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A Biosystematic Study of the Fern Genus LYGODIUM in Eastern North America

Violet M. Brown
University of Central Florida

A BIOSYSTEMATIC STUDY OF THE FERN GENUS *LYGODIUM* IN EASTERN NORTH AMERICA

BY

VIOLET M. BROWN
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THESIS

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ABSTRACT

The mainly tropical genus Lygodium differs from other ferns in that the fronds are indeterminate and are vine-like. A single species, L. palmatum is native in temperate North America. The temperate Asian L. japonicum is naturalized throughout much of the southeastern United States. About twenty years ago, L. microphyllum was introduced into South Florida and is now naturalized in several counties. The present study documents differences among spores and their generation, development of sporophytes from the fertilized egg, and in flavonoid chemistry. Hybridization experiments showed a strong possibility for cross fertility between species. Experiments with prothallial development and differentiation revealed that environment influenced variation and gametangium formation. Greater similarity in sporophyte developmental stages and in frond phytochemistry show that the native L. palmatum is phenetically closer to the tropical L. microphyllum than to L. japonicum. All three species are clearly distinct at all levels examined.

ACKNOWLEDGEMENTS

When I consider the pleasant obligation of expressing appreciation to those who have helped in this study, I find the task awesome. Far too many will remain nameless as so often the help may have been only a word that was needed. Some I remember, some are lost to me. For every kindness, for all encouragements, and for all the challenges given, I am thankful.

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The largest contribution came from my husband, Frank Brown. He has given me eight years of college with all the pleasures and liabilities associated with them, shared my successes and failures, joys and tears, triumphs and tragedies, and still kept his wallet and arms open. I think I will keep him around.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES (continued)

FIGURES PAGE

LIST OF TABLES

INTRODUCTION

Early in 1977 while I was visiting my family in Palm Beach County and collecting botanical specimens in various areas of the Everglades, my sister found a species of clinging fern. I recognized its similarity to Lygodium japonicum, but was unfamiliar with the species. Several herbarium specimens were collected and brought to Dr. Henry O. Whittier for identification. We tentatively identified it as Lygodium microphyllum and I began searching the literature to find as much as possible about it. Little was known, and I was able to find only one illustration of a fertile pinna with no illustrations for vegetative blades. Intrigued, Dr. Whittier and Dr. Miller encouraged me to learn as much as I could about the species. Each time I returned to Palm Beach County, I visited the original population, noting the changes. Later, I attempted germinating the spores, then tried doing paper chromotography of flavonoid extracts. The results were fascinating, so I then attempted parallel studies of Lygodium japonicum which had appeared as a volunteer in my yard in 1974. Upon acceptance in the Biological Sciences graduate program at the University of Central Florida, the biosystematic study a the three

species of Lygodium found in the United States seemed to be a work worthy of future study.

Taxonomic History of the Genus

The genus Lygodium is pantropical to subtropical and is comprised of 30 to 40 species. Taxa are known from the Eastern United States, South America, New Zealand, Australia, S.E. Asia, Africa, Indomalaya, the Pacific Islands, and north to Japan. Fossil records are found dating from the lower Cretaceous and Tertiary in Siberia, Europe, China and America. Copeland (1947) recognized 39 species, Holttum (1960) counted about 40 species and Tryon and Tryon (1982) reported the genus to contain about 30 species.

Lygodium is a natural genus, so homogenous because of the vine-like indeterminate fronds (Figure 1) that there is presently little agreement on division into subgroups. From 1800 to 1803 botanists around the world saw the first species of Lygodium resulting from numerous botanical expeditions into the tropics. Independently, and almost simultaneously, several authorities named them. Seven generic names were created during these three or four years and two more were designated later. The following is a summary of the nomenclatural history of the genus. (Tryon and Tryon, 1982)

Figure 1. A single frond of *Lygodium microphyllum* showing the vine-like habit characteristic for the genus.

Lygodium Sw.

Bot. Schrad. 1800(2): 106. 1802, nom. conserv. Type: Lygodium scandens (L.) Sw. (Ophioglosum scandens L.)

Ugena Cav. Icon. Descr. Pl. 73. 1801. Type: Ugena simihastata Cav. = Lygodium semihastatum (Cav.) Desv.

Ramondia Mirbel. Bull. Sci. Soc. Philom. Paris 2: 179. 1801. Type: Ramondia flexuosa (L.) Mirbel (Ophioglossum flexuosum L.) = Lygodium flexuosum (L.) SW.

Odontopteris Berhn., Bot. Schrad. 1800(2): 127. 1802. Type: Odontopteris scandens (L.) Bernh. (Ophioglossum scandens L.) = Lygodium scandens (L.) Sw. Lygodium subgenus Odontopteris (Bernh.) Reed. Bol. Soc. Broter. II, 21: 144. 1947.

Gissopteris Bernh. J. Bot. Schrad. 1800 (2): 129. 1802. Type: Gissopteris palmata Bernh. = Lygodium palmatum (Bernh.) Sw. Lygodium subgenus Gissopteris (Bernh.) Reed. Bot. Soc. Broter. II, 21: 141. 1947.

Hydroglossum Willd. Akad. Wiss. Erfurt 2 (6): 20. 1802. Type: Hydroglossum longifolium Willd. = Lygodium circinnatum (Burm. f.) Sw.

Cteisium Michx. Fl. Boreal Amer. 2: 275. 1803. Type: Cteisium panicutatum Michx. =Lygodium palmatum (Bernh.) Sw.

Vallifilix Thouars. Gen. Nov. Madagas. 1. 1808. Type: Ophioglossum scandens L. = Lygodium scandens (L.) Sw.

Lygodictyon Hook. Gen. Fil. t. 111b. 1842. Type: Lygodictyon forsteri J.Sm. = Lygodium reticulatum Schkuhr.

Species of Eastern North America

Three species of Lygodium grow naturally in eastern North America, and none occur in western North America save possibly under cultivation. Two species, L. japonicum and L. microphyllum, are introductions from Asia which have become naturalized. Lygodium palmatum is endemic to the eastern United States.

Lygodium japonicum has been known in North Carolina since 1900 and in Florida since 1932 (Wherry, 1964). It is native to Japan, but seems to escape cultivation easily and, in many areas, has become a weedy pest. It can tolerate a variety of habitats, from full sun on marginal land to deep shade in marshes. It appears to be continuously fertile and is increasing its range.

Lygodium microphyllum was collected by R. A. Long, February 11, 1958, from a nursury in Delray Beach, Florida. The first known collection from a naturalized population was made in Martin County in June, 1965 (Becker, 1968). Collections have been made as far north as Polk County, Florida. I have seen natural populations approximately one mile south of the St. Lucie/Martin County line along the Sunshine Parkway. I have noted considerable expansion of the species range since I first began observing it in 1977. This species is found throughout the old world tropics from Africa to Australia, Asia and Melanesia.

Lygodium palmatum, the North American endemic, was originally collected in Pennsylvania, and described in 1801. Since, it has been recorded in Dade County, Florida (suspected of representing a cultivated plant), Georgia, Alabama, Mississippi, Tennessee, North Carolina, South Carolina, Virginia, West Virginia, Kentucky, Ohio, Michigan, New Jersey, New York, Connecticut, Rhode Island, Massachusetts and New Hampshire. It can be locally abundant, but it is rare and population numbers are decreasing. It is usually found near the margins of acid swamps and bogs, the soil sandy or peaty, low in ordinary nutrients, and intensely acid (Wherry, 1964). When the soil is disturbed, the fern soon vanishes.

DEVELOPMENTAL STUDIES

Materials and Methods

Experimental Populations

Lygodium microphyllum was collected from a population in Palm Beach County approximately one-quarter mile south of Hypoluxo Road and one mile east of US 441. The fern is growing in a cypress dome (*Taxodium distichum*) which normally has standing water for nine to twelve months of the year. The area is difficult to reach and is used mainly by wild hog hunters. A canal was dug in 1980 approximately one-half mile to the north, which is draining the swamp enough to reduce standing water in the dome. I have checked this population two or three times each year since 1977 and have observed an expansion of the population area in all directions. Although the original area observed in 1977 was about five meters square, the area is now (1984) approximately twelve meters square. This population is at the edge of the dome, immediately inside surrounding *Melaleuca quinquenervia*. Other populations occur among the cypress to the west and north, and a large population is further west. Single plants are scattered through the dome and surrounding areas where sufficient water is available. Col-

lections made each year from 1977 through 1983 are in the University of Central Florida Herbarium. Representative specimens were deposited in the University of Florida Herbarium, Gainesville.

Lygodium japonicum volunteered in spring, 1974, under a boxwood hedge in my front yard at 1208 Leeway Drive, Orlando, Florida. By summer's end of 1975, it had covered the hedges and must be rigorously pruned to contain its spread, and individual plants dug up. Spread is in all directions. Specimens of this population are deposited in the University of Central Florida Herbarium.

Lygodium palmatum was sent in March, 1983, by Dr. C. Ritchie Bell from living material grown in the North Carolina Botanical Garden at Chapel Hill. Voucher specimens are deposited in the University of Central Florida Herbarium.

Establishment of Cultures

Lygodium microphyllum. On February 10, 1983, spores were collected from dried material gathered in October, 1982. The spores were put into a 5% Clorox solution for fifteen minutes, centrifuged at 750 RPM for five minutes, then decanted. The spores were then washed in deionized water three times and centrifuged at 750 RPM for one minute between washes. One liter of sterile deionized water

and one drop of Tween 80 was added to the spores. Tween 80 helped to suspend the spores uniformly through the water. One and one half mililiters (1.5 ml) of solution was pipetted onto each of 18 petri dishes of 100 x 25 mm size containing 40 ml Bold's Basal medium in one per cent agar.

Bold Basal medium, pH 6.6 (Nichols and Bold, 1965; also known as Bristol solution) is a mixture of six stock solutions of the macronutrients and three micronutrient stock solutions as follows:

a. Macronutrients - use 10 ml each/940 ml.

$NaNO_3$	10 g/400 ml
$CaCl_2.2H_2O$	1
$MgSO_4.7H_2O$	3
K_2HPO_4	3
KH_2PO_4	7
NaCl	1

b. EDTA - use 1 ml/l

EDTA	50 g/l
KOH	31

c. Iron - use 1 ml/l

$FeSO_4.7H_2O$	4.98 g/l
H_2SO_4	1.0 ml/l

d. Boron - use 1 ml/l

H_3BO_3	11.42 g/l

e. Micronutrients - use 1 ml/l

$ZnSO_4.7H_2O$	8.82 g/l
$MnCl_2.4H_2O$	1.44
MnM_3	0.71
$CuSO_4.5H_2O$	1.57
$Cu(NO_3)_2.6H_2O$	0.49

Difco Bacto-agar was used to prepare the sterile 1% agar solution.

The inoculated petri dishes were placed in a growth chamber set for a sixteen-hour day with 27°C day and 25°C night temperatures. Six plates were sown with dry, unwashed spores to determine if differences in the germination and growth resulted from the wash and centrifuge technique.

On May 15, 1983, more L. microphyllum was collected from the population in Palm Beach County. Five days later, thirteen petri dishes were inoculated; eleven with washed spores and two with dry, unwashed spores. This was done to try to approximate the duration of spore viability. (The first plates were inoculated with material collected in October and plated in February.)

Lygodium japonicum. L. japonicum was collected from the population at 1208 Leeway Drive, Orlando, Florida on February 11, 1983. Five days later, and using the same washing techniques, twelve petri dishes were inoculated with washed spores and six petri dishes sown with dry, unwashed spores. The inoculated plates were placed in the growth chamber.

Lygodium palmatum. L. palmatum arrived from Dr. Bell on March 11, 1983, and was plated the same day using the same techniques. As the amount of material was

limited, only four petri dishes were inoculated and placed in the growth chamber.

All plates were checked daily and sterile deionized water was pipetted onto the spores as was needed to retard drying.

Breeding Experiments

One prothallus of L. japonicum with archegonial initials was transferred into each of four petri dishes to see if selfing would occur. The same procedure was used for L. palmatum.

One male prothallus of L. microphyllum and one L. japonicum prothallus was transplanted to each of four plates. One L. microphyllum female prothallus and one L. japonicum was transplanted to each of four plates. One male L. microphyllum with one L. palmatum, one female L. microphyllum with one L. palmatum, one L. palmatum with one L. japonicum was transplanted to each of four plates for each cross.

Regeneration Experiments

Four male prothali, four female prothali of L. microphyllum, four each of L. japonicum, and four of L. palmatum were chopped up with a sterile scalpel and separately plated on growth media. The first sporophyte leaf from each species was plated using the same technique.

Results

Developmental Patterns

The usual growth and development pattern for ferns is a distinct haploid-diploid alternation of generations; a spore develops into a small, bi-planar, heart-shaped haploid prothallus which develops male and female sexual structures known as antheridia and archegonia on the unicellular wings or on the central 2 to 3 stratose pad, respectively. Many single-celled rhizoids develop at the proximal end of the gametophyte. Antheridia produce multi-flagellate sperm which must have water to swim to fertilize the egg cell located inside the archegonia. Once fertilization occurs, the diploid embryo grows into the sporophyte plant which is recognizable as a fern. The first several sporophyte leaves are of a very different shape than the mature ones, so a juvenile plant has few, if any, characters that can be used to identify its species.

Lygodium microphyllum washed and unwashed spores were inoculated onto growth media. Germination began in six days, continuing for 28 days. The filamentous protonematal cells divided longitudinally, initiating bi-planar growth. Growth and development were rapid, resulting in recognizable prothalli by day twelve. At day thirteen, antheridial initials were observed. The first periclinal division

with cell walls complete; and a mature antheridium on day fourteen. On day nineteen, several archegonia were observed in various stages of maturation, one still having a cap cell attached, but with neck cells beginning to erode inside. Many antheridia were mature at this time and sperm were swarming.

The first sporophyte leaf was produced on day 52, and sporophytes continued to emerge over a period of several weeks. When the sporophytes developed the fourth leaf, they were transferred to pots containing commercial potting soil.

When all female prothalli in a petri dish had been fertilized and transferred, some of the remaining male prothalli began enlargement which produced the usual heart-shaped prothallus at the distal end. These "rejuvenated" prothalli then began archegonial production and discontinued making antheridia. These newly female prothalli were successfully fertilized and they produced healthy sporophytes.

Lygodium japonicum germination began in both washed and unwashed spores on day 4, but very little germination was observed on washed spores for 21 days. Germination of washed spores lagged far behind the unwashed ones, and germination continued over a two-month period. The prothalli produced from washed spores took four to five weeks longer to reach sexual maturity than those which remained unwashed. Unwashed spores produced prothalli which were sex-

ually mature at 18 days. Both archigonia and antheridia seemed to arise simultaneously on the same prothallus rather than antheridial growth being first.

Sporophytes first appeared on day 39 from the unwashed spore plants, and on day 63 for washed spore plants. Crowding of sporlings delayed sexual maturity and produced prothalli with anomalous growth. Some prothallial cells dedifferentiated, producing protonematal, or filamentous, growth, usually from the apical notch, but at times, from any area of the prothallus. When the filament reached an uncrowded area of the plate, bi-planar growth was initiated, and the usual notched prothallus formed. These formed normal archegonia and antheridia, and were successfully fertilized and produced healthy sporophytes.

Lygodium palmatum spore germination began on the sixth day and continued for 35 to 40 days. Germination was limited to only 3-5% of the spores sown. It was necessary to flood the plates every other day to retard drying. Sexual maturity was observed on day 32, and the first sporophyte on day 41. This species required water every other day during its entire growth period, and continues to have a requirement for abundant moisture as a juvenile, maturing plant.

Breeding Results

Lygodium japonicum crossed with L. microphyllum male: L. japonicum stopped producing archegonia but continued antheridial growth. The prothallus proliferated, producing numerous heart-shaped prothalli from all parts of the original gametophyte. No archegonia were seen on the proliferated prothalli which were subsequently transplanted for hybridization experiments. The male prothallus of Lygodium microphyllum continued to elongate, producing a ribbon-like, many-times-folded gametophyte, with numerous proliferations. Antheridial production continued. No hybridization was possible as no L. japonicum archegonia were produced.

Lygodium japonicum crossed with L. microphyllum female gametophytes: L. japonicum stopped producing archegonia but continued antheridial growth. Proliferation from all parts of the prothallus produced numerous heart-shaped prothalli, all with antheridia. The Lygodium microphyllum female proliferated from all parts of the original gametophyte, producing heart-shaped prothalli. Archegonial production was reduced, but continued at about 10% of the usual numbers. Fourteen sporophytes developed. When the sporophytes had five or six first sporophyte leaves, they were transferred to separate 4" plastic pots filled with commercial potting soil and kept under constant illumi-

nation from grow-lites for three months. When the sporophytes were 12-16 cm tall, the pots were placed in a well-shaded area under low shrubs outside. One plant survived the extreme cold of December, 1983. In its present immature condition, it resembles neither parent at similar developmental stages. Further evaluation cannot be made until the plant matures.

Regeneration Results

Lygodium japonicum, L. microphyllum, and L. palmatum prothalli which were chopped up and plated on growth media regenerated. Each part, from any cell, began to develop into a typical prothallus within twenty-four hours. A L. palmatum regenerated prothallus developed a sporophyte on the twenty-first day. (It had probably been fertilized prior to being chopped up.) The rest of the regenerated prothalli continued to mature and, over a period of forty-five days, developed sporophytes. L. japonicum paralleled L. palmatum in its regeneration. Lygodium microphyllum paralleled the other two species in regeneration, but the results of archegoniate vs. antheridiate prothalli differed. The chopped up pieces of each sex were plated separately on growth media. Prothalli developed from pieces of either sex regenerated into prothalli, but regenerants matured into gametophytes with the same sexual ratio

observed from spore germination. No regeneration occurred from any cell of the chopped-up first sporophyte leaf from any of the three species.

PHYTOCHEMICAL STUDIES

Materials and Methods

Standard preparations for phytochemical analysis using paper chromatography (Mabry et al., 1970) were followed. Air-dried fronds of each species under study were used. The pinna blades were stripped off, leaving as much of the venous material behind as possible. Blade material was macerated with mortar and pestle. Rachises of each species were clipped with scissors into pieces approximately 1 cm long. Four grams (4.0g) of each leaf blade sample were weighed and placed into glass vials, with tightly fitting lids. Eighty milliliters (80 ml) of ethyl acetate (Mallinckrodt, reagent grade) was added and the vials placed in a dark cabinet for 48 hours, then decanted into new vials with tightly fitting lids. The second extraction was made using 80 ml of methanol (Mallinckrodt, Photrex). The same technique of extraction and decanting was used. The third extraction, using the same technique, was made with a 1:1 ratio of methanol and sterile, deionized water. 1.2 grams of rachis materials of each species were weighed and three extractions were done using 19 ml of each reagent and the same techniques. The decanted extracts were then concentrated to

approximately 5 ml using a vacuum desiccator or a Büchi Rotovap depending on the volume of extract to be concentrated.

Chromatograms

Whatman 3mm chromatographic paper was spotted with each extract. Several sheets were prepared using concentrations (or amounts) of 125 µl, 200 µl, 250 µl, 300 µl, and 375 µl. The dry sheets were placed in chromatographic chamber and a solvent of glacial acetic acid and water in a ratio of 3:1:1 was used. When the solvent front was approximately 35 mm from the lower edge, the sheets were taken out and dried in a hood. The second dimension was run in a different cabinet using a solvent of glacial acetic acid and water in a ratio of 15:85. When the solvent front was approximately 35 mm from the lower edge of the paper, the sheets were removed and dried as before. The sheets were examined in a darkroom under long-wave ultraviolet light. Spots of color were circled with pencil and the color was noted on the paper. Next, each chromatograph was fumed with ammonium hydroxide under the ultraviolet light with notation again of the size and color of each spot. Each chromatogram was sprayed with alcoholic aluminium chloride, and color and size of each spot was noted. Tracings were made of each chromatogram, and the compounds were located by R_f values on the graph. A composite tracing

was made of leaf blade compounds and rachis compounds for each species. The compounds were then numbered, and a comparison was made between species. (Figures 2-7) The amount of extract of each application to the paper was 25 µl, drying between each applicatlon. A separate sheet was used for each extract and each species.

Results

The chemical finger-prints run on the three species of Lygodium under study showed them to be distinct and separate species. Many isolates are common to all, some shared with only one other species, and some isolates are found in only the one species. (Table 1)

Lygodium palmatum has one isolate unique to blade and one unique to rachises, but a total of five isolates not found in either L. japonicum or L. microphyllum.

Lygodium microphyllum has no isolates unique to its blades alone, but three unique isolates are found in the rachis alone. Three isolates are shared in common between blade and rachis. A total of six isolates is distinctive of L. microphyllum alone.

Lygodium japonicum has no unique isolates restricted to the blades alone, but there are ten found only

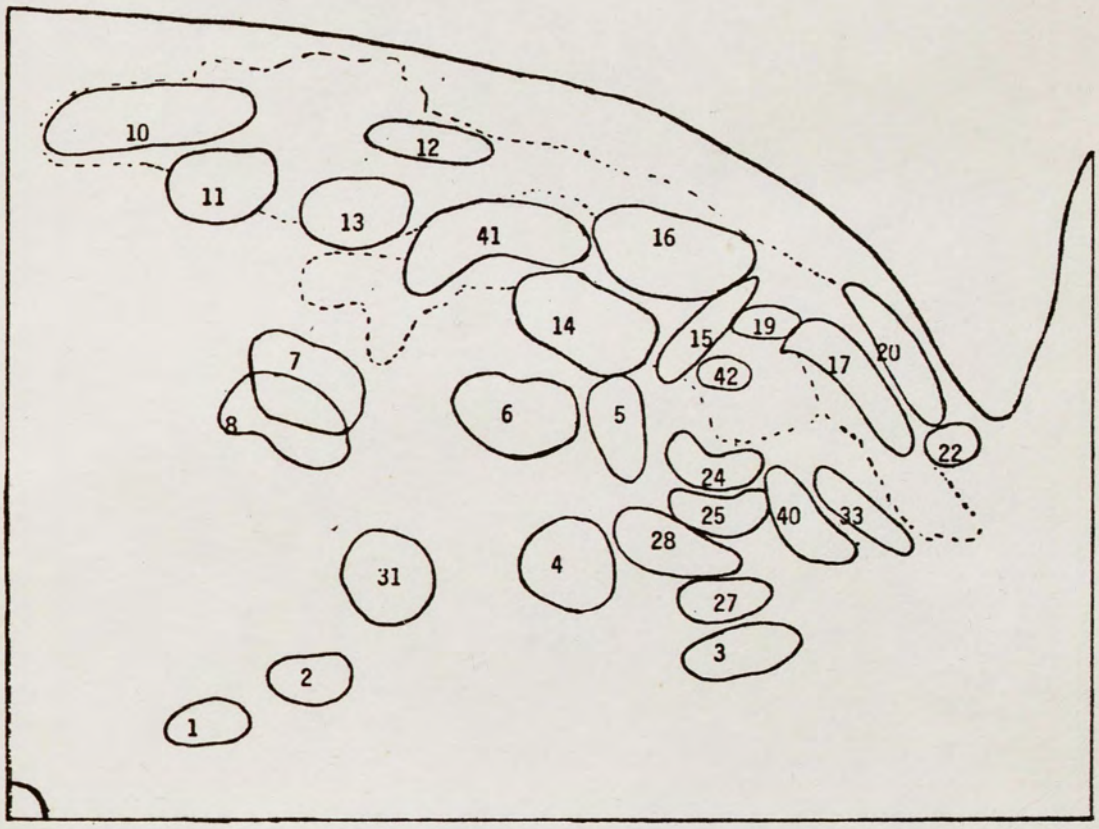

Figure 2. A composite two-dimensional chromatograph of chemical isolates from the pinna blades of Lygodium japonicum. The origin is at the lower left corner.

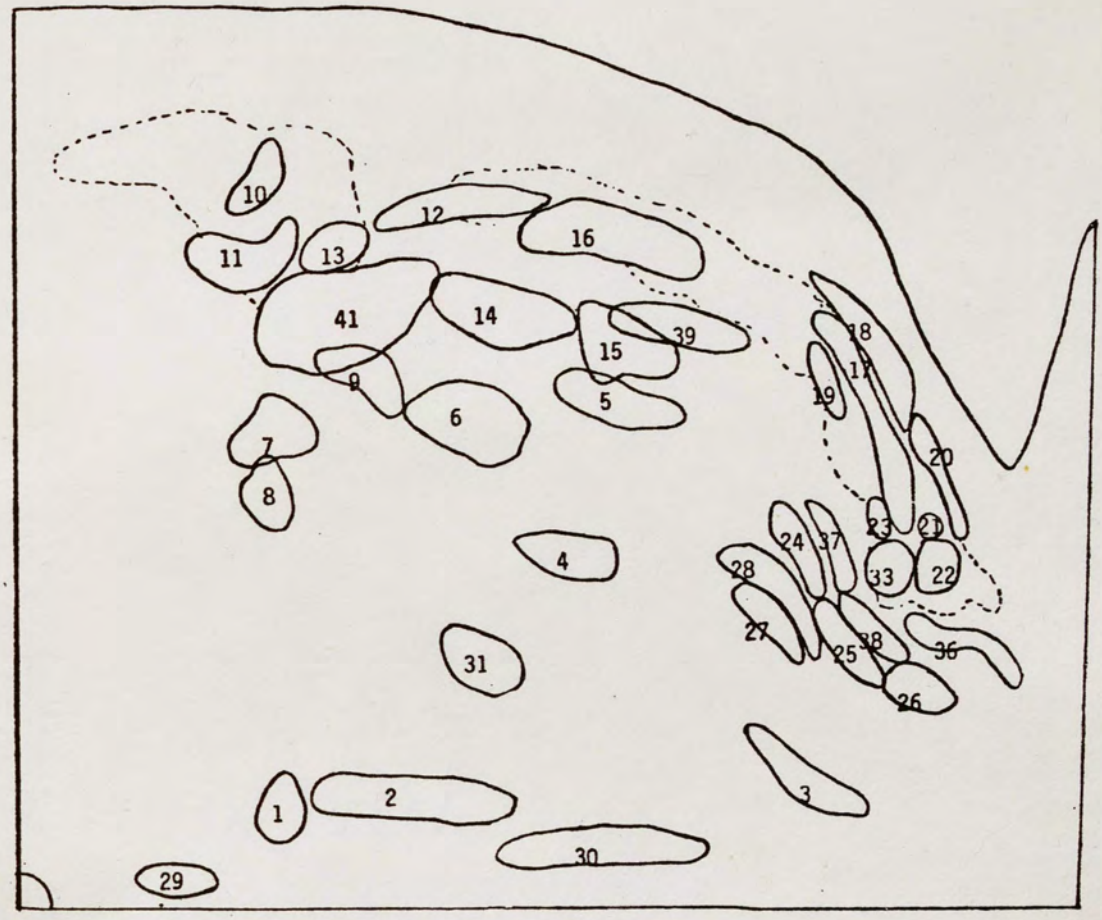

Figure 3. A composite two-dimensional chromatograph of chemical isolates from the rachises of Lygodium japonicum. The origin is at the lower left corner.

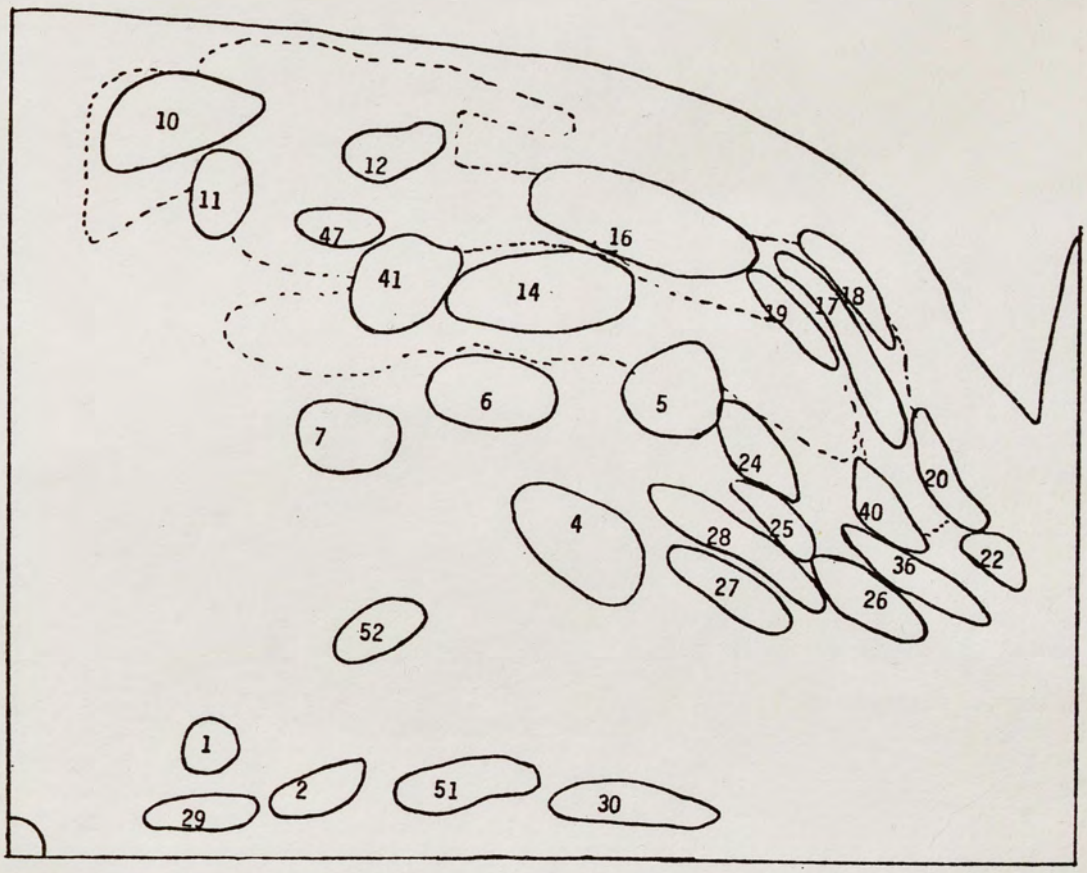

Figure 4. A composite two-dimensional chromatograph of chemical isolates from the pinna blades of Lygodium microphyllum. The origin is at the lower left corner.

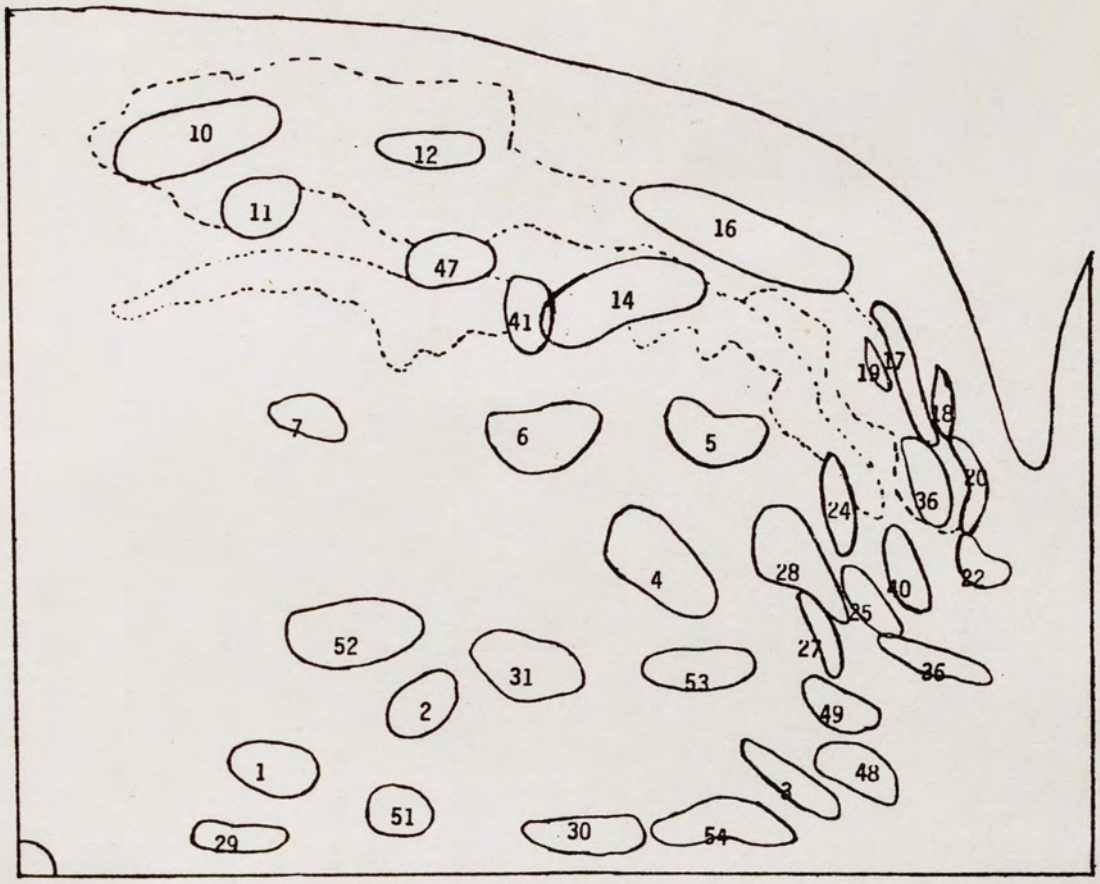

Figure 5. A composite two-dimensional chromatograph of chemical isolates from the rachises of Lygodium microphyllum. The origin is at the lower left corner.

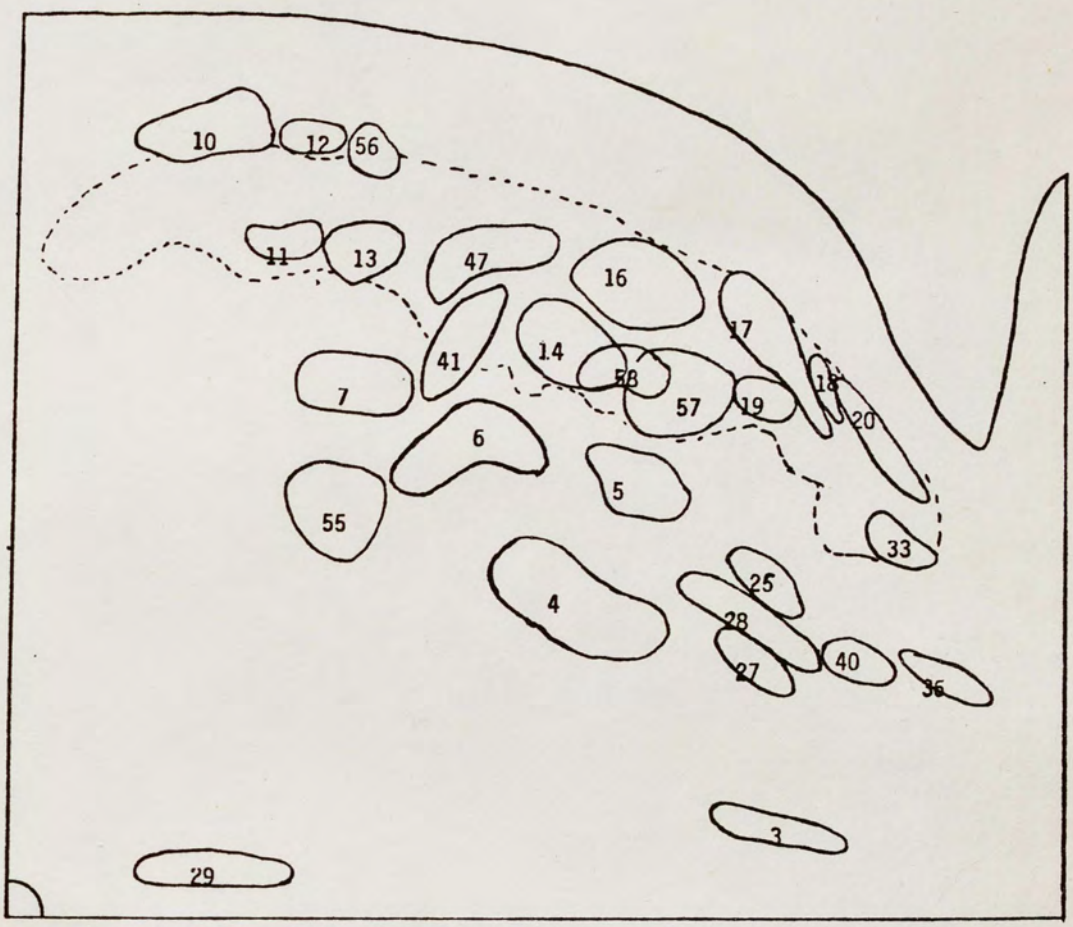

Figure 6. A composite two-dimensional chromatograph of chemical isolates from the pinna blades of Lygodium palmatum. The origin is at the lower left corner.

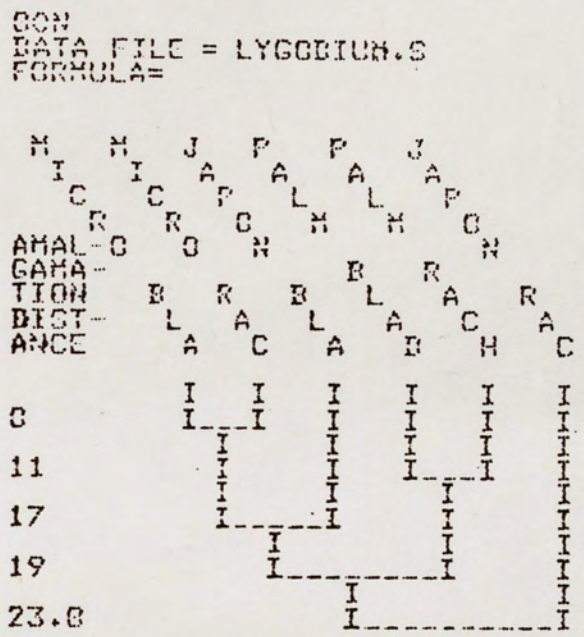

Figure 8. A computer generated dendrograph showing the relationships among Lygodium japonicum, L. microphyllum, and L. palmatum pinnule blades and rachises based upon chemical isolate similarities and differences (see Table 1).

in the leaf rachises. A total of eleven isolates are found only in L. japonicum.

Four isolates are common to L. japonicum and L. palmatum not found in L. microphyllum. Four isolates are common to L. japonicum and L. microphyllum, but not L. palmatum. Only one isolate is found in both L. microphyllum and L. palmatum, but not in L. japonicum. L. japonicum shows forty-five isolates isolated by paper chromatography; L. microphyllum, thirty-eight and L. palmatum, thirty-four isolates. There are twenty-five isolates common to all three species. Because there are so many isolates in each species, three or four isolates showing on the graphs could be artifacts and not genuine isolates. Further work will be done to determine the identity of the isolates.

Dr. Haven Sweet, Professor of Biological Sciences at the University of Central Florida, ran a computer analysis on the isolates isolated, and the dendrographs showed L. microphyllum and L. palmatum to be similar in their chemistry and L. japonicum was more remote. In juvenile stages of leaf development, a parallel situation was observed (Figure 21).

Table 1. Explanation of chromatographic spots and their distribution among *Lygodium japonicum* (japon), *L. microphyllum* (micro), and *L. palmatum* (palm), with R_f values (x 100).

Spot No.	spot R_f	japon blade	japon rachis	micro blade	micro rachis	palm blade	palm rachis
1	25,10	x	x	x	x		x
2	31,10	x	x	x	x		x
3	76,13	x	x		x	x	x
4	57,39	x	x	x	x	x	x
5	63,57	x	x	x	x	x	x
6	46,57	x	x	x	x	x	x
7	26,52	x	x	x	x	x	x
8	22,48	x	x				
9	33,61		x				
10	24,84	x	x	x	x	x	x
11	26,75	x	x	x	x	x	x
12	33,84	x	x	x	x	x	x
13	34,69	x	x				x
14	53,70	x	x	x	x	x	x
15	66,70	x	x				x
16	61,81	x	x	x	x	x	x
17	82,65	x	x	x	x	x	x
18	82,79	x	x	x	x	x	
19	77,64	x	x	x	x	x	x
20	86,75	x	x	x	x	x	x
21	87,63		x				
22	90,66	x	x	x	x		
23	83,60		x				
24	75,47	x	x	x	x		
25	77,37	x	x	x	x	x	x
26	84,37		x	x	x		x
27	73,30	x	x	x	x	x	x
28	71,39	x	x	x	x	x	x
29	14,05		x	x	x	x	x
30	56,09		x	x	x		x
31	43,30	x	x		x		
32	23,88		x				
33	83,49	x	x			x	x
34	74,29		x				
35	33,11		x				
36	93,46		x	x	x	x	x
37	83,49		x				
38	85,39		x				
39	63,43		x				

Table 1 (continued)

Spot No.	spot R_f	japon blade	japon rachis	micro blade	micro rachis	palm blade	palm rachis
40	74,48	x		x	x	x	x
41	40,79	x		x	x	x	x
42	67,67	x					
43	06,75	x					
44	21,93	x		x	x		
45	31,96	x					
46	57,74		x				
47	38,82			x		x	x
48	70,16				x		x
49	70,23				x		
50	36,92			x	x		
51	35,09			x	x		
52	33,29			x	x		
53	64,27				x		
54	63,08				x		
55	28,51					x	x
56	44,91					x	x
57	63,71					x	x
58	57,73					x	
59	12,43						x
60	38,78						x

DESCRIPTIVE STUDIES AND TAXONOMIC TREATMENT

The biosystematic study of any group should lead to better understanding of the taxonomy and is of greatest value if it is presented against a background of traditional, morphologically-based taxonomy. Without utilizing traditional taxonomy, indentifications are rendered difficult. Therefore, I have provided a key to species of *Lygodium* as well as an original description, listing of taxonomic synonyms and specimens examined for each of the species studied.

Key to Species

1. Secondary rachis-branch bipinnate, especially so on fertile portions of the frond . . . . . *L. japonicum*
1. Secondary rachis-branch pinnate 2
    2. Secondary rachis-branch terminating in a single, palmately-lobed pinnule. . . . . *L. palmatum*
    2. Secondary rachis-branch bearing pinnately arranged petiolulate, unlobed pinnules (occasionally the distal pinnule is bifid) . . . . . *L. microphyllum*

Lygodium japonicum (Thunb.) Sw.

J. Bot. Schrad. 1800 (2): 106. 1801.

Synonyms (fide Walker, 1976).

Ophioglossum japonicum Thunb. Fl. Japon. p. 328. 1784.

Lygodium microstachyum Desv. var. glabrescens Nakai. Bot. Mag. Tokyo 41: 686. 1927.

Rhizome protostelic, to 10 cm long in specimens examined, creeping below ground surface, dichotomously branched, densely covered with dark brown, branched septate trichomes. Roots approximately 10-12 cm long, many branched, fibrous. Stipe or rachis cross sections dome-shaped, two-ridged, glabrous along dome, septate epidermal hairs between ridges. Rachis bearing very short (1-2 mm) primary branches alternately arranged, ending in a dormant terminal meristem densely covered with brown, septate hairs (4-7 cells). The terminal meristem normally does not develop, but if the main rachis is broken, the terminal meristem on the next primary rachis below begins to grow and produces branches in exactly the same way as the main rachis. Primary rachis-branch branches dichotomously, one secondary rachis-branch on each side of the terminal meristem. Secondary rachis-branches narrowly winged (.01-.04 mm), the upper surface raised and covered with septate epidermal hairs (2-5 cells long); pinnules in a pinnate arrangement, petio-

lulate, ovate-deltoid in outline, 10-20 cm long, 9-12 cm wide, with 2-5 pairs of more or less elongate linear-lanceolate, lanceolate, or ovate segments, the lower ones pinnately incised or lobed, dichotomously veined, with the veins free. Margins of pinnule lobes serrulate, each serrulation ending in a hooked tooth. Upper surface of pinnule bears septate epidermal hairs (1-5 cells long) on laminar cells; lower surface bears epidermal hairs only on veins. Fertile leaflets, often smaller than sterile ones, are fringed along their edges with short, narrow lobes, each lobe bearing two rows of sporangia, each attached to a short vein and covered by a laminar flange which acts as an indusium. The sporangial lobe has a large vein through the center, with small, alternate veins bearing the sporangia. Sporangium short-stalked, more or less pear-shaped, with an annulus of a few thick-walled cells around the narrow end, dehiscing by a vertical slit, each sporangium containing 128 spores. Spores large, ranging from 72 μ to 78.75 μ, average 71.7 μ; tetrahedral-globose, trilete. (Figures 9, 10, 20, 21)

Protonemata normally turn bi-planar if grown in red or blue light, remaining filamentous if grown in far-red light (Raghavan, 1973). Crowding causes protonemata to remain filamentous. Gametophytes heart-shaped, bearing both archegonia and antheridia on same prothallus; archegonia few (3-9), located near the notch on either ventral or dorsal

side, or both. Short-stalked antheridia are borne on lamina on either surface in much greater numbers than archegonia. Rhizoids are borne on a thickened pad (2-3 cells thick) on the dorsal side along the lower median 1/3 of the proximal end.

The specimens examined are listed below.

Florida: Alachua, R .P. St. John 657, Jan. 2, 1936 (FLAS P2469); Alachua, E. West s.n., 1934 (FLAS 207); Alachua, D. B. Ward 1856, Apr. 24, 1960 (FLAS P5165); Alachua, W. G. D'Arcy and D. Griffin 2475, May 16, 1968 (FLAS P7042); Alachua, D. B. Ward and R.R. Smith 2280, Oct. 8, 1960 (FLAS P5419); Alachua, J. C. Esterday 779, Nov. 23, 1981 (FLAS P8850); Bradford, D. W. Hall and D .B. Ward 8403, Aug. 2, 1972 (FLAS P7766); Calhoun, S. C. Hood, 1575, Mary 19,1949 (FLAS P3524); Calhoun, E. S. Ford 5128d, Aug. 3, 1957 (FLAS P5584); Citrus, E. P. St. John 2287, May 5, 1938 (FLAS P2275); Collier, E. Scull s.n., Jan 20, 1935 (FLAS P208); Dade, M. W. Diddle s.n., March 1932 (FLAS 5939); Duval, M. W. Diddle 309, Jan 9, 1940 (FLAS P5940); Duval, M. W. Diddle 785, Jan. 12, 1954 (FLAS P5938); Escambia, J. R. Burkhalter 5663, Nov. 20, 1977 (FLAS P8288); Gadsden, Small, E. P. Jurz, and R. P. St. John 533-1/2, no date (old) (FLAS P3395); Gadsden, W. J. Stutts, and A. Gholson 23601, Sept. 5, 1979 (FLAS P8475); Gadsden, D. B. Ward 2958, Mar. 17, 1962 (FLAS P5690); Gadsden, A. M.

Laessle s.n., June 3, 1953 (FLAS P4623); Gulf, K. D. Perkins and J. B. Nelson 303, May 7, 1977 (FLAS P8248); Highlands, J. P. McFarlin 10168, Jan. 10, 1935 (FLAS P3270); Hillsborough, R. Garrett s.n., Sept 9, 1953 (FLAS P1634); Leon, R .K. Godfrey, 52867, Jan. 31, 1955 (FLAS P5597); Liberty, D. B. Ward 5875, Aug. 19, 1966 (FLAS P7337); Liberty, C. S. Lotspeich 308, Jan. 27, 1978 (FLAS P8348); Liberty, J. H. Beaman 344, Aug. 25, 1951 (FLAS P4265); Liberty, J. D. Blake s.n., Mar. 13, 1948 (FLAS P3302); Pinellas, J. Taylor and S. E. Taylor s.n., Nov. 24, 1962 (FLAS P5735); Polk, T. Oswalt s.n., Dec, 27, 1977 (FLAS P8246); Polk, P. F. White s.n., Sept. 4, 1968 (FLAS P 7056); Orange, V. M. Brown 2001, Mry 1, 1984 (FTU); Orange, W. R. Llewelyn s.n., Nov. 1961 (FLAS P5650); Santa Rosa, L. Dunavin s.n., Mar. 24, 1982 (FLAS P8817); Seminole, D. B. Ward and J. Beckner 4561, May 13, 1965 (FLAS P6690); Seminole, W. T. Scudder 0650, Jan. 23, 1968 (FLAS P7275); Suwannee, T. Jensen s.n., October 12, 1961 (FLAS P5616); Walton, West and Arnold s.n., June 2, 1954 (FLAS P4947); Walton, R. R. Smith 1340, May 29, 1967 (FLAS P7039); Walton, H. A. Davis 15861, Octt. 12, 1971 (FLAS P7544); Washington, D. B. Ward and D.W. Hall 8481, Oct. 16, 1972 (FLAS P7767).

Georgia: Clarke, S. B. Jones 22614, May 27, 1975 (FLAS P8327).

Louisiana: E. Baton Rouge, S. Tucker, s.n., Sept 1968 (FLAS P8143); Tammany, M. K. Rylander I, Aug. 20, 1963

Figure 9. Lygodium japonicum habit as seen on an herbarium specimen.

UNIVERSITY OF CENTRAL FLORIDA

Schizaeaceae **HERBARIUM**

LYGODIUM JAPONICUM

1208 Leeway Drive, Orlando,
Orange County, Florida

Volunteer population, first appearing in 1974, growing over Boxwood (Buxus) hedge.

Collector: Violet M. Brown
Date: 29 Feb. 1984 Number: 2001

PLANTS OF FLORIDA

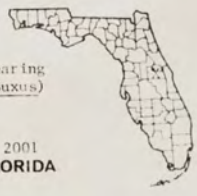

Figure 10. Lygodium japonicum detail of fertile and sterile pinnae.


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(FLAS P8321); Livingston, P. D. Cantino 1062, July 24, 1977 (FLAS P8723); Lafayette, R. Kral s.n., May 23, 1963 (FLAS P6457).

Mississippi: Adams, F. G. Meyer and P. M. Mazzeo 11703, May 29, 1968 (FLAS P7747); Jackson, S. B. Jones and M. Catchot s.n., July 22, 1969 (FLAS P7351).

South Carolina: Greenville, C. L. Ridgers and N. E. Mullens 67133, Aug. 8, 1967 (FLAS P6997).

China: Kiangsi, C. E. De Vol s.n., July 19, 1932 (FLAS P5937); Foochow, T. S. Ging 5458, Nov. 3, 1926 (FLAS P203).

Cuba: Santa Clara, R. E. Schultes s.n., Jan. 18, 1955 (FLAS 5595).

Australia: Eastern Mountains, A. MacCaskill, Jr. s.n., Jan. 1929 (FLAS P204).

Honduras: Cortez, R. P. St. John 2449, Feb. 25, 1940 (FLAS P2038).

Hong Kong: Tregunter Path, Y. S. Lau 513, Mar. 23, 1959 (FLAS P7070).

Lygodium microphyllum (Cav.) R. Br.

Prodr. Fl. Nov. Holl. p. 162. 1810.

Synonyms (fide Walker, 1976).

Ugena microphylla Cav. Icon. Descr. Pl. 6: 76, pl. 595. 1801.

Lygodium scandens Auct. nec (L.) Sw. e.g.,
C. Christ. Ind. Fil. 1905.
Lygodium scandens var. microphyllum (Cav.)
Luerss. J. Mus. Godeffroy 6: 4. 1874.

Rhizome protostelic long (to 35 cm in specimens examined), creeping, below ground surface, dichotomously branched, stipe protostelic, widely spaced, 2-4 cm from center to center, densely covered with long dark brown multicelled branched trichomes; roots to 7 cm long, occasionally branched, fibrous. Stipe and rachis slender, twining, individual fronds often 6-8 m long. Stipe cross section, dome-shaped, two-ridged, glabrous along dome, simple 1 to 2 celled epidermal hairs between ridges. Rachis bearing very short primary branches, alternately arranged, ending in a dormant meristem bud densely covered with brown, multicelled hairs. The bud normally does not develop, but if the main rachis is broken, the bud on the primary rachis-branch next below becomes meristematic and produces branches in exactly the same way as the main rachis. Primary rachis-branch branches dichotomously, one secondary rachis branch on each side of the dormant bud. Secondary rachis-branches narrowly winged, the upper surface raised and covered with multi-celled epidermal hairs; bearing pinnules in a pinnate arrangement; dichotomously veined sterile pinnules, shape variable, simple, except terminal pinnule often bifid; petio-

lulate; bases of pinnules truncate or cordate; margins crenulate; surface mostly glabrous. Fertile pinnules vary in size and shape, often larger than sterile ones, fringed along their edges with short, narrow lobes, each lobe bearing two rows of sporangia, each attached to a short vein and partially covered by a laminar flange which acts as an indusium. The sporangium lobe has a large vein through the center, with smaller, short, alternately-arranged, obliquely-angled veins, each bearing one sporangium. Sporangia large, short-stalked, more or less pear-shaped, depressed on upper surface, with an annulus of a few thick-walled cells around the narrow end, dehiscing by a vertical slit, each sporangium containing 256 spores. Spores tetrahedral-globose, trilete, ranging from 51.75 μ to 87.75 μ with an average of 70.85 μ in their longest dimension. There is a clustering of spore sizes into two relatively distinct groups, one averaging 69.6 μ, the others at 82.66 μ, average in a ratio of nine small to one large spore. (Figures 11-17, 20-21)

At maturity, prothalli are either archegoniate or antheridiate; dimorphic, with antheridiate prothalli being approximately one-third the size of archegoniate prothalli. Archegoniate prothalli are the usual heart-shape at sexual maturity, antheridiate ones continue to elongate by producing cells at the apical notch. Antheridia are produced on the continuous growth and are short-stalked. There is a

ratio of approximately one female to seven male gametophytes. Female prothalli produce 3-7 archegonia close to the notch on either dorsal or ventral side or both. Male prothalli bear antheridia all over the lamina on both surfaces. Rhizoids are borne on a thickened pad, 2-3 cells thick, at the proximal end and are one-celled.

The specimens examined are listed below.

Florida: Highlands, K. C. Alvarez 1427, May 4, 1973 (FLAS P7718); Martin, L. D. Ober s.n., June 15, 1965 (FLAS P6659); Martin, C. W. Campbell s.n., Nov. 12, 1966 (FLAS P6980); Martin, W. L. McCart 10,510, Feb. 9, 1969 (FLAS P8928); Palm Beach, V. M. Brown 325, Jan 3, 1977 (FTU); Palm Beach, V. M. Brown, 460, May 14, 1977 (FTU); Palm Beach, V. M. Brown 998, Mar. 7, 1981 (FTU); Palm Beach, V. M. Brown 1700, Oct. 10, 1982 (FTU); Palm Beach, V. M. Brown, 2015, Mar. 5, 1984 (FTU); Palm Beach, R. K. Godfrey 75935, Nov. 27, 1978 (FLAS P8308); Palm Beach, R. A. Long s.n., Feb. 11, 1958 (FLAS P5100); Polk, J. H. Willson 353, July 18, 1978 (FLAS P8440).

New Caledonia: S. Bedell 584, July 1945 (FLAS P5932).

Liberia: Monrovia, O. F. Cook 103, Feb. 1894 (FLAS P6929).

Figure 11. Lygodium microphyllum habit of pinnae on the frond.


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Figure 12. Lygodium microphyllum habit of base of plant including the rhizome and roots.


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Figure 13. Lygodium microphyllum detail of fertile pinnae.


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Figure 14. Lygodium microphyllum detail of sterile pinnae.

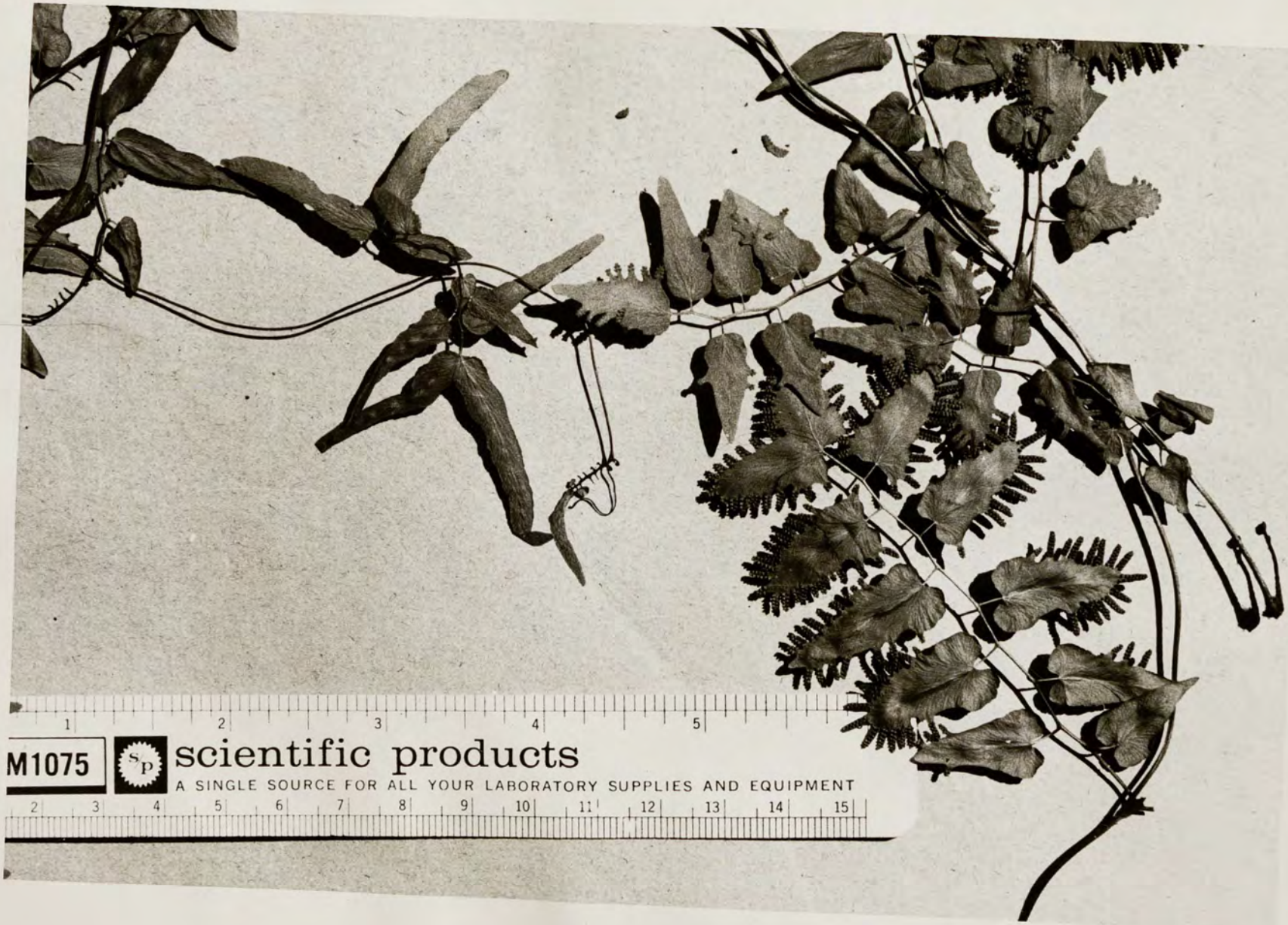
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Figure 15. Lygodium microphyllum detail of sterile pinnae.

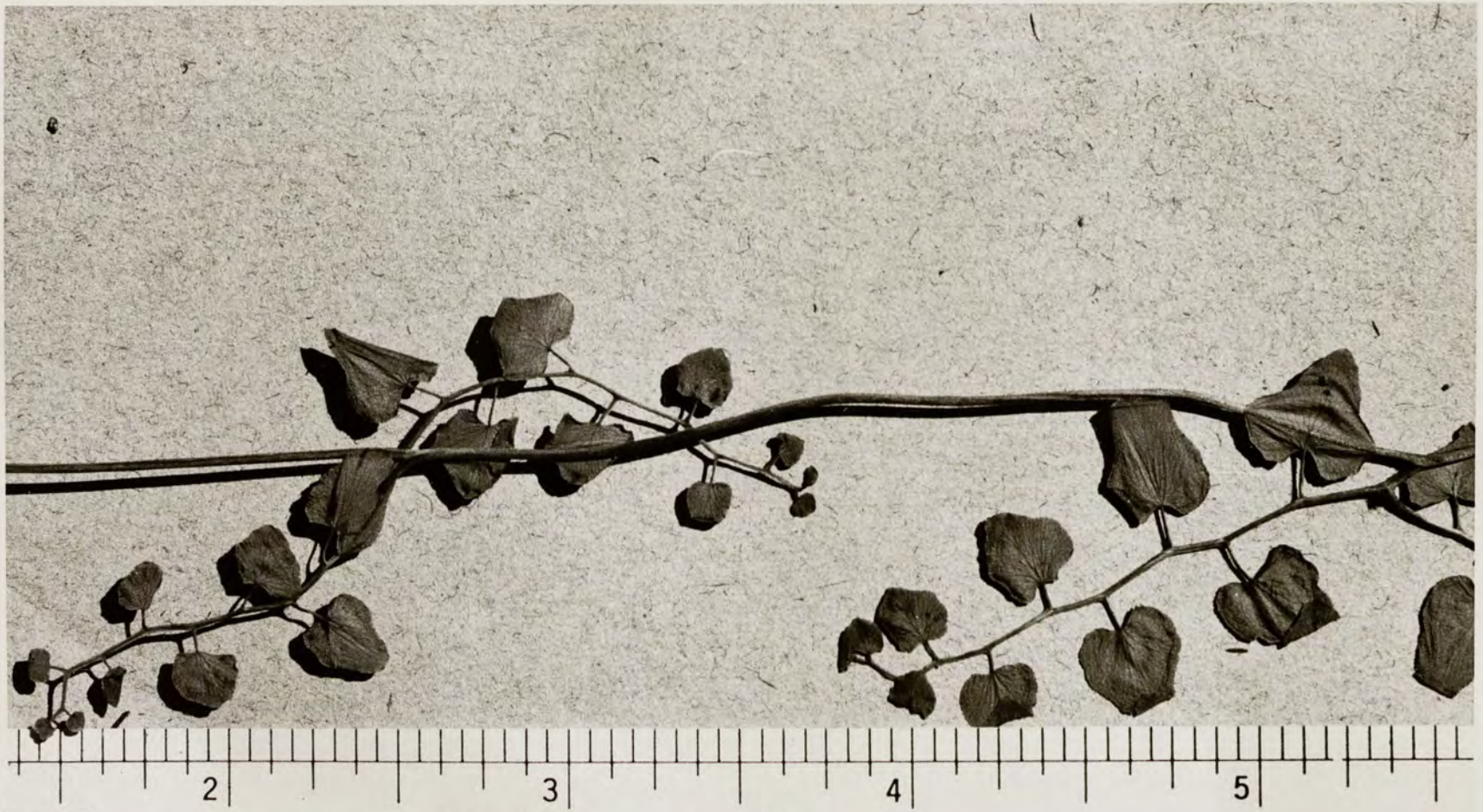

Figure 16. Lygodium microphyllum detail of sterile pinnae.


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Figure 17. Lygodium microphyllum detail of sterile pinnae.

Lygodium palmatum (Bernh.) Sw.

Syn. Fil. p. 154. 1806.

Synonyms (fide Duek, 1978).

Gisopteris palmata Bernh. J. Bot. Schrad. 1800 (2): 129. 1802.

Ramondia palmata Mirbel. Bull. Soc. Philom. 2: 179. 1803.

Cteisium paniculatum Michx. Fl. Boreal. Amer. 2: 275. 1803.

Rhizome below ground, dichotomously branched, with stipes closely-spaced from less than 1 mm to 1.5 mm from center to center, densely covered with long, dark brown multi-celled branched trichomes, protostelic; root system very well-developed, up to 25 cm long, profusely branched, fibrous. Stipe and rachis slender, twining, the whole frond often several meters long, in cross section dome-shaped, two-ridged, glabrous. Rachis bearing very short primary rachis branches, alternately arranged, ending in a dormant terminal meristem densely covered with brown, multi-celled hairs. The terminal meristem normally doesn't develop, but if the main rachis is broken, the terminal meristem on the primary rachis-branch next below begins to grow and produces branches in exactly the same way as the main rachis. Primary rachis-branch branches dichotomously, one secondary rachis-branch on each side of terminal meristem. Secondary rachis-

branches narrowly winged, the upper surface raised, and covered with multi-celled epidermal hairs or glabrous, bearing a terminal pinnule. Sterile pinnules with free-dichotomously branching veins; lobes palmate, joined at base, margins of lobes entire; upper surface mostly glabrous, lower surface bears long, multi-celled epidermal hairs (5-12 cells long) mainly on veins with occasional hairs on lamina. Fertile pinnules greatly reduced, consisting of winged veins and short, narrow lobes, each lobe bearing two rows of sporangia, each attached to a short vein and covered by a laminar flange which acts as an indusium. The sporangium lobe has a large vein through the center with smaller, short, alternately-arranged, obliquely-angled veins, each bearing one sporangium. Sporangia large, short-stalked, more or less pear-shaped, with an annulus of a few thick-walled cells around the narrow end, dehiscing by a vertical slit, and containing 256 spores. Spores tetrahedral, globose, trilete, ranging from 65.25 μ measured in their longest dimension. (Figures 18-21)

Protonemata biplanar; prothalli heart-shaped, bearing both archegonia and antheridia on same prothallus on either dorsal or ventral side or both. Archegonia develop close to notch, few (5-14); many more short-stalked antheridia than archegonia develop on laminar surface. Rhizomes

borne on a thickened (2-3 cells) pad at proximal end; unicellular.

The specimens examined are listed below.

Connecticut: Windsor, R. R. St. John s.n., Sept 24, 1934 (FLAS P2470).

Florida: Orange, V. M. Brown 1901, Mar. 22, 1984 (FTU).

Kentucky: Laurel, P. S. Preston s.n., May 2, 1984 (FLAS 8233).

Massachusetts: Amherst, M. W. Diddle 873, June 5, 1955 (FLAS P5935).

Michigan: Kalamazoo, R. W. Pippin s.n., Oct. 1969 (FLAS P7697).

New Jersey: Burlington, C. L. Pollard s.n., Nov. 12, 1899 (FLAS (1789).

North Carolina: Cumberland, R. K. Godfrey and W. B. Fox 49364, June 26, 1949 (FLAS P3824); Orange, C. R. Bell 1900, Mar.. 15, 1983 (FTU); Transylvania, M. R. Singletary s.n., Aug. 20, 1935 (FLAS P1956); Transylvania, G. L. Thomason 63, Aug. 8, 1936 (FLAS P3014); Transylvania, N. E. Mullens 67147, Aug. 8, 1967 (FLAS P6991).

Ohio: Athens, W. P. Porter s.n., Aug. 6, 1938 (FLAS P2002).

Rhode Island: West Greenich, A. Lillibridge s.n., Oct. 2, 1934 (FLAS P202).

Tennessee: Cumberland, K. Kirkman and L. Boring 1252, Sept. 29, 1979 (FLAS P8689); J. H. F. (gift of S. Rapp) s.n., no date (FLAS P1814).

Virginia: Scott, D. E. Peake 338, July 20, 1978 (FLAS P8794).

West Virginia: Nicholas, W. C. Legg s.n., Aug. 24, 1946 (FLAS P3485); Greenbrier, R. B. Clarkson 4062, Aug. 6, 1964 (FLAS P7511).

Figure 18. Lygodium palmatum habit of pinnae on the frond.


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Figure 19. Lygodium palmatum detail of fertile pinnae as seen on an herbarium specimen.


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Figure 20. Lygodium structures and development. A. Lygodium japonicum fertile lobe; B. L. palmatum fertile lobe; C. L. microphyllum fertile lobe; D. L. japonicum sporangia; E. L. microphyllum sporangia; F. L. palmatum sporangia; G. L. japonicum spores; H. L. microphyllum spores; I. L. palmatum spores; J. L. japonicum germinating spores; K. L. microphyllum germinating spores; L. L. palmatum germinating spores; M. L. japonicum prothallial development; N. L. microphyllum prothallial development; O. L. japonicum young prothalli; P. L. microphyllum young prothalli; Q. L. palmatum young prothalli.

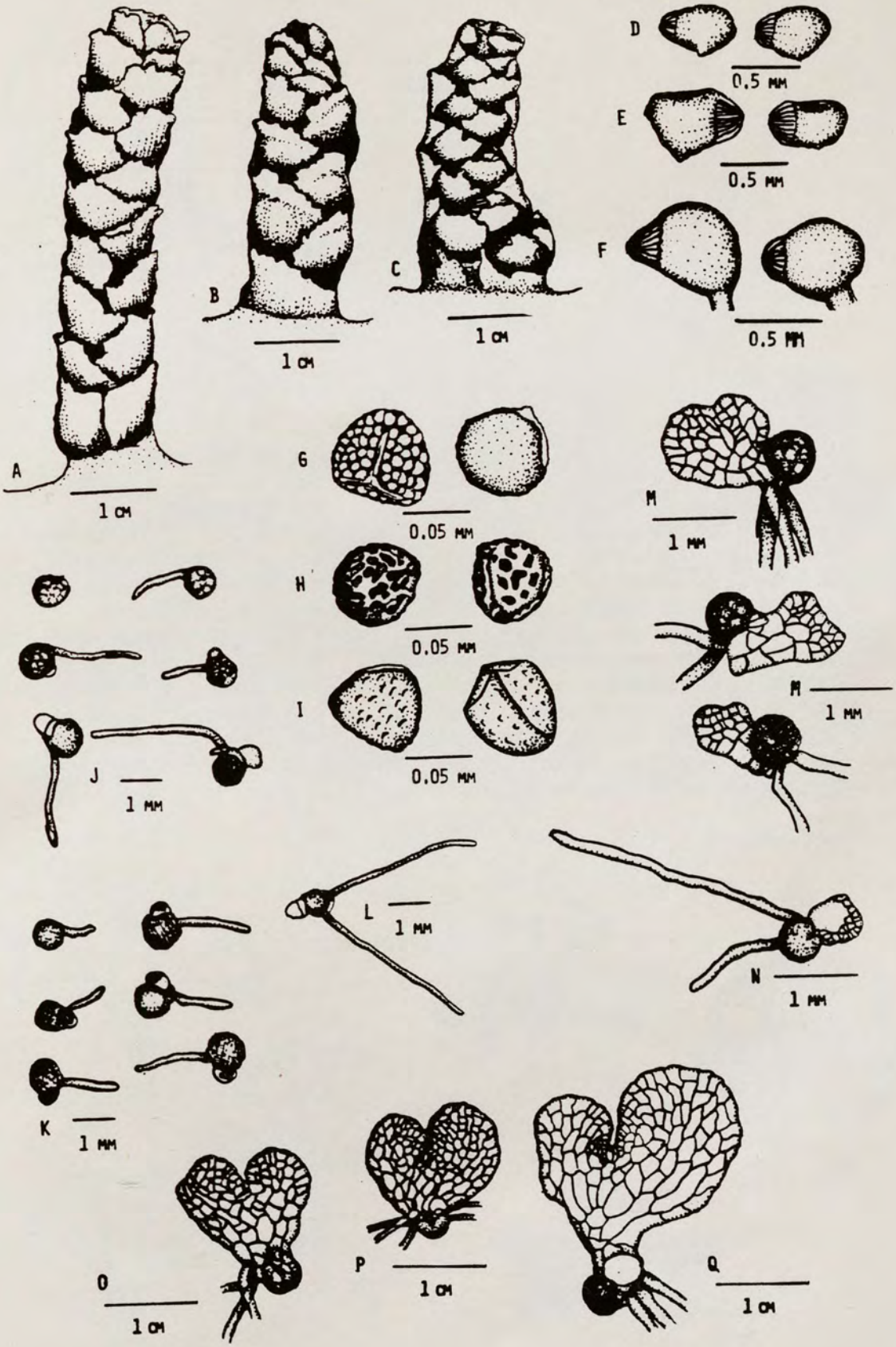
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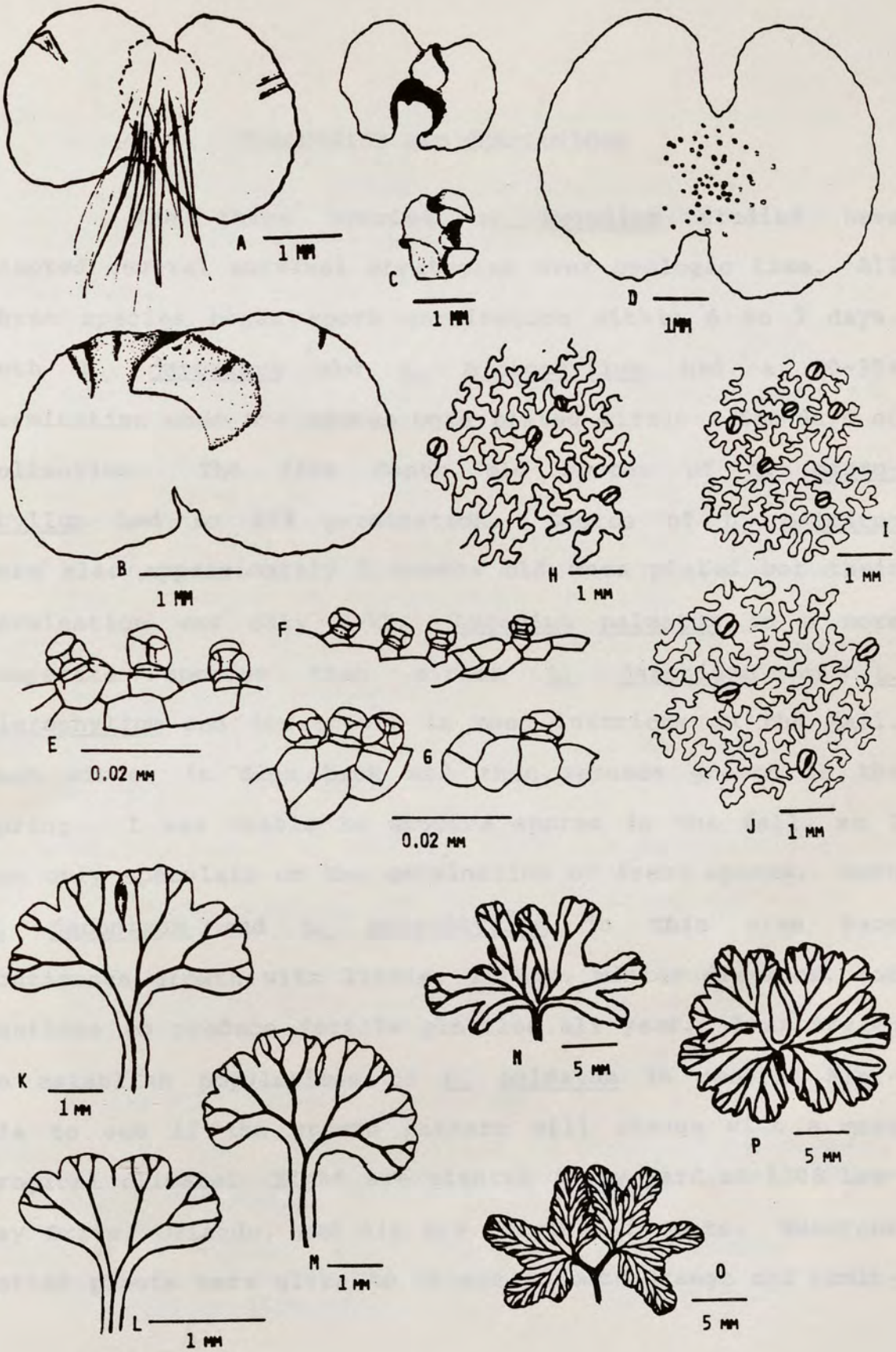
Figure 21. Lygodium structures and development. A. Lygodium japonicum mature prothallus; B. L. microphyllum mature female prothallus; C. L. microphyllum mature male prothallus; D. L. palmatum mature prothallus; E. L. japonicum stalked antheridia; F. L. microphyllum stalked antheridia; G. L. palmatum stalked antheridia; H. L. japonicum epidermal cells and stomata of first sporophyte leaf; I. L. microphyllum epidermal cells and stomata of first sporophyte leaf; J. L. palmatum epidermal cells and stomata of first sporophyte leaf; K. L. japonicum first sporophyte leaf; M. L. palmatum first sporophyte leaf; N. L. japonicum fourth sporophyte leaf; O. L. microphyllum fourth sporophyte leaf; P. L. palmatum fourth sporophyte leaf.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DISCUSSION AND CONCLUSIONS

The three species of Lygodium studied have adapted several survival strategies over geologic time. All three species began spore germination within 6 to 7 days. Both L. japonicum abd L. microphyllum had a 90-95% germination when the spores were plated within a few days of collection. The five month old spores of L. microphyllum had an 80% germination. Spores of L. palmatum were also approximately 5 months old when plated but their germination was only 3-5%. Lygodium palmatum is a more temperate species than either L. japonicum or L. microphyllum and its growth is most luxurious in the fall. Each winter it dies back and then resumes growth in the spring. I was unable to acquire spores in the fall, so I can only speculate on the germination of fresh spores. Both L. japonicum and L. microphyllum in this area have continuous growth with little, if any, winter die-back, and continue to produce fertile pinnules all year. I am trying to establish populations of L. palmatum in central Florida to see if the growth pattern will change with a more tropical climate. Eight are planted in my yard at 1208 Leeway Drive, Orlando, and six are retained in pots. Numerous potted plants were given to others in both Orange and Semin-

Nayar, B. K. 1970. A phylogenetic classification of the homosporous ferns. Taxon 19:229-236.

Nichols, H. W. 1973. Growth media-freshwater. Pp. 8-24. In: Stein, J. R. Handbook of Phycological Methods. Harvard Univ. Press, Cambridge.

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ole County. Many are surviving and will be monitored as they develop.

Lygodium palmatum seems to have a higher requirement for water than the other species studied. From spore germination through all maturation stages so far observed, the necessity for water was about three times greater for L. palmatum. Its absolute dependence on water, its decline and disappearance when disturbed, its need for very acidic soil, and its seemingly short sporulating period could account for the distribution of the species being somewhat disjunctive.

All three species have prothalli which proliferate vegetatively whereby many can be produced from one prothallus. Any injury, fragmentation or isolation of a prothallus seems to initiate growth of new prothalli. When either a single male or female prothallus of L. microphyllum is broken into pieces, the fragments dedifferentiate and regenerate the usual sexual ratio of one female to seven male gametophytes.

All three species seem to be out-breeders. With the techniques used, no evidence for self-fertilization was observed. More studies need to be done to determine if selfing is possible under other cultural conditions than those used. This would require a long and carefully controlled series of experiments.

All three species produce many large spores with thick walls. The fern fronds climb up trees, shrubs or fences and produce the fertile segments only on portions of the fronds where they receive sunlight. Thus, the sporangia are raised high enough and are sufficiently exposed for the spores to be easily wind borne. Also, all three species' usual habitat is at the margins of streams or marshes and the spores are capable of being dispersed by water for considerable distance.

In south Florida, L. microphyllum grew in association with Melaleuca quinquenervia and Schinus terebinthifolius, two species which are introductions spreading rapidly through the state. Both species are crowding out native vegetation and are often found in pure stands. Both are strongly allelopathic and few species grow under them. Even so, L. microphyllum can fluorish around and over them.

Lygodium microphyllum spores show two size clusterings. Whether this is incipient anisospory or only a result of spore maturation, or position in the sporangium is not known. Prothalli of this species being dioecious and dimorphic when grown under culture conditions suggests anisospory but more carefuly controlled studies need to be done to determine growth patterns under various conditions.

The spores of all three species germinated faster if they were sown without washing in a fungicide. They mycorrhizae apparently associated with these species (Mishra et al., 1981) could have been reduced by the 5% Clorox solution to such an extent that germination was slowed. There was no attempt in this study to isolate nor identify possible mycorrhizal oranisms.

The first three sporophyte leaves of each species are so similar as to be indistinguishable (Figure 22 K, L, M). The fourth leaf begins to show characteristics of the genus (Figure 21 H, O, P) but not of the species. Juvenile leaves of L. microphyllum are morphologically similar to the mature pinnules of L. palmatum (Figure 21 O). Beginning at about leaf number 7 or 8, L. palmatum juvenile leaves are similar to the same stages of L. japonicum. Because the L. palmatum under cultivation from spores has not yet produced the typical palmately-lobed pinnules, I am unable to determine the time sequence of juvenile to adult leaf. Lygodium japonicum and L. microphyllum which were grown from spores are now recognizable as to species, but have not yet produced fertile pinnules at one year old. These cultivated plants should not be used as a measure of age vs. maturity as they have been moved into and out of several environments. Once established in a permanent,

undisturbed environment, a more accurate evaluation of maturation age could be made.

All species under study developed both stalked and sessile antheridia (Figure 20 E, F, G). Stalked antheridia have not been reported for this genus as far as we have been able to determine.

The patterns of the epidermal cells are similar among the species but variation can be detected. Lygodium microphyllum cells are generally smaller that the others and L. palmatum has very large stomata when compared to the other two (Figure 21 H, I, J). The larger stomata correlate with the greater requirement for water observed for L. palmatum. Also, the first rhizoids and the mature root system of L. palmatum are consistent with higher water demand. Both rhizoids and roots are longer and more numerous in L. palmatum than in the other two species.

The culture studies of these three species were conducted using material from only one population for each species. No attempt was made to determine if experimental results undertaken would be consistent throughout the range of the species. It would be interesting to see if each species would remain consistent or vary within a limited bounds if sample populations were studied from over the entire distributional range.

As a result of these studies, several potentially fruitful lines of investigation have emerged. Observations on growth and development suggest that useful studies could be conducted in areas such as detailed phytochemistry of the abundant flavonoids and in developmental morphology. Testing to see if L. microphyllum will show strong sexual dimorphism when grown under natural conditions (i.e., not in a growth chamber with rigidly controlled conditions) and testing to determine if L. palmatum and L. microphyllum have endogenous inhibitors of spore germination as is found in L. japonicum (Yamane et al., 1980) would be most informative. A thorough search should be conducted in north Florida to see if a natural population of L. palmatum still exists. Longer term experimentation and cultivation could determine whether L. palmatum can grown to maturity under natural conditions in central and south Florida. As a continuation of the present work, Profesor James Wallace of Western Carolina University has agreed to study the flavonoids of all three species utilizing materials furnished by me from Florida for L. japonicum and L. microphyllum. He has access to natural populations of L. palmatum to complete the study which is part of his continuing work on the phytochemstry of Schizaeaceae. Continuation of experimental work based on cultures may follow

publication of the new results and information developed during the course of this study.

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