair of sporangia then became folded back upon a penultimate unit of the branch system and became fused with this unit." The author demonstrates this theory of stamen development in his paper by a series of illustrations, based on the study of various types of stamens, taken from plants of different families.

In some families with flowers having numerous stamens, several of the stamens are supplied by branches from one main trace, called the stamen fascicle trace, which arises directly from the central cylinder. An example of this condition may be found in the family Annonaceae (Wilson, 1937), which is closely related to the Sapotaceae (Hutchinson, 1926). Note the similarity in the sapodilla flower: The single strand departing from the stele appears to be homologous to the stamen fascicle, since it branches at the base of the tube to supply members of the whorls C, D, E, and F. In some cases, the filament trace has become fused for its lower part with the midrib of the segment to which it is attached. According to Wilson (1937), a fascicle trace may represent either lateral fusion of stamen traces (in case a flower is a compressed shoot) or, by the telome theory, the rachis of a primitive branch system with terminal sporangia. The essentially dichotomous nature of vascular branching in the tube composed of whorls C, D, E, and F, in

the sapodilla and related flowers further implies the androecial nature of this structure.

Upon morphological examination of several related species of Manilkara, Gilly (1943) found, that in some, the members of whorl C (the so-called "petals" of authors) are of long and narrow shape, while the dorsal appendages (whorl D) are broad and petaloid. An example of this type, Manilkara tabogaensis, is illustrated in Figure 20. In M. emarginata (Figure 19) the reverse is true. The shape of the staminodes (whorl E) also varies in different species of Manilkara, ranging from short and recurved to long and narrow to broad and petaloid.

The fact that the same range of variation in shape and size may be found in members of whorls C, D, and E, as well as the similarity of the nature of the vascular supply in these whorls, add further support to the assumption that the tube is an androecium, rather than a corolla.

Finally, it is noteworthy, that during the process of fruit development the so-called "corolla-tube" (whorls C, D, E, and F) is lifted or torn out of the flower. The two outer whorls (A and B), on the other hand, are persistent at the base of the ripe fruit.

SUMMARY

After experimenting with various methods of dehydration, clearing, bleaching, and staining, it was found that the most useful techniques in working with dried Sapodilla flowers from

herbarium specimens were dehydration in ethyl alcohol, clearing in xylol, bleaching in peroxide, and staining in Methyl Green and Bismarck Brown.

Although the venation in members of the two outer floral whorls is similar, the source of vascular supply to each is different. Two lateral veins, arising from a higher level, in addition to the midrib, are present in each sepal. These lateral sepal veins arise from the same level as the midrib of the petals. The entire venation of each petal originates from the single petal trace.

The traces passing into the androecial tube diverge from the main cylinder at a higher level than the petal traces. The venation patterns of the exterior staminodes (the "corolla-lobes" of authors) and of the inner staminodes are very similar, and essentially dichotomous.

In the androecium of those forms of Manilkara zapotilla, where the union between the exterior staminodes (the "corolla-lobes" of authors) and the "dorsal appendages" apparently is not entirely complete, an interesting type of accessory venation occurs. This may represent vestigial traces of the vascular supply of the dorsal appendages. In some specimens examined, the prominent accessory venation is replaced by a network of small veinlets connecting the main veins of the exterior and interior staminodes.

In specimens examined of two other species of Manilkara,

where the appendages are distinct from the exterior staminodes, each "dorsal appendage" is supplied by traces from both the exterior staminode to which it is attached, and the interior staminode adjacent to it on the other side.

In one specimen of Manilkara emarginata, stamens with three traces were found to be present, but lack of time prevented further investigation. This is an interesting phenomenon, because it was observed in but one of the flowers examined in this study.

The principal venation of the ovary consists of one dorsal and one ventral trace per carpel, the dorsal bundles extending up through the style.

CONCLUSIONS

The anatomical study reported in this paper supports morphological observations which indicate that the so-called "biseriate calyx" of authors actually consists of an outer series of sepals and an inner series of petals. Furthermore, the so-called "corolla-tube" appears to be an androecium. This assumption conforms to the telome theory of the origin of stamens.

The shape of the members of any of the sterile androecial whorls may vary, in different species, from quite narrow to very broad and petaloid.

The single traces, passing from the vascular cylinder of

the peduncle into the androecial tube and dividing to form the midribs of stamens and both the exterior and interior staminodes, may be homologous to stamen-fascicle traces in other plant families, such as the Annonaceae.

On the basis of such an interpretation of the structure of the flower of the Sapodilla and related species of Manilkara, the classical description of the family Sapotaceae cannot be applied to the subfamily Mimusopoideae. This subfamily would have to be described as polypetalous, with a highly evolved androecial tube, separate from the corolla, whereas the subfamily Sideroxyloideae is sympetalous, with epipetalous stamens. The subfamily Madhucoideae, as yet not investigated from an anatomical standpoint, appears to be allied with the Mimusopoideae rather than the Sideroxyloideae.

PLATE I

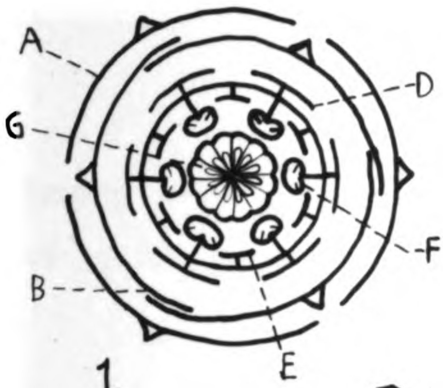
Explanation of Figures

Figure 1. Floral diagram of Sapodilla, Manilkara zapotilla (Jacq.) Gilly; the various whorls of the flower are indicated by letters A-G (see text for explanation).

Figure 2. Floral diagram of a member of the Balata-complex, Manilkara bidentata (A.DC.) Chev.; the various whorls of the flower are indicated by letters A-G (see text for explanation).

Figure 3. Diagrammatic representation of principal elements of the vascular skeleton of a flower of Manilkara zapotilla (drawn from Eyerdam 212); the flower is represented as partially dissected; two sepals, one petal, and one-sixth of the androecium are shown. The letters A to G, inclusive, indicate levels at which Figures 4-10 are drawn. Sepal midrib traces are represented by solid black; lateral sepal traces are stippled; petal traces are horizontally lined; and an androecial trace is cross-hatched.

PLATE I



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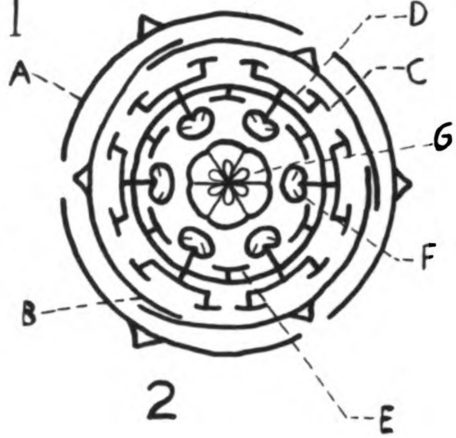

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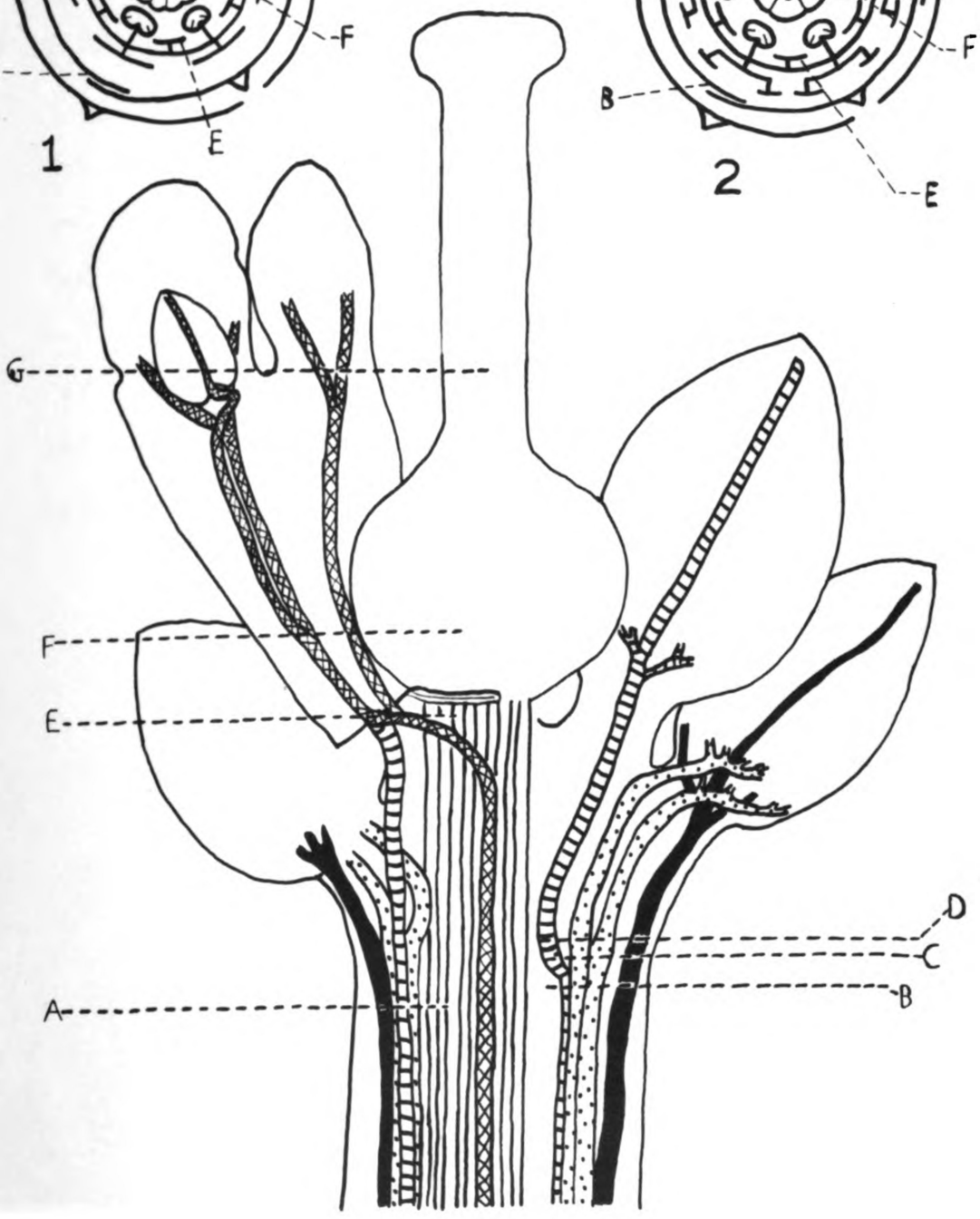

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PLATE II

Explanation of Figures

Figures 4-10: *Manilkara zapotilla*, diagrammatic cross-sections through a flower (drawn from *Alexander* s.n.); for detailed explanation, see text.

Figure 4. Cross-section from level A of Figure 3.

Figure 5. Cross-section from level B of Figure 3.

Figure 6. Cross-section from level C of Figure 3.

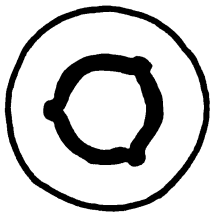
Figure 7. Cross-section from level D of Figure 3.

Figure 8. Cross-section from level E of Figure 3.

Figure 9. Cross-section from level F of Figure 3.

Figure 10. Cross-section from level G of Figure 3.

PLATE II



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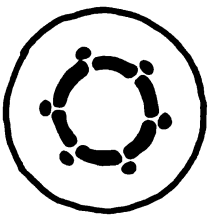

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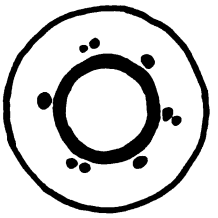

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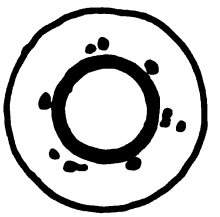

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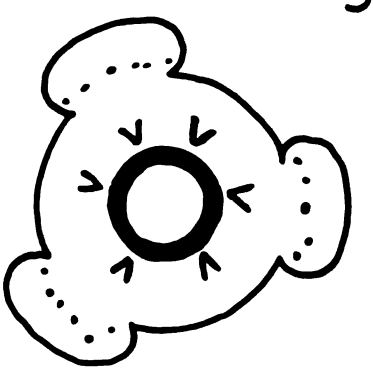

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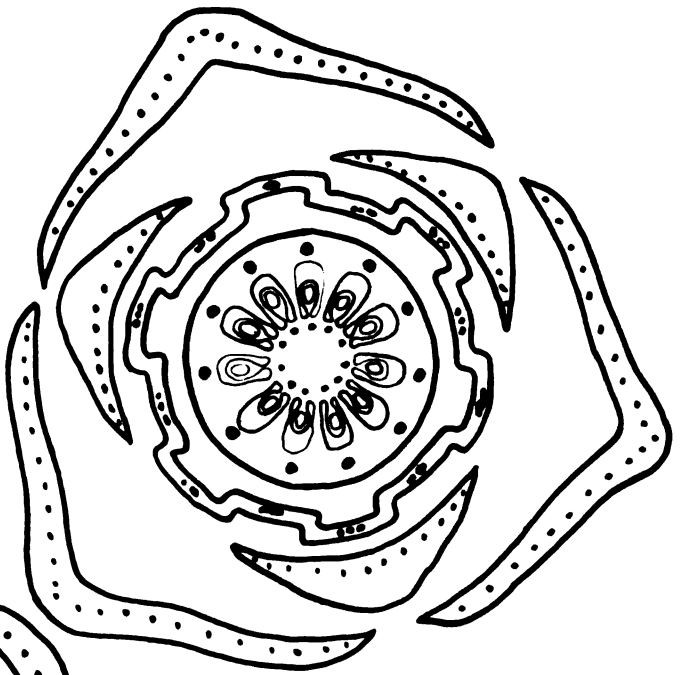

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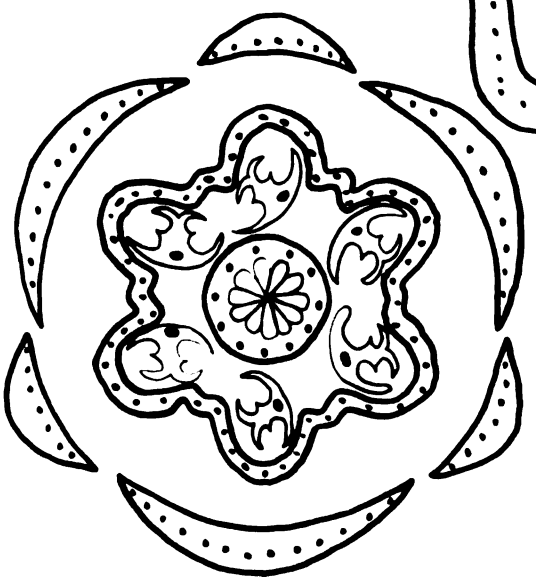

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PLATE III

Explanation of Figures

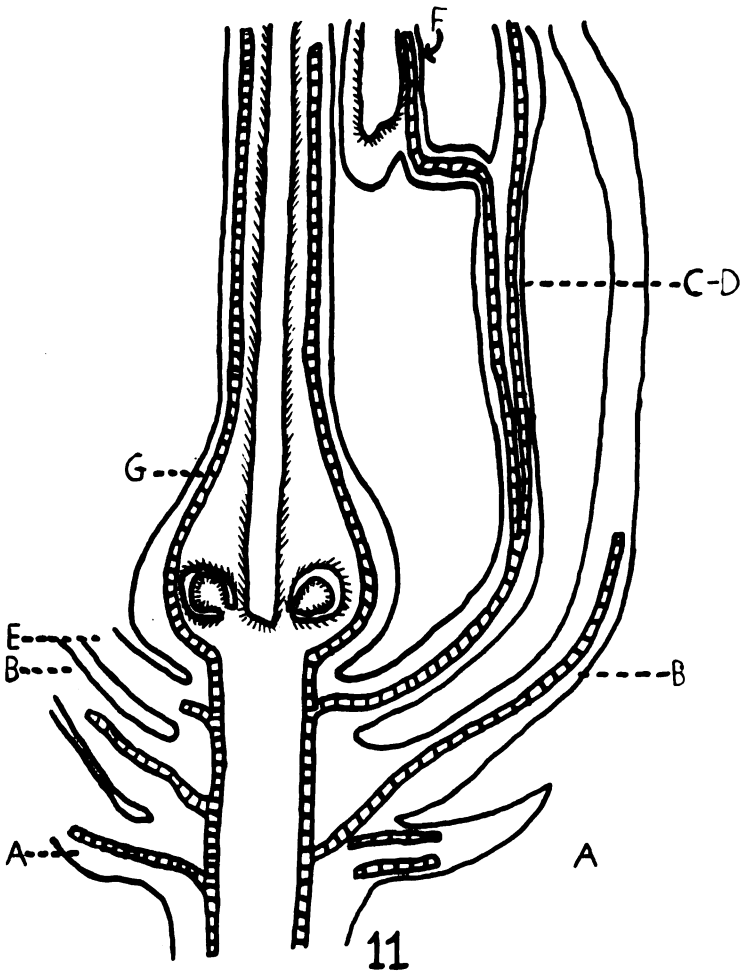
Figure 11. Manilkara zapotilla, diagrammatic longitudinal section of flower, (drawn from Broadway s.n.); apices of style, anther, androecial tube, petals, and sepals are not shown. Letters A-G, inclusive indicate floral whorls as described in text.

Figure 12. Manilkara zapotilla, a single sepal (drawn from Eyerdam 212) showing venation; A is midrib trace, while B and B' are lateral traces (approximately x 7.5).

Figure 13. Manilkara zapotilla, a single petal (drawn from Eyerdam 212) showing venation (approximately x 7.5).

Figure 14. Manilkara zapotilla, inner view of one-third of the androecial tube, showing pattern of principal venation (drawn from Eyerdam 212; approximately x 7.5).

PLATE III



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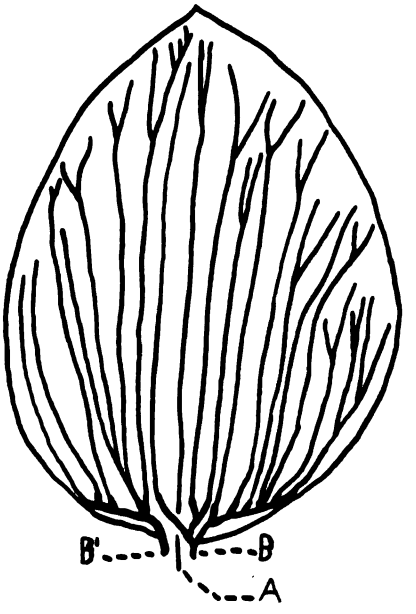

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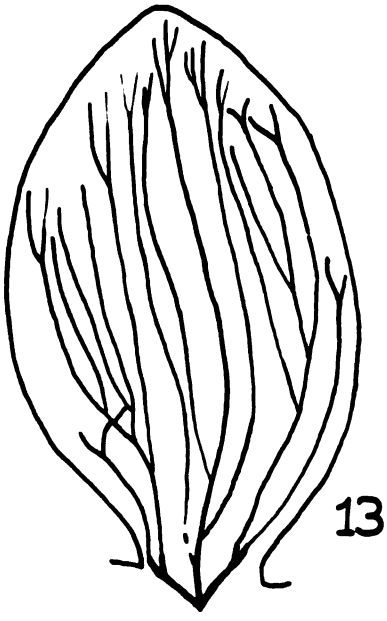

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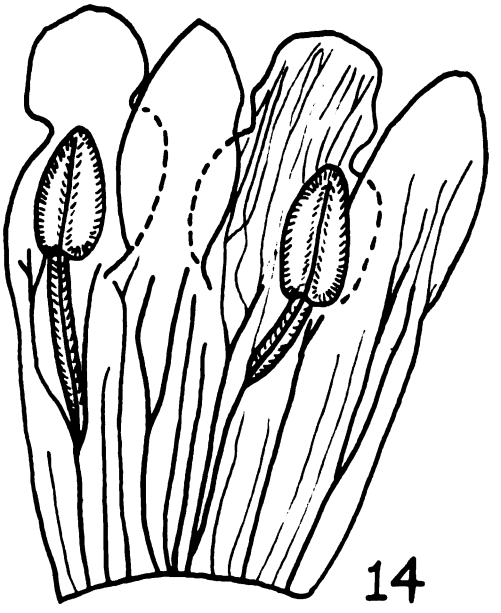

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PLATE IV

Explanation of Figures

Figures 15-20. Drawings representing inner view of one-third of the androecial tube, from flowers of the Sapodilla and related species, showing venation pattern (approximately x 7.5). One stamen and one staminode in each drawing have been cut off to expose the venation of the outer androecial segments.

Figure 15. Manilkara zapotilla (drawn from Lundell 500).

Figure 16. Manilkara zapotilla (drawn from Jack 7820).

Figure 17. Manilkara zapotilla (drawn from Harris 8634).

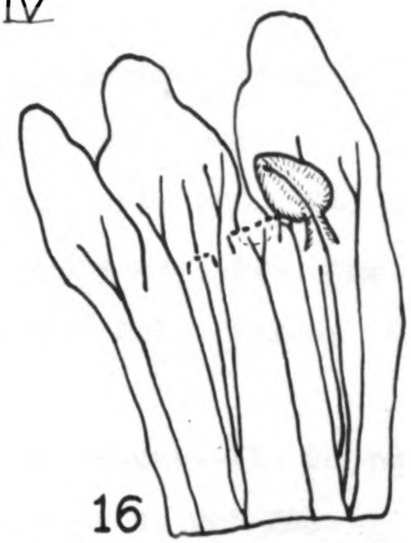
Figure 18. Manilkara zapotilla (drawn from Matuda 3254).

Figure 19. Manilkara bahamensis (drawn from Seibert 1317).

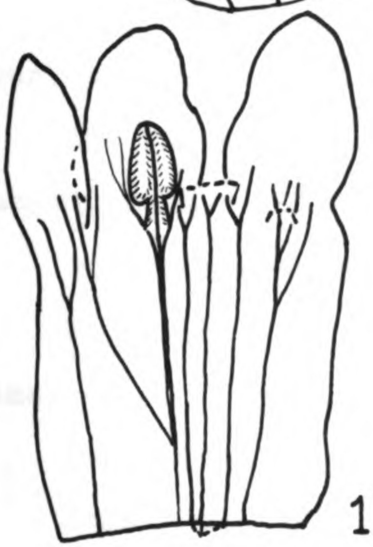
Figure 20. Manilkara tabogaensis (drawn from Woodson, Allen, and Seibert 1455).

PLATE IV

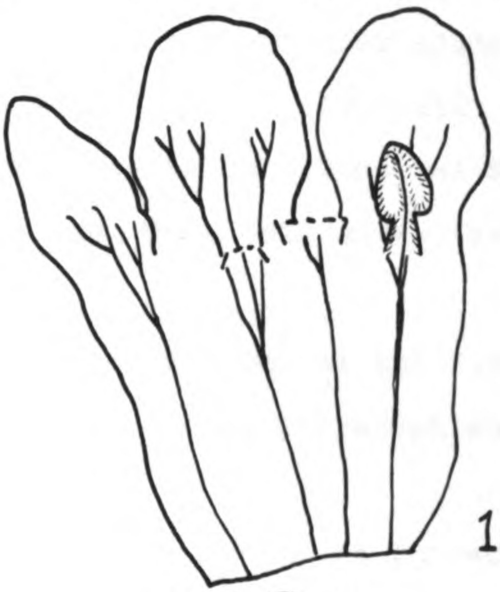
15



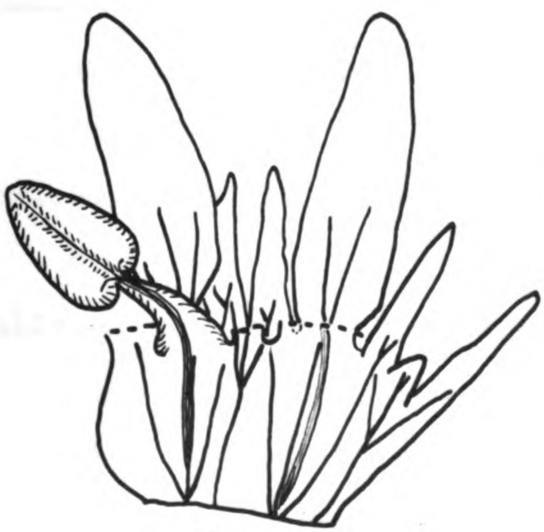
16



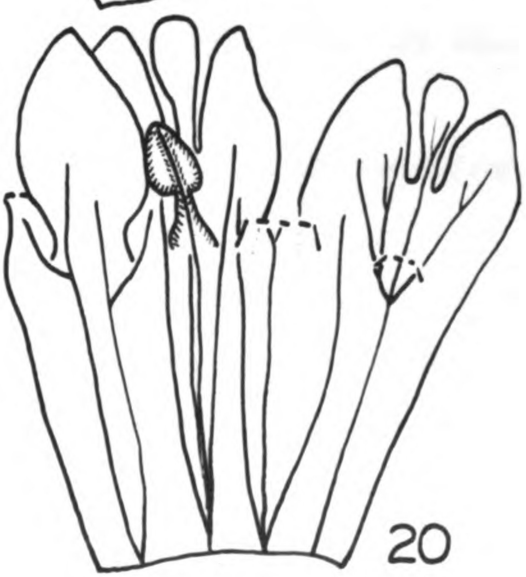
17



18



19



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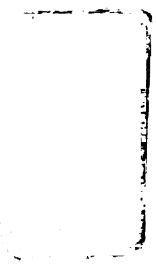

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