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A COMPARATIVE STUDY OF [illegible] [illegible] OF [illegible]
[illegible] OF THE FAMILY [illegible]

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[illegible]

A dissertation presented to the Graduate Faculty
of the University of Virginia in Candidacy
for the Degree of Doctor of Philosophy

Approved

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A COMPARATIVE STUDY OF WOOD ANATOMY OF FIFTY-FOUR SPECIES OF THE FAMILY AQUIFOLIACEAE

Maggie Thurman Pennington, B.S. M.S.
-1953-

A dissertation presented to the Graduate Faculty
of the University of Virginia in candidacy
for the degree of Doctor of Philosophy.

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A Comparative Study of Wood Anatomy of Fifty-four Species of the Family Aquifoliaceae.

Introduction

The Aquifoliaceae is a small family widely distributed over the world. It is composed of trees and shrubs, most species being evergreen. The family contains five genera: *Ilex*, the largest and most widely distributed genus of over three hundred species; *Phelline* a monotypic genus restricted to New Caledonia; *Byronia*, consisting of three species reported from the Hawaiian Islands and Tahiti; *Sphenostemon*, a monotypic genus also reported from New Caledonia; and *Nemopanthes*, a third monotypic genus common to northeastern United States.

Very little anatomical evidence of secondary growth of stems has hitherto been available to taxonomists to assist them in evaluating this family. Previous workers have confined work for the most part to the flowers, fruits, young stems, and leaves. The primary aim of the anatomist is to provide new and significant material for use in conjunction with traditional morphological data to establish relationships based upon a more complete knowledge of the plants than is afforded by a consideration of gross morphological features alone. His investigations may serve to strengthen some existing, taxonomic system or may suggest another, sounder system. A brief summary of the findings of workers studying anatomical characteristics is given here.

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The cuticle of the young stem is very thick. It is smooth in some species (e.g., I. *glabra* (L.) Gray). Cork arises in the epidermis or hypodermis and the cork cells are usually thick-walled. Xylem is commonly traversed by broad rays. Vessels have scalariform perforation plates. Pericycle contains isolated strands of fibers.

Solereder ('08) described the vessels of secondary woods as having scalariform perforations, and the parenchyma with bordered pits. Kanehira ('12) gave the upper limits of the number of vessels as 120 per mm. in tangential arrangement for Ilex. Loesener, according to Solereder ('08) states that the rays are exclusively uniseriate in Ilex verticillata. Growth rings in Ilex were described in 1927 by Coster. Wood is considered ring-porous by Metcalf and Chalk in The Anatomy of Dicotyledons ('50). These contributions by no means give a complete summary of our knowledge of the secondary xylem.

It is the aim of this study to present a more complete analysis of secondary woods of fifty-four species of this family. These species, including representatives of all genera, are listed in Tables I, III, and V on pages 8-9, 15-16, 20-21.

Materials and Methods

Materials for this study consisted of portions of stems evidencing secondary growth. These were obtained in part by collections of the writer, but to a greater extent through the courtesy of the Florida Agriculture Experimental Station, the Chicago Museum of Natural History, the Yale School of Forestry, the Blandy Experimental Farm of the University of Virginia, and Dr. W. J. Phillips of Charlottesville, who gave freely of his private collection. Those specimens received from the Chicago Museum of Natural History and the Yale School of Forestry were herbarium specimens, while all others were fresh.

Both macerations and sections were made of each of the fifty-four species studied. Macerations were made by using a modification of Jeffrey's Method as reported by Titman ('38) in a similar study of the Nyssaceae. Macerated wood elements were stored in vials of glycerin-alcohol-water which facilitated extraction of small quantities for observation at any time. Some elements of each species were also mounted in balsam for permanent record.

Fresh material was fixed in formal-acetic-alcohol. Woods were boiled prior to sectioning. Transverse, longitudinal and tangential sections were made using a sliding microtome. Sections were cut at ten to twenty microns. All sections were stained with iron-haematoxylin and counterstained with safranin according to the schedule in Johannsen's Microtechnique. One modification was necessary to obtain the desired stain. This involved a reduction of the haematoxylin time by one half and, of the safranin time from two hours to thirty minutes. All sections were cleared in oil of

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Numerous investigators have established basic criteria for the comparative study of secondary xylem. Among these, the conclusions of Frost ('30) ('31), Kribs ('35), and Gilbert ('40) are of particular significance. Frost described in detail the phylogenetic development of the vessel elements in the secondary wood of the dicotyledons. His conclusions, now classed among the important contributions to wood anatomical research, are briefly summarized here:

1. Primitive vessel segments are characterized by:
    - (a) great average length;
    - (b) small cross-sectional diameter;
    - (c) angularity of outline in cross-section;
    - (d) evenly thickened walls;
    - (e) thin walls;
    - (f) absence of a distinct end wall;
    - (g) end wall perforations scalariform with many bars and small apertures;
    - (h) lateral wall pits scalariform and fully bordered;
    - (i) absence of tertiary thickening of the vessel walls.
2. Phylogenetic development of the vessel segments is manifest in:
    - (a) reduction in average length;
    - (b) increase in cross-sectional diameter;
    - (c) uneven thickening of walls;
    - (d) acquisition of thick walls;
    - (e) change in end walls from a highly inclined position to a transverse position;
    - (f) change from angular to rounded cross-sectional outline;
    - (g) change from scalariform to porous end wall perforations by loss of bars and widening of apertures;
    - (h) gradual loss of borders of lateral wall pits;
    - (i) introduction of tertiary thickenings in vessel elements of secondary wood.
    - (j) specialization of scalariform lateral wall pits to produce first, transitional elongate-elliptic opposite pits, second, round opposite pits, third, highly specialized alternate round intervascular pits.

Using the series of vessel characteristics established by Frost as phylogenetic indicators, Kribs ('35) defined six types of medullary rays which are believed to exhibit various degrees of specialization. His conclusions may be summarized as follows. The most primitive ray type consists of a combination of multiseriate rays with high uniseriate 'wings', and numerous and high uniseriate rays composed of high upright cells. Specialization takes the form of reduction either of the uniseriate rays, leading to a type with multiseriate rays only, or of the multiseriate rays, leading to types with wholly uniseriate rays. Parallel with the reduction of the uniseriate or of the multiseriate rays, individual rays become less heterogeneous and the component cells smaller.

Gilbert ('40) concluded that the evolutionary development of secondary wood proceeds from a primitive diffuse-porous arrangement of vessel elemknts to a ring-porous condition. In the primitive condition the annual rings may be scarcely recognized, but are clearly distinguishable in the advanced condition due to the alternate arrangement of vessel areas with fiber areas.

The conclusions of Frost, Kribs and Gilbert are used as criteria in the comparative study of the secondary woods of the Aquifoliaceae.

The following is a summary of the characteristics measured and observed for each xylem element:

(a) length of element;
(b) diameter of element;
(c) wall thickness of element;
(d) angularity of vessel end walls;
(e) cross-sectional outline of vessel;
(f) presence of tertiary thickenings;
(g) end wall perforations;
(h) side wall pits;

(i) even or uneven side wall thickening;
(j) cellular inclusions.

Observations were made on xylem vessels, tracheids, fibers and parenchyma. One hundred tabulations of all observations and measurements listed above were recorded for each of the elements in all fifty-four species studied. This involved approximately 4000 readings for each species, or a total of 216,000 readings. The 216,000 individual readings are not included in this report but only means for each species are shown. These means are shown in Tables I, III, V on pages 8-9, 15-16, 20-21.

A careful weighing of evidence gained from investigations of wood anatomy in accordance with the preceding criteria should establish the level of phylogenetic development attained by any given group with respect to wood characters.

Discussion and Results

Previous workers dealing with a comparative problem of wood anatomy have restricted their efforts to the study of vessel members with little, if any, reference to other xylem elements, such as tracheids, fibers and parenchyma. This study has included all these elements and has for the most part treated each xylem member with the same interest. In this discussion, the xylem elements will be considered separately in the following order of phylogenetic importance: vessels, tracheids, fibers and parenchyma.

The most useful character in the study of vessels is generally agreed to be mean vessel length. Mean vessel diameter, types of perforations, intervascular pitting, occurrence of spiral thickenings, cross-sectional outline, and angularity of end walls are also helpful. Chalk and Chattaway ('34) showed that the overall vessel member length, including vessel 'tails', most closely approximates the length of the vessel element's cambial initial, and therefore, is a more significant measurement than length of the vessel 'body' alone. Using this method of measurement the mean vessel lengths shown in Table I, pages 8-9, were computed. The distribution of mean vessel lengths throughout the Dicotyledons has been established and shows that vessel members whose mean lengths are less than four hundred microns are designated as 'short', and those over eight hundred microns as 'long'.

As shown in Table I, only one species studied has a mean length of less than 400 microns, *Sphenostemon* sp., with a mean length of 381.90 microns. In the genus *Ilex*, there is a wide range from *Ilex cassine* with a mean length of 623.90 microns to *Ilex Berteroi*, showing a mean length of 1222.82 microns. Of the forty-nine

TABLE I

CHARACTERISTICS OF VESSELS

Species	Length in microns	Diameter in microns	Wall thickness in microns	Angle of end wall in degrees	Cross-section outline	Tertiary thickenings	SW* Pits	EW Perf.**
Byronia sandwicensis Endl.	491.31	61.32	2.49	10	Angular	Present	S*****	33
" taitensis Gray	627.60	35.41	3.13	6	"	"	S	34
Nemopanthes mucronata Trel.	631.49	37.28	2.86	15	"	"	S	39
Phelline lucida Vieill.	764.27	47.68	2.31	14	"	"	SB***	47
Sphenostemon sp.	381.50	78.85	4.98	83	Oval	"	SB	0
Ilex amplifolia Rusby	947.21	34.27	1.66	18	Angular	"	SB	40
" amygdalifolia Rusby	983.81	53.08	1.71	11	"	"	SB	59
" aquifolium L.	1007.27	51.54	1.81	18.5	"	"	SB	37
" Berteroi Loes.	1222.52	41.97	1.68	14	"	"	SB	21
" bogorensis Loes.	1098.45	48.35	1.49	31	"	"	SB	49
" caliana Cuatr.	999.31	41.41	1.63	16.5	"	"	SB	30
" casiquiarensis Loes.	1038.75	77.19	2.47	14	"	"	SB	70
" cassine L.	628.90	28.22	1.52	11	"	"	SB	59
" Congonha Mart.	698.51	39.16	1.24	17.5	"	"	SB	20
" crenata Thunb.	731.76	41.47	1.66	21	"	"	SB	37
" cymosa Bl.	938.54	40.71	1.29	18.5	"	Absent	SB	41
" decidua Walt.	1001.35	42.68	1.73	12	"	Present	S	38
" dubia Britt.	999.37	44.71	1.39	11.25	"	"	SI****	57
" ficoidea Hemsl.	1003.79	41.39	1.46	10	"	"	S	61
" formosana Maxim.	908.49	47.63	1.67	17.5	"	"	SI	45
" glabra Gray	839.47	43.27	1.93	12.75	"	"	SB	56
" integra Thunb.	967.39	50.14	1.33	15	"	"	SI	81
" Hanceana Maxim.	1031.41	59.29	1.67	13.5	"	"	SB	63
" Jenmanii Loes.	985.63	37.21	1.21	10.5	"	"	S	48
" krugiana Loes.	961.37	29.47	1.73	14	"	"	SB	39
" latifolia Thunb.	1047.03	40.76	1.65	12	"	"	SB	26
" liukiuensis Loes.	1117.31	39.41	1.79	18.5	"	"	SB	35
" lucida Terr. & Gray	994.56	41.61	1.23	15	"	"	SB	53
" Macfadenii Rehd.	1005.73	42.64	1.89	21	"	"	SB	61
" macropoda Miq.	999.31	51.43	2.01	27	"	"	S	73
" macrococca Max.	978.24	34.00	1.98	14.5	"	"	SB	38

TABLE I (continued)

CHARACTERISTICS OF VESSELS

Species	Length in microns	Diameter in microns	Wall thickness in microns	Angle of end wall in degrees	Cross-section outline	Tertiary thickenings	SW Pits	EW Perf.
Ilex mitis Radlk.	871.32	31.06	1.87	21.5	Angular	Present	SB	47
" montana T. & G.	974.61	57.21	1.81	29.25	"	"	SB	41
" myricoides Benth.	897.65	46.59	1.93	32	"	"	SB	39
" myrtifolia Lam.	979.30	39.80	2.07	19.75	"	"	SB	53
" nayana Cuatr.	961.41	47.59	1.99	23	"	"	SB	49
" opaca Ait.	697.60	29.77	1.66	15	"	"	SB	21
" othera Spreng.	841.43	34.28	1.79	17.5	"	Absent	SI	35
" panamensis Standl.	810.60	37.03	2.01	21	"	Present	SB	40
" paraguariensis A. St. Hil.	996.32	45.21	1.91	19	"	"	SB	37
" parviflora Benth.	987.56	36.73	1.86	21.5	"	"	SB	31
" pendulosa	794.40	24.78	1.69	30	"	"	SB	47
" purpurea Hassk.	1001.47	29.70	1.59	10.5	"	"	SB	29
" repanda Gris.	741.27	27.02	1.67	12	"	"	SB	40
" Rimbrachii Standl.	797.30	28.33	1.87	17	"	"	SB	29
" rotunda Thunb.	801.02	23.44	1.59	14.5	"	"	SI	31
" sandwicensis Loes.	785.09	27.05	1.92	19.5	"	"	SB	29
" Serbertii Panch.	760.20	22.30	1.73	20.5	"	"	S	46
" serrata Thunb.	809.99	28.22	1.96	15	"	"	SB	38
" subrugosa Loes.	799.39	26.68	1.79	17.25	"	"	SB	47
" uniflora Benth.	832.98	32.72	1.69	13	"	"	SB	39
" verticillata (L.)Gray	901.02	26.06	1.88	12	"	"	SB	28
" vitiensis A. Gray	756.12	21.05	1.62	15.25	"	"	SB	46
" vomitoria Ait.	751.16	35.22	1.59	11.5	"	"	SB	51

* ---SW means side wall

**---EW Perf. means end wall perforations

***--SB means bordered

****-SI means simple

*****S means scalariform

species of the genus Ilex included it is seen that thirty-six species have a mean length over eight hundred microns; nine species have a mean length over seven hundred microns; and four species a mean length over six hundred microns. In the genus Byronia, B. sandwicensis is found to have a mean length of 491.31 microns while B. taitensis is 627.80 microns. The genus Nemopanthes with a single species N. mucronata appears to be an intermediate form with a mean length of 631.49 microns. Finally, Phelline lucida is also in the intermediate position with a mean length of 764.27 microns.

In summary, we see that one species, the only representative of the genus Sphenostemon, conforms to the definition of 'short', while the majority of the species of Ilex would be considered 'long' with fourteen species of Ilex, two species of Byronia, the genera Nemopanthes and Phelline being intermediates.

Mean vessel diameters have also been computed for the Dicotyledons. Vessels are described as 'small' if their diameters are less than one hundred microns and as 'long' if their diameters are more than two hundred microns. Table I shows that mean vessel diameters of all the species included are small. The largest mean diameter recorded is 78.85 microns in Sphenostemon sp. and the smallest, 21.05 microns in Ilex vitiensis. Here again there is a wide range as seen in Table II, Page 11.

TABLE II

Distribution of Mean Vessel Diameters

Range in microns	Frequency
69.50 - 79.49	2
59.50 - 69.49	1
49.50 - 59.49	6
39.50 - 49.49	18
29.50 - 39.49	15
19.50 - 29.49	12

Mean vessel wall thickness is also shown in Table I though not generally considered an important character. Attention is drawn again to Sphenostemon sp. with a mean thickness of 4.98 microns. The smallest mean thickness is found in Ilex Jenmanii which is 1.21 microns. It is of interest to note that the genus Ilex is relatively consistent in respect to wall thickness, with only four of the forty-nine species having a thickness greater than two microns. Also it is observed that the genera Bryonia, Nemopanthes, and Phelline are all above two microns with B. taitensis having a mean of 3.13 microns. While no standard has been established for the mean vessel wall thickness, it is of interest to see the uniformity displayed in the genus Ilex and also, the difference found in Sphenostemon, which has been found to differ so vastly from all other genera as regards mean vessel length and mean vessel diameter.

Angularity of end walls of vessels appears relatively consistent in the genera Ilex, Byronia, Phelline, and Nemopanthes, while the genus Sphenostemon has an extremely large angle of 83 degrees.

Column six of Table I shows the appearance of tertiary thickenings of vessel walls. There has been considerable controversy concerning a clear definition of 'tertiary thickenings'. Metcalf and Chalk ('50) in the Anatomy of the Dicotyledons define a tertiary thickening as the spiral and annular thickenings so characteristic of vessel walls. Using this definition, it is found that all species studied with the exception of Ilex othera and Ilex cymosa possess tertiary thickenings. These exceptions show no evidence of any thickening in the vessel walls.

Still another characteristic in which great similarity is found is the outline of vessels in cross-section. All species studied are angular in vessel cross-section except *Sphenostemon* sp. which appears round.

Side wall pits in the genera *Bryonia* and *Nemopanthes* as well as five species of *Ilex* are scalariform. *Ilex dubia*, I. *formosana*, I. *integra*, I. *othera*, and I. *rotunda* have simple unbordered side wall pits. The remainder of the species of *Ilex* recorded, as well as the genera *Phelline* and *Sphenostemon* possess simple pits with borders ranging from the fully bordered condition to a vestigial condition.

End walls of all genera except *Sphenostemon* are scalariform with the number of bars ranging from eighty-one in *Ilex integra* to twenty in *Ilex Congonha*, and the size of the apertures varies proportionally to the number of bars (e.g. the greater the number of bars, the smaller the size of the apertures; the smaller the number of bars, the greater the size of the apertures). *Sphenostemon* shows no evidence of bars in the end wall but the presence of a single large aperture is a striking feature. In every vessel of *Sphenostemon* observed the apertures appear clogged with a material of undetermined nature.

It is generally believed that the ancestral tracheal element was the tracheid, a long cell with bordered pitting, and that vessels have been derived from the tracheids by the partial disappearance of the pit-membrane from some of the pitting in the overlapping walls, thus forming a vertical series of cells that gives an uninterrupted passage for water. This view was strongly opposed at first but has been supported in more recent studies. Bailey and Tupper ('18) returned to this problem and published a paper on size variation of

tracheary elements. The main conclusions reached by Bailey and Tupper can be stated very simply - that the length of the tracheal element and the cambial initial is reduced as the specialisation increases. Tracheids were observed in three species studied. These were *Ilex cassine*, I. *subrugosa*, and I. *Macfeydenii* with the mean lengths of 702.44, 980,30, and 992.43 microns respectively. The mean diameters are 7.30, 19.01 and 11.12 microns respectively. These tracheids were found rather infrequently and all displayed simple pits with well developed borders.

As is stated in a footnote on Table III, pages 15-16, it is usual to distinguish between 'libriform fibers' with simple pits, and 'fiber-tracheids' with bordered pits, but no exact definition of these two types appears to be universally acceptable. Intermediates are so numerous that it is found more satisfactory to group all types under the heading 'Fibers'. The most important characters of fibers are the nature of the pitting and the presence of septa. Of particular interest are instances where septate fibers are grouped together in a manner suggestive of parenchyma, as in many of the species of the Celastraceae. Fiber length, though little used for diagnostic purposes, is of some practical interest and is included in this work. The distribution of mean fiber length in the Dicotyledons has been established as follows: Fibers whose mean length is less than 900 microns are described as 'short', and those over 1600 microns as 'long'. As is shown in Table III six species studied are short, and all others are intermediate in length, the largest mean length being 1401.23 microns in *Ilex aquifolium*. Those species which may be considered 'short' are : *Bryonia sandwicensis*; *Sphemostemon* sp.; *Ilex cassine*; I *Congonha*; I. *glabra*; and I. *crenata*.

TABLE III

Characteristics of Fibers *

Species	Length in microns	Diameter in microns	Wall thickness in microns	Cross-sectional outline	Pits
Byronia sandwicensis Endl.**	731.50	12.36	4.98	Oval	Bordered
" taitensis Gray	973.79	12.07	4.43	"	"
Nemopanthes mucronata Trel.	937.50	11.31	4.01	"	"
Phelline lucida Vieill.	993.20	16.80	3.49	"	"
Sphenostemon sp.	712.34	18.34	6.64	"	"
Ilex amplifolia Rusby	1117.31	15.92	3.47	"	"
" amygdalifolia Rusby	1009.37	13.02	4.08	"	"
" aquifolium L.	1401.23	28.99	6.92	"	"
" Berteroi Loes.	1295.92	20.59	5.77	"	"
" bogorensis Loes.	1103.59	19.37	6.02	"	"
" caliana Cuatr.	1107.99	17.20	4.66	"	"
" casiquierensis Loes.	1052.03	11.87	4.06	"	"
" cassine L.	789.31	12.51	2.31	"	"
" Congonha Mart.	737.29	11.02	3.88	"	"
" crenata Thunb.	848.71	10.37	2.45	"	"
" cymosa Bl.**	998.20	14.07	4.48	"	"
" decidua Walt.	1032.04	12.05	3.67	"	"
" dubia Britt.	1004.04	11.72	2.47	"	Simple
" ficoides Hemsl.	1009.99	15.02	3.47	"	Bordered
" formosana Maxim.	1005.74	10.89	3.76	"	"
" glabra Gray	888.02	11.57	4.01	"	"
" integra Thunb.	987.63	13.52	3.89	"	"
" Hanceana Maxim.	1052.06	10.86	3.08	"	"
" Jenmanii Loes.	992.22	12.39	4.09	"	Simple
" krugiana Loes.	981.31	13.99	4.23	"	Bordered
" latifolia Thunb.	1086.01	18.00	3.86	"	"
" liukiuensis Loes.	1187.61	23.66	5.39	"	"
" lucida Torr. & Gray	999.81	17.07	3.88	"	"
" Macfedenii Rehd.	1021.03	16.22	7.01	"	"
" macropoda Miq.	1003.72	19.33	4.93	"	"
" macrococca Max.	999.02	12.86	4.48	"	"

TABLE III(continued)

Characteristics of Fibers

Species	Length in microns	Diameter in microns	Wall thickness in microns	Cross-sectional outline	Pits
Ilex mitis Radlk	932.56	12.40	3.99	Oval	Bordered
" montana T. & G.	999.90	17.33	4.65	"	"
" myricoides Benth.	1021.40	16.56	4.79	"	"
" myrtifolia Lam.	1037.29	16.52	4.39	"	"
" nayana Cuatr.	978.21	18.02	4.21	"	"
" opaca Ait.	1073.34	26.21	5.49	"	"
" othera Spreng.	1012.46	28.23	4.95	"	"
" panamensis Standl.	1062.22	24.48	4.51	"	"
" paraguariensis A. St. Hil.	1001.07	27.31	5.50	"	Simple
" parviflora Benth.	999.01	23.23	4.62	"	Bordered
" pendulosa	1031.00	21.13	3.72	"	"
" purpurea Hassk.	1137.00	26.03	5.23	"	"
" repanda Gris.	978.88	22.31	4.48	"	"
" Simbrachii Standl.	993.30	26.42	5.12	"	"
" rotunda Thunb.	906.20	23.02	4.79	"	Simple
" sandwicensis Loes.	909.34	19.79	4.46	"	Bordered
" Serbertii Panch.	956.31	14.68	5.50	"	"
" serrata Thunb.	973.01	25.66	3.65	"	"
" subrugosa Loes.	1008.32	29.44	7.82	"	"
" uniflora Benth.	999.54	19.21	6.12	"	Simple
" verticillata (L.) Gray	1102.45	17.88	4.76	"	Bordered
" vitiensis A. Gray	902.21	21.45	7.46	"	"
" vomitoria Ait.	1021.50	17.31	5.19	"	"

* It is usual to distinguish between 'libriform fibers' with simple pits, and 'fiber-tracheids' with bordered pits, but no exact definition of these two types appear universally acceptable. Intermediates are so numerous that it was found more satisfactory to group all types under the heading 'Fibers'.

** Two species showed evidence of septa. These are considered in detail on page 17.

The frequencies of distribution of species having intermediate mean fiber length are shown in Table IV, page 18.

Relative consistency is observed in the mean fiber diameters as well as in the mean thickness of fiber walls. All fibers appear oval in cross-sectional outline. Side wall pitting varies from simple unbordered pits through vestigial borders of pits to fully bordered ones. Five species of *Ilex* have simple pits with no evidence of borders. In the remaining species of *Ilex* as well as the genera *Byronia*, *Nemopanthes*, *Phelline* and *Sphenostemon* the pits possess either vestigial or full borders. Septate fibers are rare, but are observed in *Ilex cymosa* and *Byronia sandwicensis* and show no evidence of being grouped together as was previously described for certain members of the Celastraceae.

Characteristics of xylem parenchyma have not been used as diagnostic features in wood anatomy but are included here in order to provide a more complete summary of all tissues of the xylem. (See Table V, pages 20-21). Parenchyma is apotracheal and diffuse and varies as regards occurrence or distribution. In the genera *Byronia* parenchyma is very abundant while, in *Sphenostemon* its rare appearance is a striking feature. Parenchyma observed in the genera *Phelline*, *Nemopanthes*, and *Ilex* appears in moderate amounts.

Pitting in parenchyma cells of some species gives the appearance of being bordered (see Eames and MacDaniels 1947, p.39). Such pits are listed here as semi-bordered. This type of pit occurs in four species of *Ilex* and the genus *Phelline*. The genera *Byronia*, *Nemopanthes* and *Sphenostemon* possess simple pits.

Cellular inclusions found in parenchyma are clusters of crystals

TABLE IV

Distribution of Intermediate Mean Fiber Lengths

Range in Microns	Frequency
1399.50 - 1499.49	1
1299.50 - 1399.49	0
1199.50 - 1299.49	1
1099.50 - 1199.49	5
999.50 - 1099.49	23
899.50 - 999.49	18

and are observed in the genera *Bryonia* and *Nemopanthes* as well as in five species of *Ilex*. These inclusions are described as rare if they occur in only a few of the parenchymatous cells and, as abundant if they occur in a large number of cells. Size of these crystals varies from tiny solitary particles to very complex clusters or druses which at times seem to almost fill the entire intracellular space.

As is stated on page 8, the classification of Kribs has been followed in regards to types of rays present in the members of the Aquifoliaceae studied. These rays are shown in Plate I,II.Only three of the six types described by Kribs are present in the fifty-four species under consideration. These three are Heterogeneous Type I, Heterogeneous Type IIA, and Heterogeneous Type IIB. No representatives possess rays belonging to the types Homogeneous I, II, or III. The genus *Bryonia* contains both uniseriate and multiseriate rays belonging to the class Heterogeneous Type IIA. *Phelline* is also found to exhibit the same ray type. *Nemopanthes*, in contrast, possesses rays, both uniseriate and multiseriate, which may be described as Heterogeneous Type IIB. Multiseriate rays are quite striking in the genus *Sphenostemon* and belong to the Heterogeneous Type IIB also. No uniseriate portions are observed in *Sphenostemon*. Ray type in the genus *Ilex* is distributed among the three types, Heterogeneous Type I, Heterogeneous Type IIA, and Heterogeneous Type IIB in the ratios of 41:6:2 respectively. These species of *Ilex* which are Heterogeneous Type I have both uniseriate and multiseriate portions with the exception of *Ilex amplifolia* and *I. verticellata*, both of which exhibit only uniseriate rays. The six species belonging to the Heterogeneous Type IIA group have both and are observed in the genera *Byronia* and *Nemopanthus* as well as in five species of *Ilex*. These inclusions are described as rare if they occur in only a few of the parenchymatous cells and, as abundant if they occur in a large number of cells. Size of these crystals varies from tiny solitary particles to very complex clusters or druses which at times seem to almost fill the entire intercellular space.

As is stated on page 5, the classification of Kribs has been followed in regards to types of rays present in the members of the Aquifoliaceae studied. These rays are shown in Plate I,II.Only three of the six types described by Kribs are present in the fifty-four species under consideration. These three are Heterogeneous Type I, Heterogeneous Type IIA, and Heterogeneous Type IIB. No representatives possess rays belonging to the types Homogeneous I, II, or III. The genus *Byronia* contains both uniseriate and multiseriate rays belonging to the Kribs Heterogeneous Type IIA. *Phelline* is also found to exhibit the same ray type. *Nemopanthus*, in contrast, possesses rays, both uniseriate and multiseriate, which may be described as Heterogeneous Type IIB. Multiseriate rays are quite striking in the genus *Sphenostemon* and belong to the Heterogeneous Type IIB also. No uniseriate portions are observed in *Sphenostemon*. Ray type in the genus *Ilex* is distributed among the three types, Heterogeneous Type I, Heterogeneous Type IIA, and Heterogeneous Type IIB in the ratios of 41:8:2 respectively. Those species of *Ilex* which are Heterogeneous Type I have both uniseriate and multiseriate portions with the exception of *Ilex amplifolia* and *I. verticillata*, both of which exhibit only uniseriate rays. The six species belonging to the Heterogeneous Type IIB group have both

TABLE V

CHARACTERISTICS OF PARENCHYMA

Species	Pits	Occurrence	Inclusions
Byronia sandwicensis Endl.	Simple	Abundant	Druses rare
" taitensis Gray	"	"	Druses abundant
Nemopanthes mucronata Trel.	"	Moderate	" "
Phelline lucida Vieill.	Semi-bordered	"	None
Sphenostemon sp.	Simple	Rare	"
Ilex amplifolia Rusby	"	Moderate	Druses rare
" amygdalifolia Rusby	"	"	" "
" aquifolium L.	"	"	None
" Berteroi Loes.	"	"	"
" borgorensis Loes.	"	"	"
" caliana Cuatr.	"	"	"
" casiquiarensis Loes.	Semi-bordered	"	"
" cassine L.	"	"	"
" Congonha Mart.	"	"	"
" crenata Thunb.	"	"	"
" cymosa Bl.	"	"	Druses abundant
" decidua Walt.	"	"	None
" dubia Britt.	"	"	"
" ficoidea Hemsl.	"	"	"
" formosana Maxim.	"	"	"
" glabra Gray	Semi-bordered	"	"
" integra Thunb.	Simple	"	"
" Hanceana Maxim.	Semi-bordered	"	"
" Jenmanii Loes.	Simple	"	"
" krugiana Loes.	Semi-bordered	"	Druses rare
" latifolia Thunb.	Simple	"	" "
" liukiuensis Loes.	"	"	None
" lucida T. & G.	"	"	"
" Macfeydenii Rehd.	"	"	"
" macropoda Miq.	"	"	"
" macrococca Max.	"	"	"

TABLE V (continued)

CHARACTERISTICS OF PARENCHYMA

Species	Pits	Occurrence	Inclusions
Ilex mitis Radlk.	Simple	Moderate	None
" montana T. & G.	"	"	"
" myricoides Benth.	"	"	"
" myrtifolia Lam.	"	"	"
" nayana Cuatr.	"	"	"
" opaca Ait.	"	"	"
" othera Spreng.	"	"	"
" panamensis Standl.	"	"	"
" paraguariensis A. St. Hil.	"	"	"
" parviflora Benth.	"	"	"
" pendulosa	"	"	"
" purpurea Hassk.	"	"	"
" repanda Gris.	"	"	"
" Rimbrachii Standl.	"	"	"
" rotunda Thunb.	"	"	"
" sandwicensis Loes.	"	"	"
" Serbertii Panch.	"	"	"
" serrata Thunb.	"	"	"
" subrugosa Loes.	"	"	"
" uniflora Benth.	"	"	"
" verticillata (L.) Gray	"	"	"
" vitiensis Gray	"	"	"
" vomitoria Ait.	"	"	"

uniseriate and multiseriate portions, while the two Heterogeneous Type IIB species, *Ilex cassine* and I. *Congonha* have only multiseriate portions.

As has been stated previously, the mean vessel length has been established as the most important phylogenetic character in a comparative study of secondary woods. In order to interpret the true value of mean vessel lengths it is desirable to determine the reliability of differences between means of any two species. By doing so, the worker may see whether the species involved are truly different or whether the difference is accidental. Table VI, page 23, shows the reliabilities of differences between mean measurements of vessel lengths of ten pairs of species of the Aquifoliaceae. The data included in Table VI was determined according to standard statistical methods.

As is seen in Table VI, six pairs of species of *Ilex* were tested for reliable mean differences. After these were found to be different each member of these pairs was tested with *Sphenostemon* sp. which has been seen to appear vastly different not only in mean vessel length, but also in many other characters.

The smallest critical ratio found in these calculations was 3.40 for *Ilex vomitoria* and I. *opaca*. The largest, 57.70 for I. *pendulosa* and *Sphenostemon* sp.

According to statistical criteria any critical ratio of 2.33 or more shows that the chances are 99:1 that the differences between given groups of data are real and are not accidental.

While all differences shown in Table VI are reliable, further calculations of critical ratios must be made for the remainder of the species not included here before definite conclusions may be drawn concerning the entire family. This work is planned for a future study.

uniseriate and multiseriate portions, while the two heterogeneous Type III species, Ilex cassine and I. coriacea have only multiseriate portions.

As has been stated previously, the mean vessel length has been established as the most important phylogenetic character in a comparative study of secondary woods. In order to interpret the true value of mean vessel lengths it is necessary to determine the reliability of differences between means of any two species. By doing so, the worker may see whether the species involved are truly different or whether the difference is accidental. Table VI, page 53, shows the reliabilities of differences between mean measurements of vessel lengths of ten pairs of species of the Aquifoliaceae. The data included in Table VI was determined according to standard statistical methods.

As is seen in Table VI, six pairs of species of Ilex were tested for reliable mean differences. After these were found to be different each member of these pairs was tested with Nemopanthus sp. which has been seen to appear vastly different not only in mean vessel length, but also in many other characters.

The smallest critical ratio found in these calculations was 3.40 for Ilex vomitoria and I. opaca. The largest, 63.70 for I. pedunculosa vs Nemopanthus sp.

According to statistical criteria any critical ratio of 2.58 or more shows that the chances are 99:1 that the differences between given groups of data are real and are not accidental.

While all differences shown in Table VI are reliable, further calculations of critical ratios must be made for the remainder of the species not included here before definite conclusions may be drawn concerning the entire family. This work is planned for a future study.

TABLE VI

RELIABILITIES OF THE DIFFERENCES BETWEEN MEAN MEASUREMENTS OF VESSEL LENGTHS OF TEN PAIRS OF SPECIES.

Species	Mean Length	Differences of means	Standard Deviation	Critical Ratios
Ilex pendulosa	794.40	96.90	85.84	6.13
" opaca	697.50		206.50	
Ilex pendulosa	794.40	170.50	85.84	18.61
" cassine	623.90		114.36	
Ilex vomitoria	751.16	53.66	102.96	3.40
" opaca	697.50		206.50	
Ilex cassine	623.90	127.26	114.36	14.50
" vomitoria	751.16		102.96	
Ilex pendulosa	794.40	43.24	85.84	4.58
" vomitoria	751.16		102.96	
Ilex opaca	697.50	73.60	206.50	4.23
" cassine	623.90		114.36	
Ilex opaca	697.50	316.00	206.50	20.90
Sphenostemon sp.	381.50		53.18	
Ilex cassine	623.90	232.40	114.36	25.10
Sphenostemon sp.	381.50		53.18	
Ilex pendulosa	794.40	412.90	85.84	57.70
Sphenostemon sp.	381.50		53.18	
Ilex vomitoria	751.16	369.66	102.96	45.30
Sphenostemon sp.	381.50		53.18	

SUMMARY

1. The Aquifoliaceae does not exhibit a gradual transition from most primitive to most advanced conditions in each of the phylogenetic lines established by Frost and Kribs.

2. The genus *Sphenostemon* differs greatly from the other genera of the Aquifoliaceae as regards vessels except in regard to the presence of tertiary thickenings and side wall pitting, thus satisfying the major criteria established by Frost for phylogenetically advanced plants. It possesses only multiseriate rays of the Heterogeneous Type IIB which is considered as 'advanced' according to Kribs. No outstanding differences are noticed between fibers and parenchyma of *Sphenostemon* and those of the other genera of this family. This genus appears to be the most advanced genus of the Aquifoliaceae.

3. The genera *Byronia*, *Nemopanthes* and *Phelline* are intermediate in vessel lengths and vessel diameters, but have rather thick vessel walls. No outstanding characteristics of fibers are apparent except the presence of septate fibers in *Byronia sandwicensis*. Parenchyma is abundant in *Byronia* and *Nemopanthes* and diffuse in *Phelline*. Crystals are found in *Byronia* and *Nemopanthes* and pitting of parenchyma is simple. No crystals occur in *Phelline* and pits are semi-bordered. Rays are Heterogeneous Type IIA in *Byronia* and *Phelline* and both uniseriate and multiseriate portions are present. Uniseriate and multiseriate rays of *NemoPanthes* are Heterogeneous Type IIB.

4a. The genus *Ilex* is a variable group as regards mean vessel length, but in all species considered mean vessel diameter

is small. Wall thickness is rather consistent. Tertiary thickenings are present in all species included except *Ilex cymosa* and I. *othera*. Consistency is observed in all other vessel characters.

4b. Six species of *Ilex* have 'short' fibers, and all others are 'intermediate'. *Ilex* is consistent in fiber wall thickness and in cross-sectional outline. Pits vary from simple unbordered to vestigial borders to fully bordered pits. Both 'libriform fibers' and 'fiber-tracheids' are thus observed.

4c. Parenchyma is apotracheal, and is moderate in occurrence. Parenchyma pits are simple in all but four species. Druses occur in parenchyma in five species.

4d. Tracheids are present in three species but are few in number.

4e. Rays belonging to Heterogeneous Type I, Heterogeneous Type IIA, and Heterogeneous Type IIB occur in *Ilex*. Thirty-nine of the forty-one species included have Heterogeneous Type I rays and are considered primitive according to Kribs' classification. The remaining two species, *Ilex amplifolia* and I. *vitiensis* as well as I. *cassine* and I. *congonha* are 'advanced'. The latter belongs to the Heterogeneous Type IIB group. Six species have Heterogeneous Type IIA rays and are thus 'primitive'.

is small. Wall thickness is rather consistent. Tertiary thickenings are present in all species except Ilex opaca and I. [illegible]. Consistency is observed in all other vessel characters.

4b. In species of Ilex have 'short' fibers, and all others are 'intermediate'. Ilex is consistent in fiber wall thickness and in cross-sectional outline. Pits vary from simply unbordered to vestigial borders to fully bordered pits. Both 'libriform fibers' and 'fiber-tracheids' are thus observed.

4c. Parenchyma is apotracheal, and is moderate in abundance. Parenchyma is [illegible] in all, but more regular. Crystals occur in parenchyma in five species.

4d. Tracheids are present in three species but are few in number.

4e. Rays belonging to Heterogeneous Type I, Heterogeneous Type IIA, and Heterogeneous Type IIB occur in Ilex. Thirty-nine of the forty-one species included have Heterogeneous Type I rays and are considered primitive according to Kribs' classification. The remaining two species, Ilex [illegible] and I. [illegible] as well as I. [illegible] and I. [illegible] are 'advanced'. The latter belongs to the Heterogeneous Type IIB group. All species have Heterogeneous Type IIA rays and are thus 'primitive'.

CONCLUSION

The family Aquifoliaceae is from the standpoint of wood anatomy a natural one and well defined. The only aberrant genus is Sphenostemon, which does not fit into the holly pattern in several respects, especially in the character of the vessels. In all characteristics it fits into the advanced category as defined by Frost.

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PLATE I

Plate I

Classification of Rays*

Figure 1. Heterogeneous Type I.

A. Uniseriate ray

Usually high, numerous and composed of very large vertically elongate cells unlike the cells of the multiseriate part of the multiseriate rays.

B. Multiseriate ray

Usually with parallel sides and with very large vertically elongated uniseriate wings composed of cells identical with those of the uniseriate rays. Cells of the multiseriate portion are oval, radially elongated or vertically elongated.

Figure 2. Heterogeneous Type IIA.

A. Uniseriate ray

Usually lower and composed of cells which are unlike those of the multiseriate part of the multiseriate rays. Composed of retangular, vertically elongated cells.

B. Multiseriate ray

Sides parallel or fusiform. Cellsof the multiseriate portion being round or oval, radially elongated, and with uniseriate tips of large vertically elongated cells; or with large, vertically elongated marginals, one cell high.

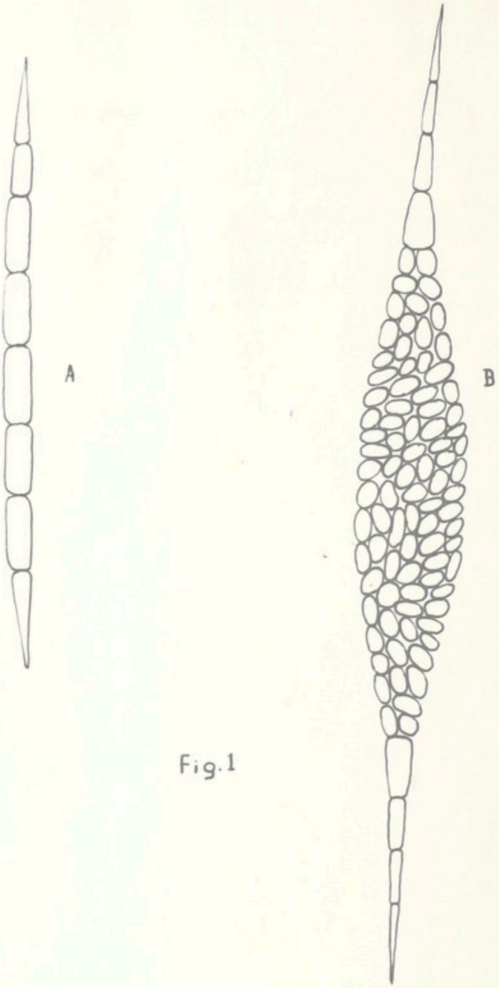

Fig. 1

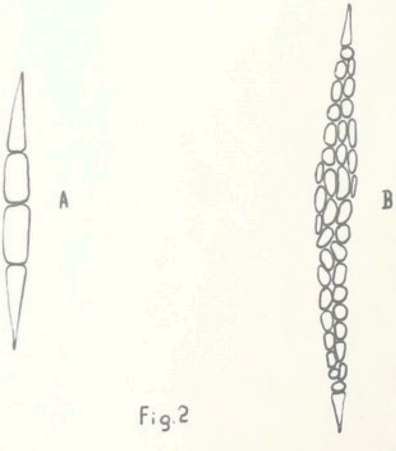

Fig. 2

Plate I

PLATE II

Plate II

Classification of Rays

Figure 3. Heterogeneous Type IIB

A. and B. Uniseriate ray

Two types of uniseriate rays; some composed of rectangular vertically elongated cells, some of cells nearly identical with those of the multiseriate portion of the multiseriate rays.

C. Multiseriate ray

Sides parallel to fusiform. Cells round to oval, radially elongated. Uniseriate tips, if long, are composed of mostly square cells.

Figure 4. Homogeneous Type I

A. Uniseriate ray

Rather low, numerous to scarce. Composed of cells identical with those of the multiseriate rays.

B. Multiseriate ray

Mostly fusiform, cells of the multiseriate portion round, radially elongated. Short to long uniseriate tips composed of cells identical with those of the multiseriate portion.

Figure 5. Homogeneous Type II

A. Uniseriate ray

Usually scarce to absent. When present low and composed of cells identical with those of the multiseriate portion.

B. Multiseriate ray

Fusiform. Composed entirely of small round, radially elongated cells. Uniseriate tips absent or extremely short.

* This classification of medullary raus is according to Kribs.

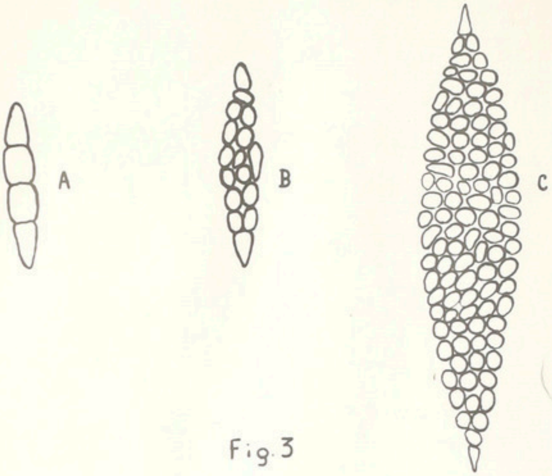

Fig. 3

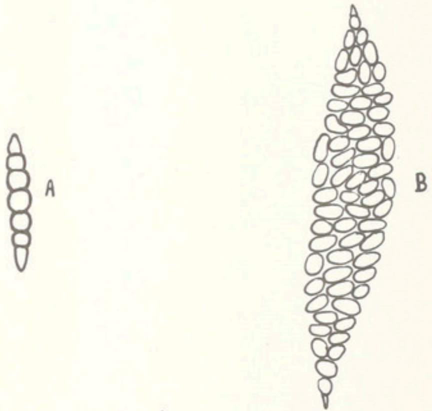

Fig. 4

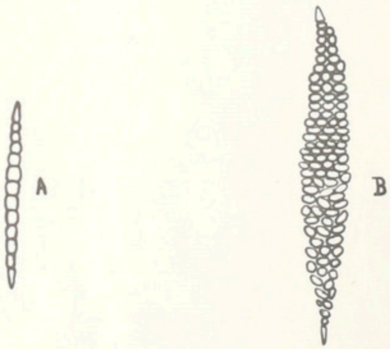

Fig. 5

Plate II