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A MONOGRAPHIC STUDY OF *LYGODIUM* SWARTZ (PTERIDOPHYTA: LYGODIACEAE)

by

JUDITH GARRISON HANKS

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1998

This manuscript has been read and accepted for the Graduate Faculty in Biology in satisfaction of the dissertation requirement for the Degree of Doctor of Philosophy.

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ABSTRACT

A MONOGRAPHIC STUDY OF *LYGODIUM* SWARTZ (PTERIDOPHYTA: LYGODIACEAE)

by

Judith Garrison Hanks

Chairman: Dr. John T. Mickel

A monographic study of the pantropical fern genus *Lygodium* has been undertaken, based on the gross morphological comparisons of approximately five thousand herbarium specimens and on phytochemical analysis and spore architecture. The biogeography and ecology, paleobotany, cytology, and gametophyte morphology are summarized. Interspecifically the genus is homogeneous, and yet, intraspecifically it is polymorphic. To resolve some of the problems created by this inherent variability, phytochemical, spore, and sporophyte characters were included in a cladistic analysis to reflect species relationships.

Twenty-six species of *Lygodium* are treated along with three hybrids. The taxonomic treatment includes a review of the nomenclature and a key to the species. General morphological features found to be most consistent in *Lygodium* are type of branching pattern, size of pinna-stalk, articulation, pulvini, margin pattern, venation pattern and degree of dimorphism. Characteristics that are most variable are indument amount, segment shape and size, and size of sorophores.

The phytochemical analysis resulted in the isolation of twenty-two hydroxycinnamic acid conjugates from *L. japonicum*. A methodology for the extraction, purification, and preliminary identification of these compounds has been developed. The compounds elucidated, thus far, are β-1-O-caffeoylglucose, 4-O-(E)-caffeoylglucose, 4-O-*p*-coumaroyl glucose, and an as

yet unnamed compound that is new to natural product chemistry. The co-occurrences of these compounds in other species of *Lygodium* were then analyzed to determine the importance of these chemicals in the taxonomy and phylogeny of *Lygodium.*

Spore morphology studies indicate that the overall pattern is constant intraspecifically and a very useful character in species identification. The spore architecture revealed four basic surface patterns in *Lygodium*: tuberculate, ridged-verrucate, reticulate and granulate. All twenty-six species were surveyed resulting in a key based entirely on spore morphology.

The cladistic analysis included 42 characters with 111 states. *Lygodium flexuosum, L. japonicum, L. kerstenii* and *L. venusum* represent the most ancestral clade. The rest of the taxa are divided into two monophyletic groups with *L. polystachyum* as the intermediate species between them and the ancestral group. Based on outgroup analysis the following are considered pleisiomorphic in *Lygodium*: short-branching rhizome, pinnate branching pattern, pubescence, entire segments, and lack of articulation or pulvini.

DEDICATION

This thesis is dedicated to Dr. Rupert Barneby who has graciously shared his systematic and botanical knowledge with me as well as enlightened me with his humor and philosophy.

ACKNOWLEDGMENTS

I would like to thank Dr. John Mickel for serving as my mentor in this endeavor. He shared his enormous knowledge of ferns with me and never dissuaded me from asking questions (of which I had an enormous number) or interrupting his own studies. Dr. Mickel's gift of enthusiasm for botany and specifically, ferns, is infectious. Many individuals are learned, few are enthusiastic, encouraging, and willing to share their experience and knowledge. The years spent in the Fern Herbarium as part of the fern family have been a happy and rewarding time indeed! I would also like to gratefully acknowledge the support and assistance of Mrs. Carol Mickel. There were many instances when her kind words and reassurances provided me with the timely encouragement I needed.

My initial motivation to pursue this degree was the result of a Biochemical Systematics course taught by Dr. P. Mick Richardson. Throughout the years Mick has provided me with not only his expertise but also his unending encouragement and understanding: for this I remain ever grateful. He has come to New York for all the exams and interim meetings as a member of my doctoral committee and I am indebted to him for his review of this dissertation and guidance throughout the years.

Dr. Dennis Stevenson, another member of my doctoral committee, has provided me with both technical and financial assistance as Director of the Harding Laboratory. Dennis helped support my phytochemical and SEM studies while at the same time providing continuous intellectual stimulation. His knowledge of plant anatomy and morphology, as well as cladistics provided the backbone for much of this work. Anyone who has spent time in the lab

understands the incomparable botanical knowledge that Dennis possesses as well as an often, much needed, sense of humor.

Dr. Barbara Meurer-Grimes taught me Phytochemistry from a technical and academic standpoint. Due to her expertise we were able to isolate, purify and identify 22 compounds, three of which are new to natural product chemistry. I am grateful for her critical, careful, and supportive review of my dissertation. The structural elucidation of the compounds is largely the result of her expertise. The timeliness with which Dr. Meurer-Grimes continued the chemical analyses and then read this dissertation was remarkable considering she had just moved to Australia and started a new career. I am indebted to Dr. Ruth Stark as Director of the Nuclear Magnetic Resonance Facility of the College of Staten Island. Dr. Stark and Dr. Cherryl Tihal are responsible for the experimentation and analyses that lead to the structural elucidation of the six compounds isolated in this study.

Dr. W. H. Wagner, as a committee member, also provided me with inspiration, enthusiasm and renewed commitment whenever he visited New York. His review of my dissertation and comments were gratefully appreciated. I am also grateful to Dr. Robbin Moran for all the fern conversations, biogeographical expertise, and encouragement since his arrival at the Garden. His comments helped improve this study and I thank him for serving on my committee.

I wish to thank Dr. Thomas Jensen who generously gave me access to the SEM facility at Lehman College and supported an important facet of this dissertation. Dr. Jensen has served as a member of my committee since its inception and has always been supportive of the study. I would especially like to thank Mr. Michael Baxter of Lehman College for generously sharing his expertise and experience in the preparation and use of the SEM.

Dr. Rupert Barnaby has helped mold my ideas on systematics, botany, and life over the past 8 years. I am grateful for his critical but encouraging review of this dissertation.

The staff of the Herbarium at NYBG managed the many loans and provided me with support and friendship throughout the years. I am especially grateful for my friendship with Sarah Hunkins and Eileen Whalen. Without the help and support provided me by Drs. Patricia Holmgren and Jacqueline Kallunki in providing me space to store my specimens and an area to work, this monograph would have been impossible. Dr. Kallunki has always been especially encouraging and has on numerous occasions helped me keep my perspective. My friendship with Lisa Campbell has been an invaluable asset both botanically and personally. I have many fond memories of the Sorting Room and thank all who have helped make the last years enjoyable.

I would also like to thank Dr. William Wayt Thomas for employing me on the 'Coastal Forests of Brazil' project for 8 years as his Curatorial Assistant. He has always been supportive of my research and generous with his time, knowledge and facilities. I learned much about curating and working with him was an enjoyable and knowledgeable experience.

Many staff members of the Garden have given me support over the years. I would especially like to thank Ms. Sandy Guiot for all her help in many facets of this degree; Ms. Muriel Weinerman for cheerfully helping with the photographic work; Ms. Mobee Weinstein for the "growing advise" and diligently tending to *Lygodium*; and Ms. Sondra Lebost for her support and friendship. The Fern Herbarium has many volunteers with whom I have had happy experiences, especially Ms. Ruth Russell, who also helped confirm my German and French translations. Ms. Eth Williams taught me the art of growing ferns from spores. I am grateful for having known and worked with

the late Joseph Beitel. Dr. Ivan Valdospino remains a good friend and colleague. As fellow graduate students in the Fern Herbarium, we spent much time learning and providing support and encouragement to each other. I cherish those days and am grateful that we were both students at the same time. Gracias, mi amigo. I remain most grateful for the nomenclatural knowledge imparted to me by Dr. Michael Nee. Michael also provided me with space when I desperately needed it, listened "cheerfully" to my endless verbiage in the writing stage of this work, and recounted numerous dissertation defense stories to alleviate my nervousness. I would like to thank Mr. H. Fukuda for the wonderful species illustrations. Ms. Amy Ferriter and Dr. Robert Pemberton provided me with valuable information on the spread of *L. microphyllum* in Florida and current methods under study to control this introduced species.

I am most grateful for the continued support of Ms. Joan Reid at the CUNY Graduate Center: her guidance, advise and encouragement were always appreciated.

I am indebted to the following herbaria[1] who made their collections available for study: B, BH, GH, K, MICH, MO, P, S, and UC.

I would like to thank my mother for her unending, selfless, quiet support. Lastly I would like to most sincerely and simply thank my husband, Jess Hanks, who provided both botanical and technical assistance as well as continual personal encouragement: without his help this dissertation would not have been completed.

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[1] Herbaria acronyms are given according to Holmgren, et al. (1988).

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CHAPTER I

INTRODUCTION

Lygodium is a pantropical genus of leptosporangiate ferns that is unusual in its vining growth habit. Indeterminate rachis growth, delayed pinna expansion, circumnutation and a dormant pinna-bud which begins growth when damage to the rachis occurs, enable *Lygodium* to climb trees 30 m tall or create thicket-like entanglements. It is this combination of unusual growth characters that makes *Lygodium* unique and enables most botanists to readily recognize the genus. Twining leaves are found in *Salpichlaena* but that genus lacks a dormant pinna-bud. The leaves of members of the Gleicheniaceae and *Phanerosorus* possess dormant rachis and pinna-buds but do not have a twining growth habit.

Sporangia occur on marginal projections of blade segments on the highest, sunniest portions of the vine. These sporangia occur individually in two rows on either side of the midvein and are covered by an indusial flap. The lamina of the fertile pinnule may be almost completely suppressed giving the fern a delicate, highly dissected appearance. In many areas *Lygodium* is considered a weedy unwelcome member of the vegetation, often scrambling over shrubs and producing dense thickets in disturbed areas or at the margins of forests. At the other extreme, the native North American species, *Lygodium palmatum*, was the first plant in the United States to be considered endangered. Its beautiful palmate, evergreen leaf segments provided decoration as garlands at Christmas in Northeastern communities. In 1869 Connecticut passed laws to protect *L. palmatum* and in 1875 the law was codified. D. C. Eaton (1879) indicated that this was the first time in the United States that a statute

designated a plant to receive special status because of its beauty (Montgomery and Fairbrothers, 1992).

Lygodium consists of 26 species in this current treatment. As originally perceived by Prantl (1881) there were 22 species and a subsequent revision by Reed (1946) had 41 extant species and 15 fossil species. No complete study of the genus has been published since Reed and no in-depth study since Prantl, with the exception of an unpublished doctoral thesis in 1956 by Dr. Elizabeth Valentine (University of Pennsylvania). Dr. Valentine's work followed the scheme of Prantl, included 32 taxa, and based all of the net veined species on hybridization events. However, many regional treatments of the genus have added not only numbers of taxa but also important information on distribution, ecology, and morphological variation (e.g., Copeland, Philippine Islands, 1947; Christensen, Madagascar, 1932; Holttum, Flora Malesiana, 1959; Duek, Tropical America, 1978; and Singh and Panigrahi, India, 1984). The tremendous morphological variation in *Lygodium* has been the primary contributor to the number of species described. The resulting taxonomic inflation has been based on the acceptance of these variants as distinct species [e.g. in Genera Filicum (1947), Copeland cited 39 species; Duek (1978) reported 49 species]. This study recognizes the plasticity of many characters (e.g., pubescence, segment shape and size) within a species and thus the number has been reduced to 26 species.

Lygodium is pantropical with the exception of one temperate North American species and subtropical outliers in New Zealand, Australia, and Japan (Fig. 1.1). The majority of the species are found in the Old World tropics, with seven taxa occurring in the New World. There are African/American, Asian/African, and Australasian alliances. The fossil record dates the genus to

Figure 1.1. The global distribution of *Lygodium*.

the Jurassic/Cretaceous boundary with a distribution that extended throughout Northern Europe, Asia and North America (Fig. 1.2)

The genus is placed by some pteridologists in the Schizaeaceae *sensu latu*, a diverse family of 5 or 6 genera: *Anemia, Mohria, Schizaea, Actinostachys, Lophidium* (excluded in some treatments) and *Lygodium* (e.g., Prantl, 1881; Christensen, 1905; Copeland, 1947; Alston and Holttum, 1959; Duek, 1978; and Tryon and Tryon, 1982). The genera are extremely diverse morphologically as can be seen by the comparison of *Anemia, Lygodium* and *Schizaea* and *Actinostachys* in Table 1.1. The members of the Schizaeaceae *s.l.* are thought to be held together by the characteristic sporangium with subapical annulus, sporangia borne singly on the abaxial leaf surface and a long fossil history dating the family back at least to the Cretaceous. The sporangium may not be a reliable phyletic character as this type of sporangium may have evolved more than once (Bierhorst, 1971; Jennings and Eggert, 1977). Others have raised the genera to family status, Lygodiaceae (*Lygodium*), Anemiaceae (*Anemia* and *Mohria*), and Schizaeaceae *sensu stricto* (*Schizaea* and *Actinostachys*) in the order Schizaeales (Nakai, 1937; Bierhorst, 1971; Reed, 1946; del la Sota and Morbelli, 1987). This hierarchy was adopted in the recent Flora of North America series (1993). Cladistic analyses of rblc and morphological data sets hypothesize that the Schizaeaceae *s.l.* represents a monophyletic group that diverged very early on, thus, partially supporting an elevation of the genera to familial status (Hasebe *et al.*, 1995; Pryer *et al.*, 1995).

Nomenclatural and taxonomic problems are inherent in the study of any pantropical genus that has a long botanical history and numerous geographically distinct treatments. The current study tries to combine new methodologies (phytochemistry, spore ultrastructure, and cladistics) with the enormous contributions of an array of botanists to produce a modern

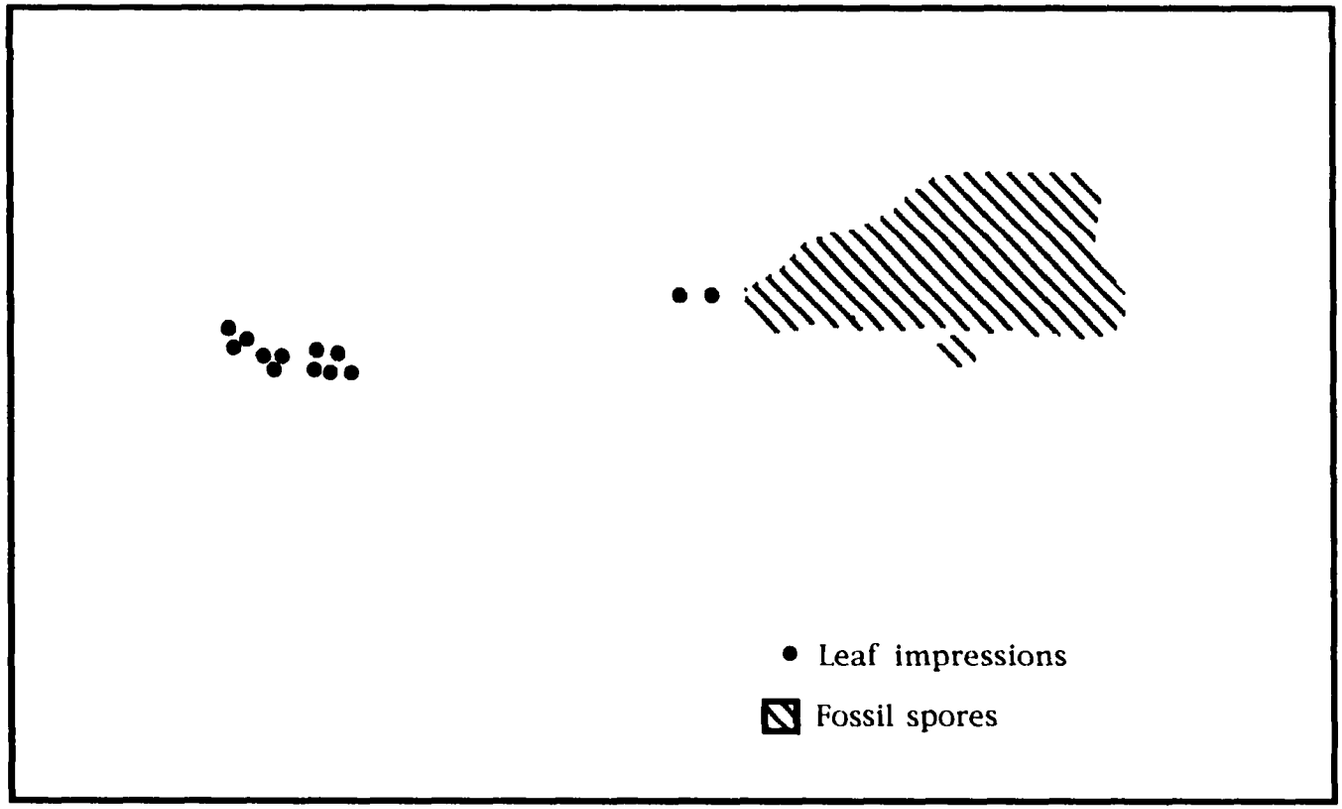


Figure 1.2. The distribution of fossil material of *Lygodium* from the Cretaceous geologic period. Dots are sites of leaf impressions. The shaded areas are the distributions of fossil spores (Bolkhovitina, 1959).

Table 1.1. A comparison of Schizaeaceous genera.

	Lygodium	*Actinostachys* & *Schizaea*	*Anemia*
Distribution	pantropical subtropical S.A., N.Z. & Japan, 1 spp. temperate U.S.	pantropical mostly Old World 1 spp. temperate U.S.	tropical mostly New World
# Species	26	± 45	± 120
Ecology	open forest (borders), shrubby savannahs, disturbed vegetation	diverse: open sandy soil; rocks, bogs, shaded forest	open habitats, well-drained sites; rarely forest
Rhizome	short, creeping	short, creeping or ascending	short, creeping horizontal
Indument	black hairs	brown hairs	dark orange or brown hairs
Stele	medullated protostele	medullated protostele	dictyostele
Fronds	climbing rachis indeterminate; pinna axis short - dormant apex + 2 pseudodichotomous pinnules or pinnate; pinnules palmately lobed or cordate	erect, undivided to dichotomously, forking, grasslike	erect, simple, pinnate to tripinnate
Fertile Parts	hemidimorphic sporangia on finger-like projections on ultimate segments;	hemidimorphic sporangia on flaglike proj. or at apex of div. vein fork ± hairs	hemidimorphic sporangia on erect, lower-most pair of pinnae to fully dimorphic
Venation	free to rarely net	free	free to rarely net
Stomata	diacytic anomocytic	hypocytic	pericytic desmocytic polocytic
Indument	hairs; single- to multi-celled	hairs; multi-celled	hairs; single- to multi-celled

Table 1.1 (continued).

	Lygodium	*Actinostachys* & Schizaea	*Anemia*
Sporangia	oblong with subapical annulus covered by basally attached indusial flap; laterally attached	oblong with subapical annulus; basally attached	oblong with subapical annulus; basally attached
Spores	tetrahedral 53-102 μm verrucate, tuberculate, reticulate	bilateral 30-100 μm smooth to verru. to striated	tetrahedral 65-120 μm striated. parallel ridges, with or without spines
Base Chromosome #	28,29,30	77,94,96,etc.	38
Gametophyte	cordate	filamentous, tuberous	asymmetric cordate
Antheridiogens	Yes	? to No	Yes
Paleobotany	Jurassic (*Klukia, Stachypteris*)	Lower Cretaceous trilete; *Schizaeaopsis;* no monolete spores before Quaternary	Lower Cretaceous *Ruffordia*

taxonomic revision of the genus. Morphological variability and subtle intergradations of characters, combined with polyploidy produce intrinsic problems in the taxonomic analysis of this genus. There can be many approaches to this issue, from producing a treatment of pantropical polymorphic taxa in which characters are extremely variable, to one containing specialized species with narrow ranges and single characters to differentiate them. This treatment lies somewhere between these two extremes.

History of the Genus

Many botanists are responsible for unravelling the nomenclatural problems associated with *Lygodium.* At the generic level gratitude must be given to Dr. Rudolfo Pichi Sermolli who in 1956 published his research on the nomenclature of the Schizaeaceae *s.l.* Between the years 1753 and 1842 *Lygodium* received 10 generic names: *Ophioglossum* Linnaeus (1753), *Lygodium* Swartz (1801), *Ramondia* Mirbel (1801), *Ugena* Cavanilles (1801), *Odontopteris* Bernhardi (1801), *Gisopteris* Bernhardi (1801), *Hydroglossum* Willdenow (1802), *Cteisium* Michaux (1803), *Villifilix* Thouars (1808), and *Lygodictyon* J. Small (1842). As can be seen, five of the names appeared in 1801. Pichi Sermolli (1956) presents a sequence for the names historically as well as selects lectotypes for the genera. Even though *Ugena* Cav. and *Ramondia* Mirbel have priority, *Lygodium*, almost universally adopted from the time of its publication, was conserved in 1954 (Taxon 3:69-70, 156, 233. 1954). Swartz originally described four species in his genus: *L. scandens* (L.) Sw., *L. flexuosum* (L.) Sw., *L. pedatum* Sw. and *L. japonicum* (Thunb.) Sw. Christensen (1913) selected *L. flexuosum* as the lectotype of the genus and this choice was followed by Reed (1946), whereas Underwood (1899) and Copeland (1947) selected *L. scandens.* Since Swartz confounded two species under the

name *L. flexuosum* (*Ophioglossum flexuosum* L. and *Ophioglossum circinnatum* Burm.), Pichi Sermilli (1956) felt that it should not serve as the lectotype for Swartz's genus. *Lygodium pedatum* was also rejected as it is not only a synonym of *L. circinnatum* (Burm.) Sw., but was renamed*L. longifolium* by Swartz himself in 1803. Thus, from the remaining*L. japonicum* and *L. scandens*, Pichi Sermolli chose the latter to conform with Underwood (1899). *Lygodium scandens* (L.) Sw. was included as the type of the genus when *Lygodium* was placed in the list of *Nomina Generica Conservanda* (1954). The nomenclatural complexities of the genus were further elucidated by Alston and Holttum (1959), who realized that the basionym, *Ophioglossum scandens* L., really represented a combination of 5 plants. These include a specimen of *L. volubile* Sw. from Brazil (Linnaeus' first quoted reference from Hortus Cliffortianus, 1737), a specimen of *Ophioglossum flexuosum* L. from Ceylon(*Hermann 374*, the sterile version of *Hermann 375* on which Linnaeus' based his description of *O. flexuosum*), two drawings of *L. venustum* from Brazil (Breyne, Cent. 185, t. 96 and a figure by Morison copied from Breyne) and a drawing of *O. flexuousm* L. (Rheede, Hort. Malab. 12: 65, t. 33). This last drawing is difficult to interpret: it is fertile and many of the segments have basal lobes that would indicate *L. flexuosum.* However, rarely does *L. flexuosum* have as many segments/pinna-branch. In general, I concur with Alston and Holttum's interpretation that Rheede's drawing most closely resembles *L. flexuosum.* None of the specimens cited by Linnaeus in his description of *O. scandens* are the species we currently recognize by that name. Since Swartz had segregated two of the plants Linnaeus based his description on as other species (*L. volubile* and *L. venustum*), the sterile *Hermann* specimen (375) became the best choice to be designated as the lectotype of *O. scandens* L. (Alston and Holttum, 1959). Thus, *O. scandens* L. is a

synonym of *O. flexuosum* L. and *L. scandens* (L.) Sw. becomes a synonym of *L. flexuosum* (L.) Sw. For this reason, Panigrahi and Singh (1983) proposed to change the type of *Lygodium* from *L. scandens* (L.) Sw. to *L. flexuosum* (L.) Sw. This petition was denied by the nomenclatural committee (1986) who did not feel it was necessary to change the lectotype simply because it was a synonym.

The genus *Lygodictyon* J. Sm. (1842) segregated the net-veined *Lygodium reticulatum* into its own genus. Presl (1845) followed this scheme by adopting *Hydroglossum* Willd. for the net-veined taxa and *Lygodium* Sw. for the free veined species. Presl's treatment included 41 species and 5 varieties. Hooker and Baker (1868) returned the taxonomy to one genus (*Lygodium*) and reduced the net-veined and free-veined taxa to subgenera (Eulygodium, free veins and Hydroglossum, net veins). Their final treatment contained 18 species. Prantl (1881) realizing the lack of phyletic significance of net-veins divided *Lygodium* into three subgroups based on vein emergence from the base of the segments resulting in 23 taxa and 6 varieties or forms.

Terminology

Lygodium has presented the fern taxonomist with terminology problems ever since descriptions of ferns have been written. The indeterminate rachis growth, one of the distinctive features of this genus, has made the use of standard fern terminology difficult. This vining growth habit makes the terms frond, rachis, pinnae, and pinnule confusing. Many botanists have used numerical ranks such as primary, secondary, and tertiary branches to describe the hierarchal divisions of the pinna and diminuitives as petiolule and rachilla to describe leaflet appendages. Recently, Anderson and Ollgaard (1996) presented a terminology for Gleicheniaceae, recognizing that the pseudodichotomous branches and indeterminate leaves presented special problems. Similarly, I am presenting a terminology here to account for the

structures of *Lygodium* with comparisions to some of the major terms used by previous taxonomists (Fig. 1.3). Some of the terms employed are those used by Holttum (1959) and subsequently adopted in varying degrees by Tryon and Tryon (1982).

In *Lygodium* the frond is indeterminate, specifically the rachis of the frond. The rachis bears alternate pinnae which immediately divide, pseudodichotomously, into opposite pinnules subtended by a dormant "pinna-bud" in the axil. The "pinna-stalk" is used for the reduced pinna, and primary pinna-branches bear either segments or secondary pinna-branches. Therefore, the pinna-branch with its divisions represent the pinnule. The pinna-branches may end in segments, divide dichotomously and end in segments, or be pinnate. The segment may be attached to the axis (pinna-branch) by a segment petiole. All degrees of dissection are described from the primary pinna-branch. The ultimate leaflets are called segments. The midvein of the segment is the costa. The stipe is the portion of the frond from the rhizome to the first pinna-branch or dichotomous division in juvenile plants. All sizes of fronds are approximate. Axes refer to any branch or non-laminar division.

Fertile divisions in *Lygodium* may resemble the sterile divisions (monomorphic) with the sporangia borne on fingerlike projections from the segment margins or have stages of dimorphism in which there is a reduction of the lamina on fertile segments to a complete suppression of leaflet tissue. Prantl (1881) used the term sorophore for these projections: the term indicates a portion of a leaflet bearing sori. He regarded the single sporangium as a monangial sorus. These terms have been adopted in this treatment. Reed (1946) used the terms sporangiophore. The flap-like

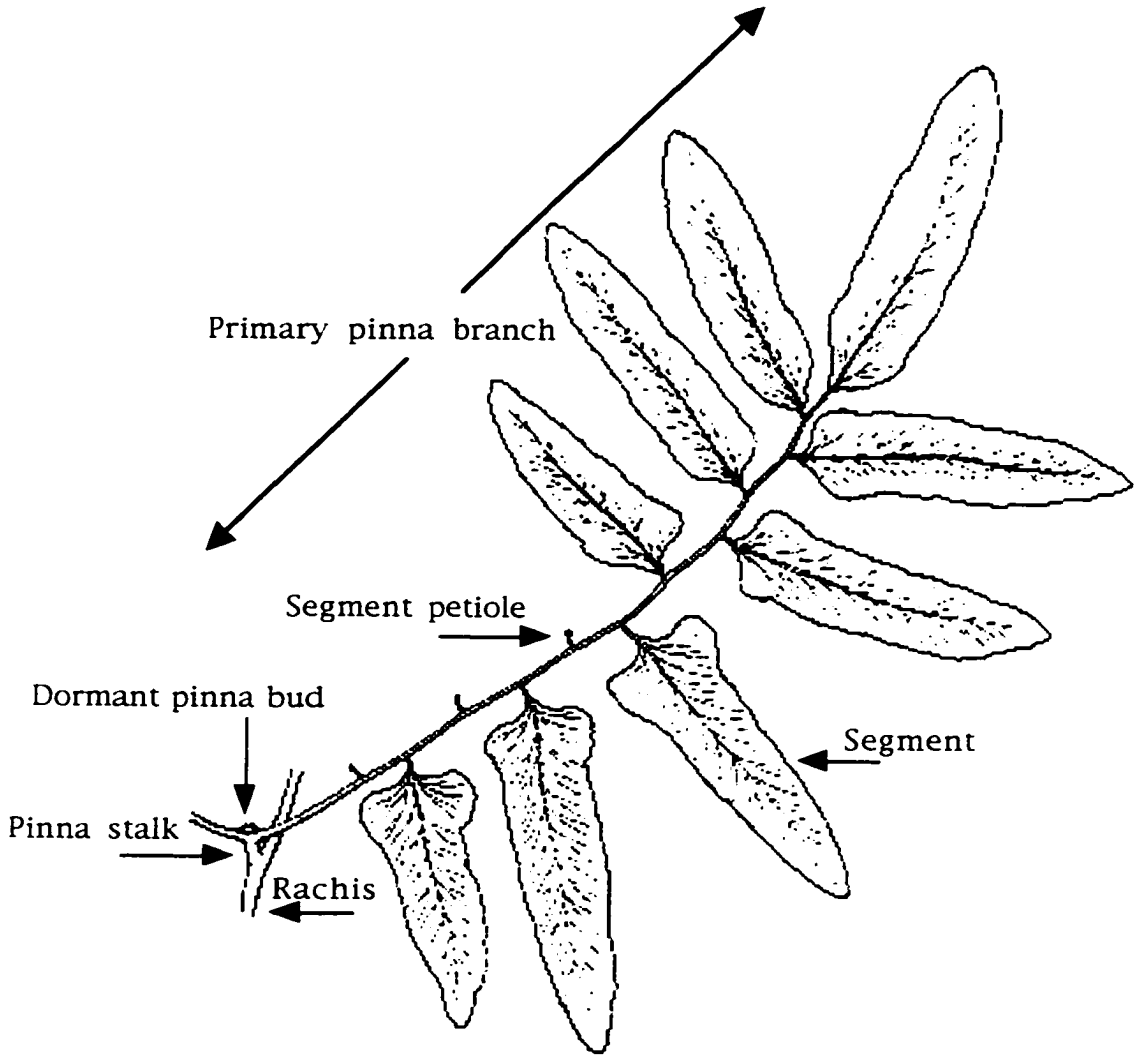


Figure 1.3. Illustration of the terminology used in descriptions of *Lygodium*.

covering over the monangial sorus is an outgrowth of the sorophore or lamina of the segment and therefore fits the definition of an indusium.

In pteridology a useful descriptor is the number of times the frond is pinnate. All taxa of *Lygodium* are at least bipinnate due to the fact that the pinna apex is dormant. In this treatment the number of times pinnate refers to pinnate from the primary pinna-branch. Therefore, *Lygodium microphyllum* is always once pinnate from the pinna-branch whereas *L. japonicum* is 2-3 times pinnate (refer to Figs. 10.36A and 10.34E,F respectively).

The following is a short summary of some of the more common terms used in this treatament and their equivalents in prior treatments.

rachis:
- primary axis (Prantl, 1881; Tryon and Stolze, 1982)
- primary rachis (Maxon, 1909)

pinna stalk:
- primary branch (Duek, 1978; Maxon, 1909)
- primary rachis branch (Holttum, 1959; Singh and Panigrahi, 1984)
- dwarf branch (Prantl, 1881, Copeland, 1958)
- primary petiole (Hooker, 1874)
- pinna rachis (Tryon and Tryon, 1982)

pinna-bud:
- abortive bud (Copeland, 1958)
- stalk (Lellinger, 1989)

primary pinna-branches:
- secondary pinna (Maxon, 1909; Proctor, 1989)
- rachises of pinna (Copeland, 1958)
- secondary petioles (Hooker, 1874)
- secondary rachis branches (Holttum, 1959; Singh and Panigrahi, 1984)
- secondary branches (Lellinger, 1989, Duek, 1978)
- secondary segment (Prantl, 1881)

segment petiole:
- stalk of leaflet (Hooker, 1874; Duek, 1978)
- leaflet petiole (Lellinger, 1989; Proctor, 1989; Tryon and Stolze, 1982)

segment:
- ultimate divisions = pinnule (Hooker, 1874)
- half pinnae, pinnules, ultimate pinnules (Copeland, 1958)
- leaflet (Holttum, 1959; Duek, 1978)
- tertiary segment (Prantl, 1881)
- pinnules (Singh and Panigrahi, 1984)

sorophore: marginal spike (Copeland, 1958)

indusium: outgrowth of sporangium (Copeland, 1958)
laminar outgrowth or flange (Tryon and Tryon, 1982)

Economic Uses

In many Old World tropical areas the rachis of *Lygodium* is used for its strength: weaving handbags in the Philippines and Thailand, making roofs for huts in Borneo, for thatching and making hammocks in New Zealand by the Maori, and wiring together canoes in Malaysia. In Borneo an endemic bird species uses the vines to make nests.

The plants are also used for their chemical components to treat an assortment of ailments. In Bihar and Orissa, *Lygodium flexuosum* (from dry/moist deciduous forests at 300-800 m) is used to treat malarial fever (root), rabies, to conrol dysentery and treat cholera (Girach and Aminuddin, 1989). Since many tribes use it for the same disorders there is some potential for concrete medicinal properties. *Lygodium circinnatum* leaves are used topically to treat pain in the Nicobar Islands off the coast of Sumatra, while the rhizome stele is used as straw (Dagar, 1989). *Lygodium japonicum* was found to be active against the Sindbis virus (a single-stranded, enveloped RNA virus of animals) with the activity increasing 10-100 fold on exposure to ultraviolet light (Taylor *et. al.*, 1996). The chemical studies done in this work produced many UV active compounds. Antibacterial properties were reported for this taxon in an earlier study (Mitscher, 1978).

CHAPTER II

FOSSIL *LYGODIUM*

The distinctive fertile foliage and indeterminate growth habit have aided paleobotanists in recognizing fossil species of *Lygodium.* The species was widespread in the Eocene in Europe, North America, Australia and Asia. Alliances based on macrofossil evidence are found in the Jurassic and Upper Cretaceous. The earliest well-documented macrofossil ancestor of *Lygodium* is the Jurassic genus *Klukia* , and possibly *Stachypteris* (van Konijnenberg-van Cittert, 1981).

The earliest fossils attributed to the Schizaeaceae *s.l.* are the late Devonian/Early Carboniferous *Senftenbergia*, (Radforth, 1938, 1939). Macrofossil studies of the fertile pinna have suggested this genus is Schizaeaceous due to the characteristic arrangement of single sporangia in two rows abaxially on each side of the vein. However, the flask-shaped sporangium with subapical annulus has been the character used most in determining Schizaeaceous ancestry. In *Senftenbergia*, the sporangia contain an annular patch of thick-walled cells (2+ cells deep) encircling the apex, with a longitudinal dehiscence. Each sporangium is attached by a short stalk. The genus was placed by Reed (1946) into its own family, Seftenbergiaceae, in the order Schizaeales. Radforth (1939) demonstrated a hypothetical sequence in the placement of the annulus through a series of positional changes ending in the subapical, single row type that exists in extant Schizaeaceae s.l. His developmental sequence considers the most primitive type *Senftenbergia sturi* (Sterzel) Radforth from the Lower Carboniferous in Britain with an irregular patch of cells around one side of sporangium. Then *S. ophiodermatica*

(Geopert) Stur in the Upper Carboniferous had a 2-3 celled annulus with an irregular arrangement. *S. plumosa* (Artis) Radforth from the Upper Carboniferous had an annulus moving apically and containing 2-3 rows of cells. Finally, the most recent, *S. pennaeformis* had an apical annulus of 1-2 layers.

Senftenbergia pennaeformis Brongn. contained an estimated 1300 spores/sporangium. The spore surface contained anastomosing ridges (reticulate) with projections from the ridges and the size ranged from 52-70 μm. It is difficult to find genera in the Schizaeaceae to which these fossil taxa are closely allied. *Senftenbergia* has a short-stalked sporangium as does *Lygodium* (*Lygodium* may have a few cells present while the sporangia of *Anemia* are sessile). Its 2-3 rowed annulus may be more closely related to the 1-rowed annulus of *Lygodium* which is often irregular so that a transitional 2-cells may be present. However, the sporangium in *Lygodium* is laterally attached which is not the case in *Senftenbergia*. The spores are more like those of *Anemia*, although both *Anemia* and *Lygodium* have taxa with reticulate spores. Radforth (1939) felt the alliance was closest to *Anemia*; however, an extant Schizaeaceae family alliance was difficult to assign.

Recently the alliance of *Senftenbergia* with the Schizaeaceae *s.l.* has come into dispute. Jennings and Eggert (1977) suggest the sporangial character alliance is superficial. The classic subapical annulus can be found in extant *Stromatopteris* and *Gleichenia*, while in the Osmundaceae, the annulus is apical early in development and, at maturity, becomes displaced laterally by differential growth. Anatomical studies on the rachis, pinna and sporangia of partially petrified specimens of *Senftenbergia* by Jennings and Eggert (1977) and sporangial and spore studies by van Konijnenburg-van Cittert (1981) place the genus with the extinct Tedeliaceae (genera *Ankyropteris* and

Clepsydropsis). The vascular strand contains two peripheral loops which do not occur in the Schizaeaceae. According to Jennings and Eggert (1977), to go from the looped vasculature of *Senftenbergia* to the simple stele types of the extant Schizaeaceae would have involved more than one reduction. The Tedeliaceae, on the other hand, have stalked sporangia, an annulus of 2-4 rows of cells, and a petiolar anatomy similar to that found in *Senftenbergia*. Galtier and Phillips (1996) suggest that the Tedeliaceae evolved a sporangium with an apical annulus early in the Namurian. However, they are not directly related to modern Schizaeaceae as they differ anatomically, but could represent the fossil sister group to the extant Schizaeaceae. This would remove *Senftenbergia* from any direct relationship with modern Schizaeaceae. Jennings and Eggert (1977) point out that it is difficult to base the ancestral alliance of a fossil genus on one character (e.g., sporangial morphology). Bierhorst (1971, pg. 266) agrees that any affinities based on this type of sporangium is superficial:

> "The sporangia found in the extant Gleicheniaceae, Stromatopteridaceae, Schizaeaceae, Anemiaceae, and Lygodiaceae form a structural continuum so finely gradate that none of these families may be characterized on the basis of this organ. Attempts to so characterize them in the past are based on characteristics that, it is becoming increasingly clear, are particulary unconservative."

Dispersed spores of *Klukisporites*, and the macrofossil genera, *Klukia* and *Stachypteris*, are presumed *Lygodium* ancestors in the Jurassic (possibly the late Triassic). Macrofossils and spores of *Klukia exilis* (Phillips) Raciborski are recorded from the Jurassic for a large area in North America, Western Europe and Eastern Asia. The macrofossils (England) were twice pinnate, the fertile pinnules bore 6-14 sporangial pairs and the sporangium contained a single row of annular cells. The sporangial attachment was at the base rather than at the side as in extant *Lygodium*. Spores from the Lower Deltaic of England of *Klukia exilis* studied by van Konigpenberg-van Cittert (1981) partially

resemble reticulate spores of extant species, *L. microphyllum* or *L. reticulatum*. However, in the fossil, the proximal face architecture is verrucate. The extant reticulate spores of *Lygodium* have either smooth or reticulate proximal faces. Fertile segments of *Stachypteris spicans* Pomel of the Jurassic resemble those of *Lygodium* whereas the spores, recently restudied using SEM by van Konijnenberg-van Cittert (1981) differ in being less reticulate distally (many unfused ridges) and granulate proximally. There are more discrepancies in assigning an alliance of this genus with extant *Lygodium* than there are with *Klukia*.

Many spore collections from the Jurassic have been attributed to *Lygodium* and assigned species status (Bolkhovitina, 1959). The fossil spores are trilete with tuberculate, verrucate and reticulate surface patterns.

In the Cretaceous of Europe and North America, the Schizaeaceae *s.l.* is represented by *Ruffordia, Pelletixia, Schizaeopsis, Schizaeangium, Schizaeopteris* and material identified as *Anemia*-like (Collinson, 1996; Dettman and Clifford, 1992). Most of these alliances are based on the distal annulus, with the exception of *Pelletixia* and *Schizaeopsis*, whose alliances are based on the inrolled pinnules and striate spores, the latter character indicative of extant *Anemia* and *Mohria*. Thus, *Ruffordia, Pelletixia* and *Schizaeopsis* are considered *Anemia*-like fossils. There are two Late Cretaceous taxa of *Lygodium, L. pumilum* (Brown, 1943) and undescribed material from New Jersey (Radcliffe *et al.*, 1995).

Tertiary fertile and sterile foliage of the fossil *Lygodium kaulfussii* suggest affinities with extant *L. palmatum* (Manchester and Zaveda, 1987). Sterile segments were palmately lobed, the base obtuse to cordate and the margins entire to undulate. An articulation occurred at the petiole/segment junction. This articulation is missing in extant *L. palmatum*. In *L. kaulfussii*

the fertile frond segments are nonlaminar, bearing single sporangia in rows covered by indusia. Spores are 50-70 μm and psilate. This species is considered conspecific with the fossils, *Lygodium binervatum* (Lesq.) Berry (Mississippi) and *L. trilobatum* Berry (Tennessee). These fossils were widespread during the Eocene and Miocene in North America, Europe, and Northern Asia, with similar species in Australia, New Zealand and Chile (Halle, 1940). *Lygodium kaulfussi* had strongly dimorphic fertile segments. Since fossils with monomorphic forms have been found only in the Miocene (*L. mioscandens* Matsuo, fertile pinna from Neogene of Japan resembling extant, *L. microphyllum*; Matsuo, 1963), this suggests that the ancestral form of *Lygodium* was strongly dimorphic (Manchester and Zaveda, 1986). Sterile leaflets of the fossil, *L. pumilum* Brown (Upper Cretaceous, Wyoming) also resemble those of *L. palmatum:* sterile pinnules are palmately lobed, veins twice dichotomous (Taylor and Taylor, 1993) and spores reticulate (Chandler, 1955). *Lygodium skottsbergii* Halle from Eocene deposits in Southern Chile, also allied with *L. palmatum*, confirms a wider geographic distribution than is known today. The number of spores/sporangium in this fossil species were between 120 and 169, suggesting the number of 256, which is common in extant taxa (including *L. palmatum*). *Lygodium scottsbergii* has palmate leaflets without the articulation found in *L. kaulfussii* and scabrate to granulate spores (Reed, 1946).

Collinson (1996) suggests that the extinct sister group of the Schizaeceae is the Tedeleaceae (Early Carboniferous to Permian). The earliest *Lygodium*-like fossils appeared in the Jurassic (*Klukia* and *Stachypteris)* whereas *Lygodium* fossils occurred in the Cretaceous. The genus was widely distributed and abundant in the Cenozoic.

CHAPTER III

CYTOLOGY

The first chromosome studies in ferns, including *Lygodium*, were conducted by Manton and co-workers (Manton, 1950; Manton and Sledge, 1954; Roy and Manton, 1965), who recognized the significance of polyploidy in fern evolution and taxonomy. *Lygodium* has chromosome base numbers in an aneuploid series, n=28, 29, 30, with tetraploid and hexaploid variants. Often ploidy levels differ intraspecifically as is the case with *Lygodium circinnatum* *L. japonicum*, and *L. microphyllum*, which have both diploid and tetraploid forms. Table 3.1 summarizes the ploidy level of those taxa of *Lygodium* that have been studied. It is readily apparent that polyploids are common (7 of the 12 species) and diploids, in which no other cytological levels are present, are less frequent (5 of the 12 species).

There are some discrepancies in chromosome counts: one reported by Brownlie (1961) for *Lygodium articulatum*, in which $2n$ = 70 to give a base number of 35 (not known in *Lygodium*); and also a report of $2n$ = 60 for *L. flexuosum* by Abraham and coworkers (1962) which would introduce a second base number for this taxon (all other data confirms n=29). Clearly additional specimens must be studied.

The high chromosome numbers in homosporous ferns (most have numbers greater than 25; Pichi Sermolli, 1987) may be the result of low-numbered ancestors that underwent hybridization, chromosome doubling and subsequent aneuploidy (Pichi Sermolli, 1987). Roy and Manton (1965) in discovering n= 29 in *Lygodium*, suggested that species with n=28 and 30 were likely polyploid derivatives of ancestors with n= 14 and 15, respectively.

Hybridization and doubling could give the modern n = 28, 29, 30, or n=29 could have arisen via addition (n=28) or deletion (n=30) creating the present aneuploid series. In this scenario, n=29 does not represent the primitive form. Wagner and Wagner (1980) suggest, on the other hand, that the ancestral ferns had high chromosome numbers as a result of paleopolyploidy. In support of this hypothesis they point out that in homosporous ferns chromosomes are all relatively uniform in size, and secondarily those groups with low numbers today are the heterosporous ferns with the most specialized morphology.

Haufler (1989) suggests that primary speciation in ferns may be occurring primarily in diploids where variability in response to environmental and ecological pressures (with subsequent isolation events maintained by outcrossing) produce new species. Secondary speciation employs allopolyploidy and tertiary speciation returns genetic diploidy via reciprocal gene silencing (silencing of homologous loci in different individuals). Such silencing would produce phenotypically distinct variants within polyploid lineages. Klekowski (1979) suggested that polyploidy is selected for because it maintains genetic variation in lieu of the homozygotizing effects of self-fertilization common in bisexual gametophytes.

Only three hybrids with abortive spores have been found in *Lygodium*: *L. venustum* × *heterodoxum*; *L. venustum* × *volubile*; *and L. kerstenii* × *lanceolatum*. In all the specimens examined no other abortive spores were observed and yet there are numerous tetraploid variants and one hexaploid variant. In this study, since vouchered material of polyploids was not found, I was unable to find any recognizable character that would correlate with increased ploidy level (e.g., spore size, stomatal size, etc.).

Walker (1985) in studying the chromosome lengths of *Lygodium micans* (= *L. volubile*) and *L. venustum* found that the former exhibited a normal distribution whereas *L. venustum* showed a bimodal distribution suggesting "... the presence of two structurally different genomes and hence an alloploid origin..."pg. 163). This situation is paralleled in *L. flexuosum* from Borneo in which Roy and Manton (1965) report the presence of two size classes of chromosomes in the tetraploid variant and uniform lengths in the diploid form. It is unknown whether the ploidy levels observed in *Lygodium* are the result of auto or allopolyploidy.

Apomixis has not been observed in this study or reported in the literature for *Lygodium*. This form of asexual reproduction is most often associated with dry habitats. Three-quarters of apogamous ferns are polyploids, chromosome doubling occurs in sporogenesis producing gametophytes that have the same chromosome number as the sporophyte. Instead of fertilization occurring, the sporophyte develops directly by proliferation. Until more gametophytes are observed, preferably in the field, apogamy is probably not a mechanism occurring in *Lygodium*.

From a practical standpoint, it is impossible to know the ploidy level of most of the specimens one examines and since no correlation has been found between stomatal or spore size and increased ploidy level, it is speculative, at best, to discuss the extent that hybridization events or unreduced spore production in *Lygodium* have contributed to speciation. This is one area where more taxa and populations must be surveyed and observed for characteristics that might be different between diploid and tetraploid forms.

Table 3.1. Chromosome ploidy levels in *Lygodium*.

Species	Chromosome Base Number	Ploidy Level	Locality	Reference
L. circinnatum	29	2*n*=58 (2x) 2*n*=116 (4x)	N. Borneo Ceylon	Manton & Sledge, 1954
L. flexuosum	28	2*n*=112 (4x)	N. Borneo	Roy & Manton, 1965
L. japonicum	29	2*n*=58 (2x) *n*=58 (4x) *n*=58 (4x)	Japan Japan Ceylon	Nakato, 1990* Mitui, 1968 Manton & Sledge, 1954
L. longifolium	28	2*n*=112 (4x)	Malaya	Roy & Manton, 1965
L. microphyllum	30	2*n*=60 (2x) 2*n*=120 (4x)	Japan Japan	Mitui, 1967* Roy & Manton, 1965
L. palmatum	30	2*n*=60 (2x)	Michigan	Wagner, 1963
L. reticulatum	30	2*n*=60 (2x)	Fiji	Takamiya, 1995
L. salicifolium	28	2*n*=56 (2x)	Malaya	Roy & Manton 1965
L. trifurcatum	28	2*n*=56 (2x)	New Guinea	Roy and Manton, 1965
L. venustum	29	2*n*=116 (4x)	Trinidad	Walker, 1966
L. volubile	29	2*n*=58 (2x) 2*n*=184(6x)	Trinidad Jamaica	Walker, 1985 Walker, 1966

*cited in Takamiya, 1995

CHAPTER IV

ECOLOGY, DISTRIBUTION AND BIOGEOGRAPHY

Ecology

Most taxa of *Lygodium* grow in sun in relatively disturbed areas where there is access to shrubs and trees which provide the support for their vining behavior. Holttum called them "sun ferns" (1959). Once established, the plants spread rapidly by rhizome branching. Each frond contributes to the formation of a thicket by outgrowth of the dormant pinna-buds. *Lygodium* can quickly move up into the canopy and laterally spread over vegetation. The establishment of new colonies by spore dispersal is variable depending on the site of colonization and gametophyte vigor. The gametophyte requires a moister environment than the sporophyte and new sporophytes take long periods of time for rhizome establishment. Once established, the sporophyte rhizome branches rapidly. Thus, many taxa of *Lygodium* are geographically rare but locally abundant (e.g., *L. palmatum*).

Many species prefer moist habitats whereas a few taxa do well in areas with significant dry seasons. *Lygodium venustum* grows throughout the New World in the wet forests of the West Indies and Mexico to the forest margins of Bolivia forming dense thickets. In many areas it is regarded as a weed. It can also be found in Costa Rica growing in very dry oak forests (R. Moran, pers. comm.). One species, *Lygodium microphyllum*, can grow in standing water, its rhizome at the soil surface. It has been introduced into the New World and grows in the cypress swamps on the east coast of Florida, primarily in Palm Beach and Martin Counties. It grows up cypress trees readily reaching the highest areas, spreading laterally over the tree canopy. Since cypress are deciduous and *L. microphyllum* is evergreen, in the winter months it increases its growth

without hindrance from cypress leaves. When new cypress leaves emerge they are shaded by the fern. Fire is a natural part of the cypress swamp ecology and *L. microphyllum* acts as a wick bringing the fire to the top of the cypress, which destroys the tree while the fern rhizome (moist below) soon produces new fronds. This same species has now expanded its territory to include the saw grass community. In Florida, *L. microphyllum* is considered an alien with a high priority for control: it is causing a reduction in natural species diversity of the Everglades. In studying dispersal by spores, *L. microphyllum* spores have been obtained from air samples on the West coast of Florida where isolated populations are now established. *Lygodium* forms spores on the uppermost portions of the frond: spores are easily carried by the wind great distances since they enter the air column from the greater heights of the tree canopy. This same taxon in Africa grows in mangrove swamps tolerating salt water. Some species of *Lygodium* do grow in shaded wet forests, e.g. *L. radiatum* in Panama. In these taxa spore dispersal cannot be as far and most probably the spores germinate close to the parent plant.

Distribution and Biogeography

Lygodium is a tropical and subtropical genus with temperate outliers in North America and Japan (refer to Fig. 1.1). Paleobotanical evidence indicates a more widespread distribution in the Cretaceous and Jurassic with a subsequent decline in range in the Paleocene (refer to Fig. 1.2). There are a number of narrowly endemic taxa: *Lygodium articulatum* - New Zealand; *L. cubense* - Cuba and Jamaica; *L. hians* - New Caledonia; *L. oligostachyum* - Hispaniola; *L. lanceolatum* - Madagascar; and *L. palmatum* - eastern United States. Both *Lygodium kerstenii* and *L. smithianum* are restricted to Africa, *L. merrillii* to the Philippines and southern China and *L. versteegii* to Malaysia. The rest of the species have wider, often overlapping ranges (Table 4.1). The

Table 4.1. Distribution of *Lygodium* species in geographic regions of the world.

New World

United States - *L. palmatum, L. japonicum* (alien), *L. microphyllum* (alien).

Mexico - *L. heterodoxum, L. venustum, L. volubile.*

Mesoamerica - *L. heterodoxum, L. radiatum, L. venustum, L. volubile.*

South America - *L. radiatum, L. venustum, L. volubile.*

West Indies - *L. cubense, L. oligostachyum, L. venustum, L. volubile.*

Old World

Africa - *L. microphyllum, L. smithianum, L. kerstenii.*

Madagascar - *L. lanceolatum, L. kerstenii.*

India - *L. circinnatum, L. flexuosum, L. japonicum, L. longifolium, L. microphyllum, L. salicifolium.*

China - *L. circinnatum, L. japonicum, L. flexuosum, L. longifolium, L. merrillii, L. microphyllum, L. polystachyum, L. salicifolium.*

Japan - *L. japonicum.*

Indochina (Vietnam through Thailand and Burma) - *L. auriculatum, L. circinnatum, L. flexuosum, L. japonicum, L. microphyllum, L. polystachyum, L. salicifolium.*

Malay Peninsula and Sumatra - *L. borneense, L. circinnatum, L. flexuosoum, L. longifolium, L. merrillii, L. microphyllum, L. salicifolium.*

Philippine Islands - *L. auriculatum, L. circinnatum, L. flexuosum, L. japonicum, L. microphyllum, L. versteegii.*

Borneo - *L. auriculatum, L. borneense, L. circinnatum, L. flexuosum, L. longifolium, L. merrillii, L. microphyllum, L. salicifolium.*

New Guinea - *L. circinnatum, L. flexuosum, L. japonicum, L. kingii, L. longifolium, L. microphyllum, L. salicifolium, L. trifurcatum, L. versteegii.*

South Pacific Islands - *L. auriculatum, L. circinnatum, L. hians, L. microphyllum, L. reticulatum, L. salicifolium, L. trifurcatum.*

Australia - *L. flexuosum, L. japonicum, L. microphyllum, L. reticulatum.*

New Zealand - *L. articulatum.*

greatest diversity occurs in the Old World tropics (19 of 26 taxa) whereas seven taxa are found exclusively in the New World (Fig. 4.1; Table 4.1). There is some morphologic evidence to hypothesize that at least three taxa represent the geographic isolation of one pantropical species (e.g., *L. japonicum, L. venustum,* and *L. kerstenii).* There are also parallel taxa between the New and Old World that may be the result of allopatric speciation or convergence. *Lygodium volubile, L. kingii, L. smithianum,* and *L. cubense* have some characters in common and yet differ in other attributes.

The distribution of *Lygodium* follows a pattern typical of pteridophytes in general. According to Kornas (1993) the majority of fern taxa (65%) occur in southeast Asia and adjacent islands (including Australia and New Zealand). Some 27% occur in South America, Central America, the West Indies and North America and only 8% are found in Africa. In Africa and Madagascar only 4 taxa of *Lygodium* are found: *L. lanceolatum* is endemic to Madagascar, *L. smithianum* is endemic to Africa; *L. kerstenii* is found both on the continent and in Madagascar; while *L. microphyllum* occurs from Africa to southeast Asia. Such low diversity in Africa is typical of ferns in general. On that continent much of the flora represents disjuncts with America-Africa or African-Asian distribution patterns. For example, *Asplenium monanthes* L., *Asplenium lividum* Mett. ex Kuhn, *Polypodium polypodioides* (L.) Hitchc. and *Trichomanes rigidum* Sw. (Kornas, 1993) represent bicontinental species with American-African distributions. Species with Africa-Asian distributions include *Adiantum incisum* Forssk., *Microsorum scolopendria* (Burm. f.) Copel., and *Pteris vittata* L. (Kornas, 1993). These two discontinuous distribution patterns (Africa-America and Africa-Asia) represent widely recognized disjunctions (Thorne, 1972, 1978). In Africa, extinctions of a once rich pantropical flora were due to a shift in the vegetation zones from north to

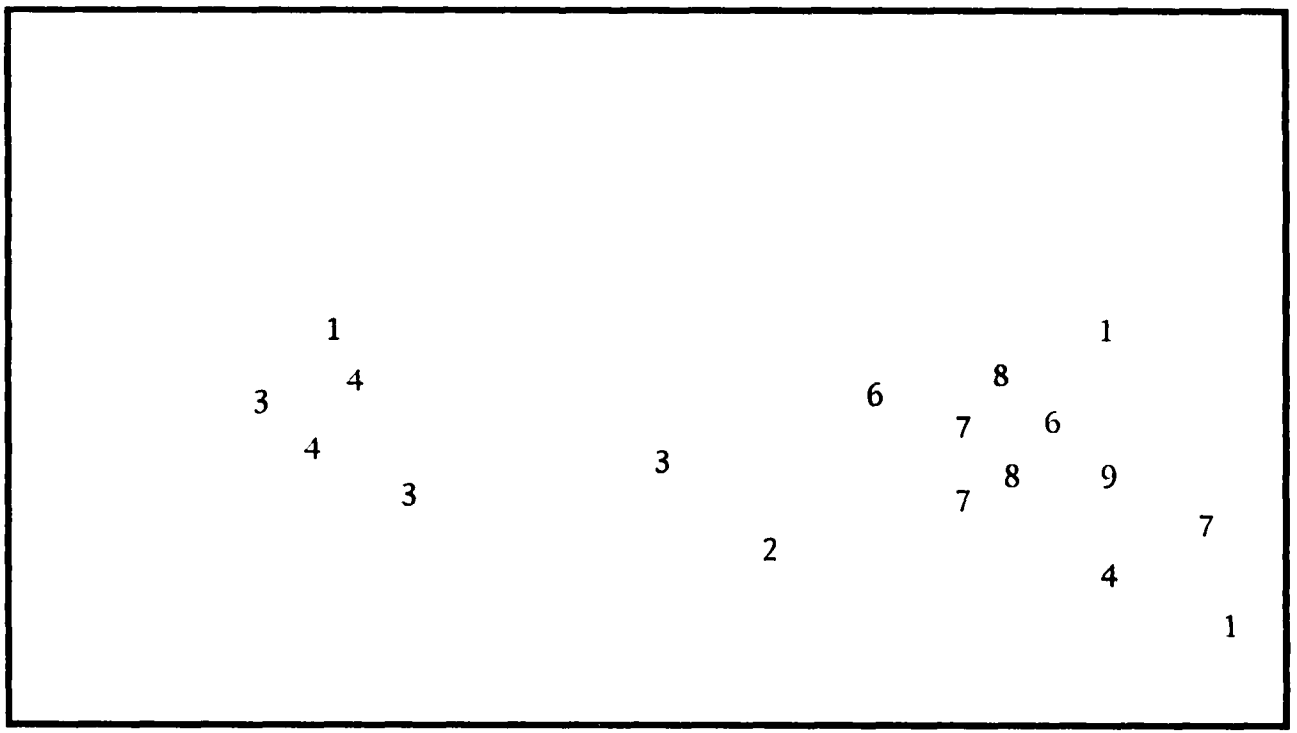


Figure 4.1. Distribution of *Lygodium* in various geographic regions of the world. The numbers represent how many species are found in each region.

south (resulting from Tertiary continental drift) and a progressively drier climate with greater climatic oscillations in the Pleistocene.

Many of the African-American fern species are differentiated into infraspecific taxa. If this approach were taken with *Lygodium* then *L. smithianum* would become a variety of *L. volubile* and *L. kerstenii* a variety of *L. japonicum* or *L. venustum*. However, geographic isolation has occurred long enough to create some consistent characters to separate the taxa. Species with disjunctions of greater than 850 miles have a considerable potential for geographic isolation according to Tryon (1972). The presence of *Lygodium microphyllum* in Africa is probably the result of spore dispersal with subsequent opportunistic colonization from its Asian range. Its presence in the United States and Guyana is most likely due to the spread by spores from a cultivated plant. Madagascar has three taxa of *Lygodium*, one of which (*L. boivinii*) is a sterile hybrid between the other two (*L. lanceolatum* and *L. kerstenii*). *Lygodium kerstenii* on Madagascar has a unique combination of segment characters: the overall segment shape is like *L. japonicum* whereas the central lobe is pinnatifid as in *L. polystachyum* (both of the latter being Asian taxa).

The greatest variety of *Lygodium* species and variation within species is found in New Guinea (9 species), Borneo (8 species) and the Philippine Islands (6 species; Fig. 4.1). These islands are close enough to other areas to permit establishment from spore dispersal. The taxa that are present exhibit a large degree of variability in characters such as indument, segment size and segment shape.

In the New World tropics Tryon (1972) cites three primary geographic areas for the concentration of fern species: Mexico, the Andes and Brazil with secondary areas in Central America and Guyana. Most taxa of *Lygodium*

present in the New World tropics have a wide distribution pattern: *L. volubile* and *L. venustum* in South America, Central America and the West Indies; *L. heterodoxum* in Mexico south to northern Venezuela, *L. radiatum* in Costa Rica to northern Venezuela. Two are endemics to the West Indies (*L. cubense* and *L. oligostachyum*).

The only temperate New World taxon is *Lygodium palmatum* whose range in the United States extends from Vermont south to Alabama, west to Kentucky and Ohio with disjunct populations in Michigan.

There are no extant species of *Lygodium* in Europe, where it once ranged widely in the Cretaceous and Jurassic. As the paleoclimate became drier and cool-temperate species were forced south, and with the Pleistocene glaciation, most fossil taxa became extinct or moved into tropical regions. Because of the east-west direction of the Alps in Europe no taxa were able to escape southward during glaciation. In North America, the north-south mountain direction allowed migration and subsequent reintroduction and a continuity of the mixed deciduous forest, of which *L. palmatum* was a part.

Some temperate taxa are present in Japan, where the Cenozoic climate changes were modified by the insular environment so that the flora persisted. *Lygodium japonicum*, for example can survive moderately cold tempertures.

In trying to explain biogeographical distributions, morphologic variability and speciation in *Lygodium*, a few facets of the homosporous fern life cycle become relevant. These aspects concern intra- and inter-gametophytic fertilization, hybridization and autopolyploidy. *Lygodium* produces bisexual gametophytes and also antheridiogens. The former permits self-fertilization and thus, the establishment of a colony from one gametophyte (one spore) with a resultant increase in homozygosity and potential lack of variability in phenotype within the newly established colony. On the other hand, the

production of antheridiogens encourages cross fertilization which increases genetic diversity. Hybridization events usually lead to reproductive isolation and sterile hybrids. However, the ability to produce fertile hybrids by chromosome doubling (allopolyploidy) becomes an important mechanism of speciation in ferns in general and possibly in *Lygodium* in particular. This may have been an important facet of the Eocene/Miocene speciations. These individuals have the ability to reproduce with each other or potentially backcross with a parent. Apogamy has not been observed in *Lygodium*, however, other mechanisms of autopolyploidy may be an explanation for some of the ploidy levels in *Lygodium*. Again there is the possiblity of back-crossing with a haploid spore to produce a triploid or diploid spore. Triploids that undergo chromosome doubling produce hexaploids: *Lygodium volubile* has hexaploid forms and thus, is the result of a $2n$ and n fertilization event, the $2n$ arising either by auto- or allopolyploidy.

The last consideration in speciation in relation to biogeography concerns spore dispersal. In general there are few endemic species at distances up to 500 miles while endemism increases at distances greater than 1000 miles. Therefore, spore dispersal is common up to 500 miles and then decreases with increasing distance. Taxa with disjunctions of 800-1000 miles have a significant potential for genetic isolation of their disjunct geographical variants, and the potential for speciation is increased. *Lygodium hians* (endemic to New Caledonia) most probably represents spore dispersal from the allied New Zealand taxon *Lygodium articulatum*, with subsequent isolation and speciation.

CHAPTER V

SPOROPHYTE MORPHOLOGY

Introduction

The comparative anatomy and morphology of the Schizaeaceae *s.l.* has been studied by many botanists (e.g., Boodle, 1901, 1903; Bower, 1926; Bierhorst, 1971, 1973, 1977; Campbell, 1940; Goebel, 1900, 1905; Ogura, 1972; Prantl, 1881; and de la Sota and Morbelli, 1987). *Lygodium*, however, has been of particular interest because of its indeterminate rachis growth and dormant pinnae. As a result of this unique combination of characters, various specialized studies have been undertaken: shoot and leaf morphology has been investigated in *L. japonicum* and *L. microphyllum* (Mueller 1982a,b, 1983) and in *L. flexuosum* (Bhambie and Madan, 1979); leaf structure and phloem tissue in *L. palmatum* was compared to other homosporous leptosporangiate ferns (Warmbrodt and Evert, 1979); and tracheary elements were studied in *Anemia schimperiana* Pr. and *L. japonicum* (Lal and Bhambie, 1983). More recently root anatomy in ferns was the topic of a paper by Schneider (1996). Comprehensive studies on *Lygodium*'s sister taxon, *Anemia,* are an important source of comparative information (Mickel, 1962, 1981). Life cycle observations have contributed information on the anatomy and physiology of the gametophytes, including the ontogenic development of antheridia and archegonia, and subsequent sporophyte development (Atkinson, 1973; Atkinson and Stokey 1964; Näf, 1960, 1961; Nayar and Kaur, 1971; Rogers, 1923; Stokey, 1951; and Twiss, 1910). All of this research has represented an invaluable morphological and anatomical resource in this study.

The rhizome is subterranean, ca. 1-5 cm (closer to surface in *L. microphyllum*), dichotomously branching and short- or long-creeping. Fronds emerge in two rows (distichously) from the upper rhizome surface. Bower (1926) points out that the two rows are nearly coincident, therefore, appearing as if in a single row. This "single row" has been observed in *L. microphyllum*. In some species with short-creeping rhizomes, the fronds may become so congested that three rows appear to be present (*L. radiatum*, *L. venustum*). Anatomically, the rhizome consists of a simple protostele with the central xylem core (septate and branched sclariform tracheids; Lal and Bhambie, 1983) containing scattered parenchyma (medullated or vitalized protostele). Among the other Schizaeaceous genera, *Anemia* and *Mohria* exhibit an amphiphloic siphonstele or dictyostele, whereas *Schizaea* has a medullated protostele. No leaf gaps form in the protostelic ring. The rhizome cortex contains three zones: an inner zone of 3-5 layers in which some cells are suberized, often with intracellular spaces for aeration, a middle zone of thick-walled sclerotic cells and an outer zone of thin-walled parenchyma cells containing silica nodules (Boodle, 1901).

Roots are numerous and are produced on the lower and lateral surfaces of the rhizome. They are protostelic: a diarch xylem core is surrounded by phloem, pericycle, endodermis and cortex (comprised of schlerenchyma and parenchyma). Schneider (1996), in a comprehensive study on fern roots, reports that the rhizodermis is usually one cell layer thick. This layer is made up of elongate cells in most ferns, except in species of *Schizaea* and *Mohria* and in some species of *Anemia* and *Lygodium* , where both long and short cells are interspersed. Root hairs are unicellular in the Schizaeaceae *s.l.* except in some species of *Schizaea* where the root hairs may be septate (this variation also occurs in the Marattiaceae). Schneider considers the most systematically

useful root character to be the cortex, which comprises 2-4 zones and differs in complexity in the various fern groups. Taxa of *Lygodium* and some members of the Pteridaceae (*Adiantum, Coniogramma, Onychium* and *Pityrogramma*) possess a cortex in which the innermost cell layer of large parenchyma cells is combined with a schlerenchymatous inner cortex and a parenchymatous outer cortex [Schneider (1996) refers to this arrangement of cell layers as the "*Lygodium*-type"; the schlerenchymatous inner cortex was also observed by Bierhorst (1971)]. This suggests an interesting alliance between *Lygodium* and the Pteridaceae (including Adiantaceae), a relationship that has been proposed in the past and also in some current fern phylogenies (Mickel, 1962, 1974; Wagner, 1969; Holttum, 1973; Kramer, 1990).

The fronds are indeterminate and grow as high as 20 m. They are initiated from single initial cells in the zone of prismatic surface cells of the shoot apex, and their subsequent ontogenic development has been studied by Mueller (1982a,b). He concludes that the complex leaves and simple stem morphology are specializations of function within the shoot and not an indication of poorly differentiated organs.

The rachis branches to produce widely spaced alternate pinnae which immediatedly divide into two opposite pinna-branches subtended by a dormant pinna-bud. In a juvenile plant the first branches are dichotomous and this gradually changes to anisotomous and finally lateral pinna formation (Mueller, 1982b). The dormant bud consists of a solid strand of procambium which branches laterally from the rachis procambium. The strict use of the term dichotomous has been partially disputed by Mueller (1982a,b) who suggests that since the branching of the shoot in *Lygodium* involves a cessation of growth in the central region of the apex, this "dichotomy" may actually be derived from some form of lateral or sympodial branching (1982

a,b). Due to the continuous apical growth, both Prantl (1881) and Bierhorst (1971) concluded that deviations from the basic dichotomous branching pattern would occur.

Vascular tissue differentiation is in an acropetal direction and two protoxylem poles develop alternately at successive pinnae from each of the two lateral protoxylem strands of the rachis. The two protoxylem strands diverge into the two opposite primary pinna-branches. There is little vascular tissue differentiation in the dormant bud when mature (Mueller, 1983). If the original apex is injured, the dormant buds of the next proximal pinna grow out, enabling the scrambling vine to continue growing without having to replace itself from the rhizome below.

The rachis circumnutates and has been compared by Mueller (1983) to the "searcher" morphology produced by many twining shoots of flowering plants. This behavior is characterized by precocious rachis elongation and delayed leaflet expansion. In *L. japonicum* the new frond grows to 20-40 cm before the apical 10-15 cm begins to circumnutate. Rapid elongation continues as long as support is present. The rachis spirals in a left-handed direction (counterclockwise): this direction has been noted to be opposite on occasion (Mueller, 1983; Prantl, 1881). Ornduff (pers. comm.) reports that *L. circinnatum, L japonicum* and *L. lanceolatum* all twine in both directions whereas *L. flexuosum, L. microphyllum, L. reticulatum, L. venusutm* and *L. volubile* are all counterclockwise.

The pinna-branches either end in segments or dichotomously divide and end in segments or are 1-3 times pinnate. The segments are sessile or borne on petioles. The axes in *Lygodium* are usually grooved or narrowly winged, rarely terete.

Fertile segments are borne on distal portions of the frond. The fertile segment lamina may be reduced to varying degrees, in some cases suppressed entirely, producing the extreme dimorphism seen in *Lygodium palmatum*, *L. articulatum*, and *L. trifurcatum*. In other cases the fertile segment resembles the sterile one with sporangia borne on marginal projections (sorophores). There are two rows of single sporangia (the "monangial sori" of Prantl, 1881) alternating to the right and left of the midvein, and covered by a flap-like indusium. The sporangia originate as an outgrowth of a marginal series of cells that are continuous with the apical cells of the leaf segment (Bierhorst, 1971). The slow growth of the segment lamina forces the marginal sporangia into a superficial position. In *Mohria* sporangia are borne on the lower surface of what otherwise resembles sterile lamina, submarginally, associated with veinlets and protected by a revolute margin. The sporangia in *Anemia* occur in 2 rows along ultimate veins on fertile pinnae. The lamina, if present, may grow over the sporangia. In *Anemia* the fertile pinnae are generally the basal pair of pinna of the frond.

Bierhorst (1971) describes the ontogeny of the indusium as a "lip" of lamina that has grown around as a flap on the lower side of the leaf. Prantl (1881) describes the indusium as an accessory surface outgrowth of the underlying segment lamina which arises at the same time that the sporangia are established.

Materials and Methods

The tannic acid/ferric chloride leaf staining technique was used (Foster, 1934) after clearing in sodium hydroxide and chorox.

Stomatal measurements were made on the long axis at 200 x. A minimum of 20 stomates were measured in each segment. A total of 5+ collections were sampled for each taxon.

For scanning electron microscopy, gametophytes or leaf tissue were air-dried and directly placed on double-sided tape mounted on stubs and gold-coated. (See Chapter VI for SEM methodology).

Results and Discussion

The following represents a summary of the observations made in this investigation from leaf clearings, scanning electron micrographs and the morphological examination of growing or dried specimens of *Lygodium* combined with evidence available from the literature. Leaf clearings were observed to determine stomatal type and size, venation pattern and relation of the vein to the marginal layer, indument morphology, and differences in epidermal topography.

Stomata

Stomata are present abaxially on the leaflets as well as on the lamina of winged axes, on indusia and sorophores. Both anomocytic and diacytic types were observed (terminology of van Cotthem, 1973) with the latter predominant (Fig. 5.1a-c). The sizes ranged from 24.7-50.1 μm (longest axis), with the majority being 30-38 μm. The largest stomata were found in *Lygodium radiatum* (51.2 μm), which also possesses some of the largest spores. No chromosome counts have been made on this species to determine if the large spore and stomata sizes have any relationship to ploidy level as has been shown for *Anemia* (Mickel, 1962). The smallest stomata are found in *Lygodium palmatum* (26.7 μm), which also has relatively small spores. The largest range in size within a species occurs in *L. venustum* with specimens from Guyana, Brazil, and Bolivia having stomata averaging 35 μm whereas those from Jamaica and Trinidad average 26 μm, and in *L. volubile* with specimens from South America averaging 29-34 μm and those from the West Indies with larger

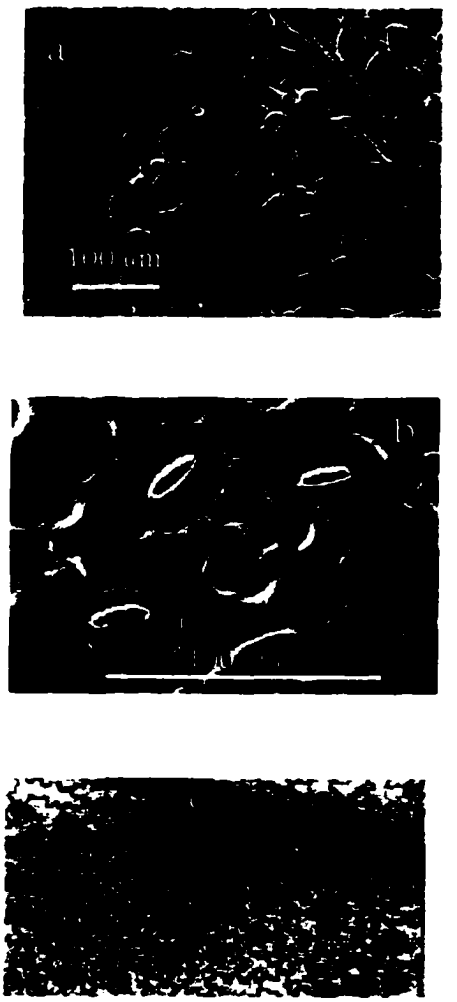


Figure 5.1. Anomocytic stomates of *Lygodium*. a. *L. palmatum* (SEM); b. *L. japonicum* (SEM); c. *Lygodium palmatum* (light microscope at 100x).

stomata (39 μm). Both these species have large ranges and exhibit variability in many morphological characters. Walker (1966, 1985) has reported tetraploids in *L. venustum* (Trinidad) and diploids and hexaploids in *L. volubile* (Trinidad and Jamaica respectively). Thus, these islands would be good sites to study a correlation between spore size and ploidy level in these two species. More studies on stomatal size and ploidy levels should be done on taxa in which both tetraploids and diploids occur (*L. circinnatum, L. japonicum, L. microphyllum*). In the other taxa the sizes of the stomata are within narrow ranges indicating size to be a consistent character (e.g., *L. microphyllum*, 32-34.3 μm; *L. japonicum*, 32-34.8 μm; *L. cubense*, 35.6-37.3 μm). The stomata of the two hybrids, *L. heterodoxum x volubile* (±30 μm) and *L. heterodoxum x venustum* (±33 μm) are both within the normal range of the parent taxa (abortive spores were observed in both these hybrids).

Indument

In *Lygodium* there are five types of hairs (Figs. 5.2a-h; 5.3a-c):

a) rhizome hairs;

b) swollen, multicellular-based pinna-bud hairs;

c) unicellular, club-shaped, glandular hairs (axes and blade);

d) unicellular, acicular, erect, eglandular hairs (axes and blade); and

e) multicellular hairs of varying lengths (axes and blade).

Rhizome hairs

Rhizome hairs are usually 1-1.5 mm long, consisting of 4-6+ thick-walled cells arranged linearly. They are lustrous, dark-brown to red-brown to black, flattened in dried specimens and appressed to the rhizome. These hairs are most prevalent covering the growing tips, especially at areas of forking.

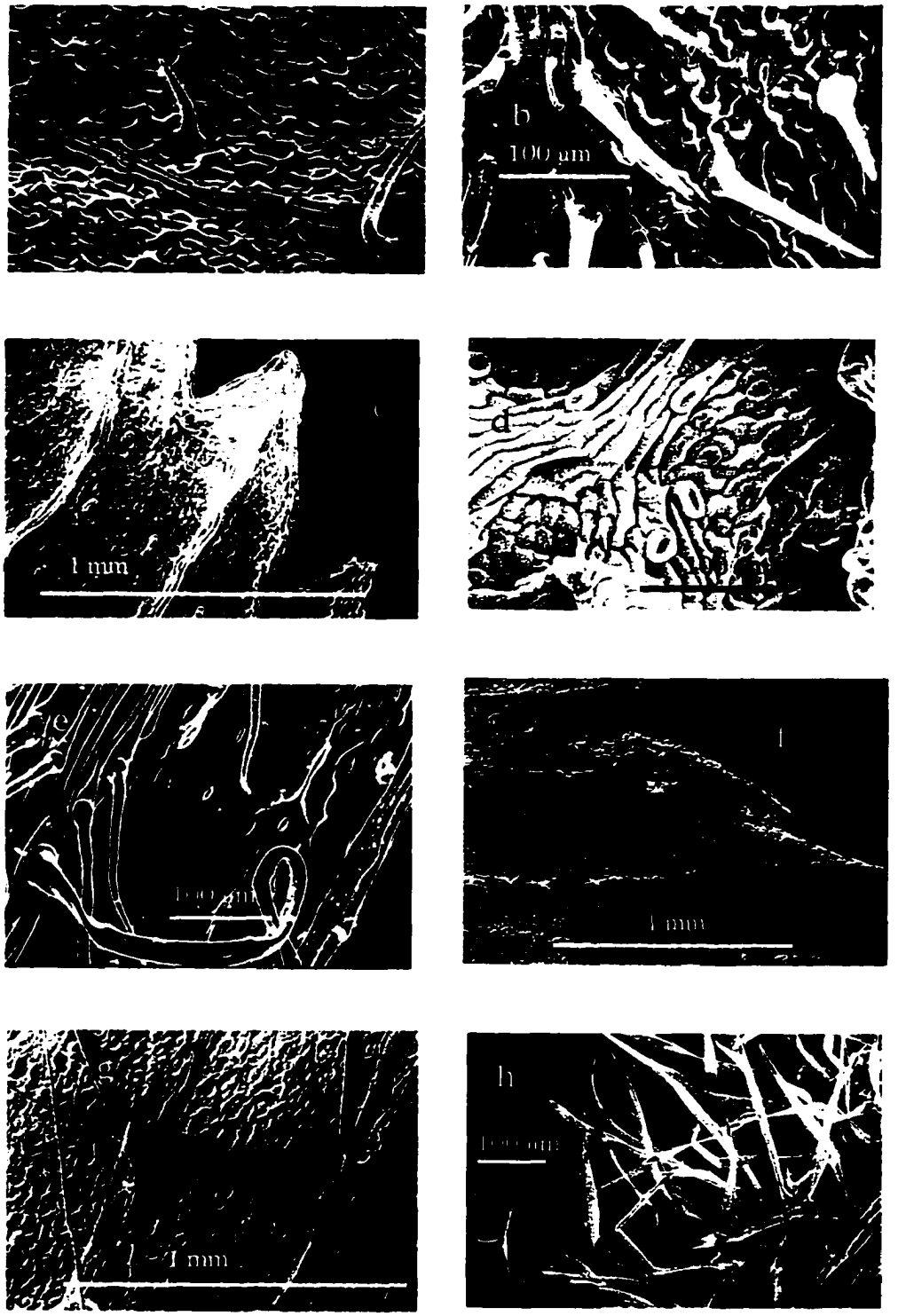


Figure 5.2. Hair types of *Lygodium*. a. unicellular hairs, *L. polystachyum*; b. club-shaped glandular and unicellular hairs, L. *polystachyum*; c. club-shaped glandular hairs, *L. radiatum*; d. club-shaped hairs (collapsed), *L. microphyllum*; e. multicellular hairs, *L. japonicum*; f. 2-celled, erect hairs, *L. japonicum*; g. multicellular hairs, *L. palmatum*; and h. petiole multicellular hairs, *L. venustum*.

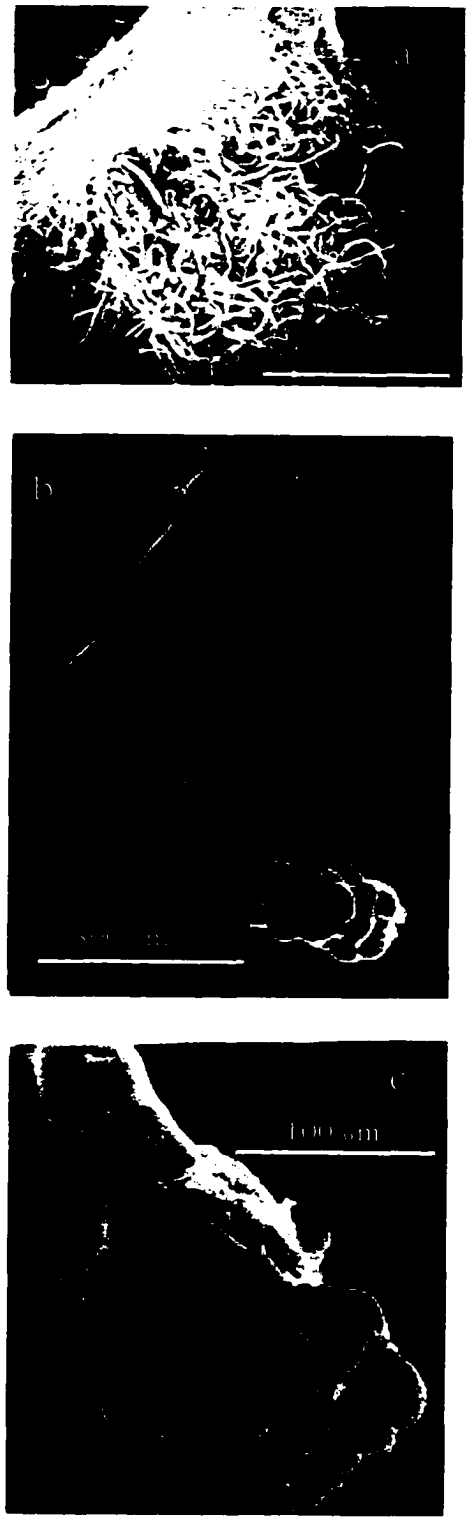


Figure 5.3. Pinna-bud hairs in *Lygodium*: a. pubescent pinna-bud in *L. microphyllum*; b, c. polycellular-based pinna-bud hairs in *L. salicifolium*.

Multicellular-based pinna-bud hairs

Swollen multicellular-based pinna-bud hairs are present in only scattered taxa from the Pacific, Asia, Indochina, and Africa. The hairs have bulbous bases, which are divided into 2-5 rows, each row comprising 4-7 cells. The rows taper to 2 adjacent cells and finally one cell to which are attached 4-6 cells, the terminal cell acute or acicular (Fig. 5.3b,c). No other cells of *Lygodium* were observed with this "scale-like" behavior. The cells are usually in the central region of the dormant bud with more simple multicellular hairs at the periphery or intermixed in a homogeneous manner. A transition between simple linear, multicellular hairs and the swollen multicellular-based hairs can be seen in *Lygodium flexuosum* and West Indian specimens of *Lygodium volubile*, in which the swollen base is less prominent consisting of 2-3 vertical rows of paired cells.

Club-shaped (clavate) glandular hairs

Club-shaped, presumably glandular, hairs are small 50-130 μm long (about twice the size of the stomata) and present in most species. However, in some species, they are present to a very obvious degree, on the abaxial segment surface (Fig. 5.2c, *L. radiatum*). These hairs are basally or peltately attached to the epidermal cell. The hairs may be present abaxially on the lamina surface, the veins, surrounding sporangia on sorophores, rarely on indusia, and often on axes (Fig. 5.2b-d). Juvenile fronds often have increased numbers of these glandular hairs. Glandular hairs are found more frequently on young leaves since these areas are more vulnerable to insect and pathogen attack (Duke, 1994). In angiosperms these glandular hairs have been found to contain a number of secondary metabolites, including phenolics (Duke, 1994). Phytochemical studies have shown that *Lygodium* produces a very large

number of different phenolic compounds which may be present in these glandular trichomes.

Roux (1992), in a study on the morphology of *Mohria*, describes two types of unicellular, glandular hairs: clavate and naviculate trichomes. The clavate hairs are thin-walled, obovoid or clavate and borne on randomly dispersed epidermal cells. More are found on the abaxial surface than the adaxial, and they are frequent on juvenile fronds. In some taxa of *Mohria* they are restricted to the veins. Clavate trichomes are basally attached and are 30-76 μm in length. They have no taxonomic value in *Mohria.* The naviculate trichomes are also present in all taxa of *Mohria* and are thin-walled, ellipsoidal to oblong. They are attached basally or subbasally to small epidermal cells and are found on the lamina abaxially, often associated with the margins or lobe apices of fertile pinna in large numbers. They are also present on the stipe and rachis and are 50 - 225 μm long. These also have little taxonomic value. It is difficult to determine if both clavate and naviculate hair types as determined by Roux (1992) are present in *Lygodium.* It appears that those hairs often found on the axes and veins, being more elongate, fit the naviculate type of Roux, whereas those present most frequently on the abaxial lamina are more similar to clavate trichomes. *Anemia* has both types of unicellular glandular hairs: the naviculate are 78-172 μm and the clavate are 32-70 μm (Mickel, 1962). The latter type of hair in *Anemia* has been correlated with size of stomata and both reflect the ploidy level (Mickel, 1962). This presents another avenue for further study in *Lygodium:* a correlation between the unicellular hairs and stomata. Mickel (1962) considers glandular hairs the primitive type and eglandular hairs derived whereas Roux (1992) hypothesizes the reverse polarity in *Mohria* . Mickel (1962) also suggests in his study on *Anemia* that hirsute lamina is pleisiomorphic and glabrous

conditions apomorphic. These unicellular glandular hairs in *Mohria*, *Anemia* and *Lygodium* may represent synpleisiomorphies for these members of the Schizaeaceae *s.l.*

Unicellular acicular hairs (eglandular)

These cells are 95-150 μm long with the thick-walled hair cell separated from the large epidermal cell by a cross-wall (Fig. 5.2a,b). They are barb-like, acicular, and are erect on the axes and lamina of some taxa (e.g., *L. polystachyum*, *L. venustum*, *L. japonicum*). When present, these hairs are useful taxonomically because of their consistent occurrence within a taxon and their sporadic occurrence in the genus.

Multicellular hairs

Multicellular hairs in *Lygodium* are extremely variable in size and number of cells. The longest ones are often covering the dormant bud (Fig. 5.3a). The cells are septate, and the septa are often dark-brown or reddish in color. The septa are transverse and no sinuate-walled (ossiform-type of Roux, 1992) cells common in *Mohria* have been observed. The cells may be thin-walled or thick-walled and the basal cells are often shorter, with longer apical cells. The terminal cell may be rounded, slightly hooked to acicular: there have been no branched cells observed. Multicellular hairs may be present on the axes (e.g., often between the grooves on the rachis), dormant bud, lamina and veins (Figs. 5.2e-h, 5.3a). In many cases the pubescence is greatest on the petioles and on an articulation zone, if present. The number of hairs is always greater on fertile segments, especially at the base of the sorophore and underneath and between the sporangia (Fig. 5.5a,b). Sorophores will often possess hairs adaxially associated with the midvein as well as abaxially.

Marginal hairs are rare as are lamina hairs. The hairs may be appressed or slightly erect. Erect hairs are usually only 2-4 cells long. The abundance of hairs is variable in any species and dependent on population genetics and local environmental characteristics.

Two-celled hairs range from 48-175 μm up to 320 μm (in which the basal measures 50 μm and the apical cell 270 μm) in *L. japonicum*. Three-celled hairs range from 175-730 μm, four-celled hairs up to 800 μm and those with 5 + cells can be over 1 mm in size. Often the hairs contain small basal cells and longer apical cells so that a hair may have 8 or 10 cells with the first 3-4 cells only 100 μm in total while the last 4-5 cells are 900 μm (e.g., *L. radiatum*).

Dormant bud hairs usually contain 2-3 short basal cells with the apical cells longer (often 10-12 cells in total). The cell walls are thick (especially the basal cells) and the septa red or brown. The cells are either reddish, brown or stramineous.

Multicellular hairs are occasionally found attached to the longitudinal marginal layer of cells (e.g., *L. polystachyum* and *L. venustum*): these are usually 2-3 cells in length. Hairs also are found on the epidermal surface of the segment (on the lamina); however, the majority of hairs are present on the veins and axes. When present on the lamina, the hairs are more often 1-3 celled, not longer.

Indument in *Lygodium* varies extensively with habitat and population genetics. The difference in pubescence is most dramatic in *Lygodium venustum*, in which some forms can be so covered with hairs that the axes are not visible and other forms only slightly puberulent to glabrous. This is also observed in *Lygodium flexuosum* in which Philippine populations are pubescent with many unicellular erect hairs on the lamina whereas those from China and other areas in Malaysia are relatively puberulent.

Frond Architecture and Segment Morphology

The rachis branches at varying intervals to produce pinnately arranged pinnae. The only visible remnant of the pinna is the stalk, which ranges from 0.5 to 10 mm in length. The pinna divides pseudodichotomously to produce two pinna-branches with a dormant "bud" in the axil. Mueller studying pinnae initiation in *Lygodium japonicum* (1982b) found that initiation is lateral and begins with peri- and anticlinal divisions in submarginal cells of the meristem. One or two marginal cells at the center of the "pinna" edges enlarge and produce lateral lobes, which become the pinna-branches whereas another large marginal cell in the central lobe divides to produce a lenticular cell. This central lobe forms the dormant pinna-bud. The two pinna-branches grow monopodially by means of an initially continuous marginal meristem. Lateral segmentation and lobing of the meristem produces the final pinnate form.

The pinna stalk is shallowly grooved adaxially and its length is a stable taxonomic character. The dormant apex or pinna-bud is prominent or sunken in the base of the pinna-branches. It is always covered by multicellular, septate, hairs but the degree of pubescence is species dependent. Some dormant apices have sparse hairs, e.g., *L. palmatum,* whereas others are extremely pubescent, e.g., *L. microphyllum* (Fig. 5.3a). Interspersed among the multicellular hairs may be swollen, multicellular-based hairs. Originally documented by Holttum (1959), these hairs are present in some of the Old World taxa (*L. auriculatum, L. kingii, L. salicifolium, L. longifolium* and *L. smithianum*). Only the transitional two cells wide stage has been observed in New World species.

Injury to the rachis apex causes the dormant pinna-buds to grow into twining apices that create entangling thickets. In *Lygodium heterodoxum* the

dormant buds were growing out in almost all specimens observed indicating some type of persistent mechanical injury or a lack of complete suppression of pinna-bud dormancy (similar to apical suppression of lateral growth in higher plants). Some authors (e.g., Reed, 1946; Shaver, 1954) failed to observe the dormant pinna-bud in *Lygodium palmatum*, which is inconspicuous, hidden in the grooved bases of the pinna-branches and often with few hairs (Fig. 10.29D,E). It is interesting to note that in this study the pinna-bud in *L. palamtum* has never been observed to resume growth in either the field or herbarium specimens.

Primary pinna-branches may end in segments, divide dichotomously and end in segments or be once, twice or three times pinnate. Flexuous pinna-branches represent a transitional stage between dichotomous and pinnate branching patterns. Suppression of one branch and continuing growth in the other will, in turn, produce a pinnate branch with an angular or straight axis. These types of branching patterns are clearly seen in *L. cubense* with a flexuous pinna-branch and *L. volubile* with a straight pinna-branch. Some specimens of both species are intermediate. Dichotomous branching is easily seen in the highly dimorphic fertile portions of *L. articulatum*, *L. palmatum*, and *L. trifurcatum*.

The segments may be entire, lobed (equally or unequally, the lobes being discrete), palmate, subpalmate, or pinnatifid. There is a large degree of variation in segment morphology. The pattern of segment venation is closely coupled to segment morphogenesis and also branching architecture. Prantl (1881) considers the branching pattern (of both pinna-branches and segment venation) as the most important taxonomic and phylogenetic character and uses this to separate *Lygodium* into subgroups. He points out "... in the case of ferns the exact study of the branching of the leaf and what is most closely

connected with it, the arrangement of the veins, constitutes the chief concerns of scientific classification." (translated from Prantl, 1881) and he illustrates this segment groundplan in a series of schematic drawings. In *L. articulatum* pinna-branches divide dichotomously with lanceolate segments bearing pinnate lateral veins from the costa. The fertile segments are the result of repeated dichotomies. Thus, in this species there is a combination of dichotomous branching and pinnate branching. In his scheme, Prantl regards none of the segments as truly palmate and in *Lygodium palmatum* finds that the veins at the segment base branch dichotomously, with each dichotomy producing symmetrical branches to the right and left. He calls this type of branching dichotomo-pedate. In species such as *L. venustum*, which appear palmate, Prantl feels that the first forking branches are bundled and not readily recognizable as dichotomous. In this study, I find that all exhibit dichotomous branching with subsequent symmetrical or asymmetrical side branches. Practically, from an identification perspective, there are palmate forms as well (*Lygodium radiatum*, and some of the very symmetrical specimens of *L. venustum*).

Almost all treatments of *Lygodium* that discuss subgenera use the branching characteristics and segment shape as primary features. These criteria have been adopted in this study. Morphogenesis in adult segments results from differential growth in marginal and apical meristems (Mueller, 1982b). These lateral meristems produce the multilobed segments found in the *Lygodium japonicum* group, and suppression of these meristems creates segments associated with the *L. volubile* or *L. microphyllum* allies. There is great plasticity in this character. It is very easy (and we can see the intergradations in specimens) to go from the basal lobed segments of *Lygodium flexuosum* to the auricled lanceolate segments of *L. salicifolium*

(via suppression) to either the linear segments of *L. volubile* or the multilobed segments of *L. japonicum* (via increased lateral meristem activity). This polymorphism creates an intergradation among taxa that make species delineation difficult.

Articulations

Various taxa of *Lygodium*, usually those with simple segments or subpalmate segments, exhibit an articulation zone at the segment/petiole junction (Fig. 5.4a). This zone is composed of thin-walled cuboidal cells in oblique rows. The cells form a thickened, often pubescent, semicircular area at the base of the segment. When the segment abscises, cells in this layer remain attached to the petiole. This is obvious in *Lygodium microphyllum, L. reticulatum, L. articulatum, L. hians, L. salicifolium* and *L. kingii.* In dried specimens this articulation zone forms a depression, indicating the exact area of abscission (Fig. 5.4b). There are also those taxa in which there is a swelling at the petiole/segment junction or on the pinna-branch at the points of dichotomy or petiole divergence. These swellings (pulvini) are prominent in some taxa and useful taxonomically (e.g., *Lygodium lanceolatum,* Fig 10.37C), but often variable in other taxa (e.g., *L. cubense*). These areas consist of regularly arranged small thick-walled cells and, as a result, do not exhibit the same zone of separation. Articulation is another useful taxonomic character (e.g., in separating *L. smithianum* from *L. salicifolium*). However, Prantl (1881) does not recognize articulation as a character having systematic importance because he believes that the branching pattern is complete with or without the articulation area and, therefore, not integral to the taxonomy.

Segment Venation Patterns and Margins

The veins in most taxa of *Lygodium* are free. Of the 26 taxa, five possess reticulate venation (Figs. 10.28J, 10.32B,F, 10.36K, 10.37C). The presence of net

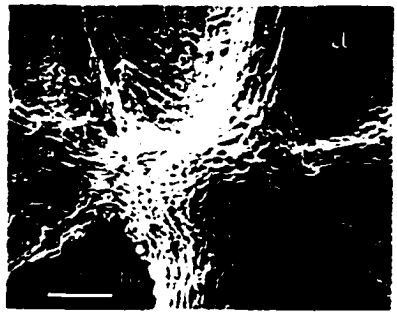


Figure 5.4. Articulation in *Lygodium*: a. articulation zone at petiole/segment junction in *L. microphyllum*; b. articulation zone in *L. articulatum*.

veins is an important taxonomic character but it is not representative of species alliances. This character appears to have risen in *Lygodium* four times: in the *L. reticulatum-L. lanceolatum* subset, in *L. heterodoxum*, in *L. merrillii* and in *L. vertseegii.* The reticulate form of venation is the simplest type of anastomosis: the pinnate veins exit the costa, merge laterally and interconnect. There are no included veinlets. The adaptive value of reticulate venation is not obvious according to Wagner (1979) but it is somewhat correlated to leaf size: most of the taxa of *Lygodium* that exhibit this pattern have large segments (e.g., *L. merrillii, L. versteegii*). However, the species with the largest segments do not have reticulate veins (e.g., *L. borneense*). In net veined species, anastomosings may be absent in the fertile segments where the lamina is reduced (e.g. *Lygodium x boivini*). This character has also enabled us to recognize hybrids that form between the reticulate and non-reticulate taxa, e.g. *L. heterodoxum x L. venustum, L. heterodoxum x L. volubile* and *L. x boivinii*, which is a hybrid between *L. lanceolatum* and *L. kerstenii.*

Free veins divide 2-3 times depending on the width of the segment. The angle at which the veinlets exit the costa has been measured in all taxa. This character was used by Duek (1978) to separate the New World taxa, *Lygodium volubile* and *L. micans.* However, this angle has proven to be too variable a character to be taxonomically useful. In most cases where the venation angle is very different there are other characters that are more obvious and, therefore, have been used in this treatment.

Veins end at the margin, often merging into a longitudinal layer of cells, or in rare cases end before the margins. This marginal zone consists of 1-5 cell layers and is an important taxonomic character. *Lygodium circinnatum* has an obvious thickened margin (4-5 cell layers) and can be separated from its close ally, *L. longifolium* by this character.

In a number of taxa the veins extend beyond the margins as papillate extentions of the serrations (e.g., *Lygodium japonicum* and *L. venustum*).

Juvenile Growth

When only juvenile pinnae of *Lygodium* are available it is difficult to impossible to identify the species. The primordial leaves (Prantl's term for the first divisions of the juvenile frond) are dichotomous, each dichotomy ending in a palmate, multilobed segment with laciniate margins. No dormant pinna-bud is present in the axil of the dichotomy. The frond may produce a series of 3-8(-16) determinate primary segments or pinna-branches before initiating adult indeterminate growth. In *Lygodium japonicum*, each successive segment exhibits an increase in size and often changes from palmate to anisotomously lobed segments to eventually pinnately arranged segments. The first few climbing leaves may bear one or two determinate pinna at their bases but subsequently produce adult pinnae which have the classic two opposite pinnules and a dormant pinna-bud (Mueller, 1982a). The juvenile plants of *Lygodium polystachyum* resemble a determinate twice pinnate fern and enable us to envision what the ancestral form might have looked like before the climbing form evolved.

Fertile structures

Sporangia arise singly on either side of the midvein in acropetal order (Fig. 5.5a,b). These monangial sori are placed on closely aggregated, pinnately arranged veins. The sporangia are usually borne on fingerlike projections or sorophores that are confluent with the margin of the fertile segment. In some taxa the fertile lamina has been suppressed so that sporangia appear at the ends of the secondary veins (e.g. *Lygodium articulatum, L. palmatum, L. trifurcatum*; Figs. 10.27H, 10.29C, 10.27E,F,

respectively). In some species the laminal width varies within the species. For example, some forms of *Lygodium circinnatum* are monomorphic while others have the fertile lamina reduced to 3-6 mm (Fig. 10.31B,C). The number of sporangial pairs is extremely variable and is based partially on the size of the fertile segments, the age of the plant, the height at which fertile segments are induced, and other undetermined environmental and physiological factors. It is of little taxonomic value.

Sporangia originate from marginal cells, each occupies a terminal position on a fertile vein. The marginal initials are continuous with the prismatic layer of apical cells of the leaf. The sporangial initial comes into being after the stalk is formed, it divides periclinally to establish the outer wall initial and an inner cell. The inner cell becomes the tapetum, inner wall layer, and sporogenous tissue (Bierhorst, 1971).

The indusium forms as a secondary outgrowth of lamina which grows around the sporangium. Observations show that the sporangium develops slightly before or concommittantly with the elongation of the sorophore. Prantl (1881) suggests that the indusium is the result of intercalary growth of the free margin. Mettenius' definition of indusium precludes the presence of stomata. Since stomata are present on the indusia of *Lygodium*, many authors have chosen not to use the term for this flap-like covering.

The sporangium is flask-shaped, laterally attached by a 1-2 rowed stalk (Fig. 5.5c,d,f). An subapical row of cells, one cell deep, comprises the annulus (Fig. 5.5d). The apical cell layer may occasionally consist of two transitional cells giving a two-rowed appearance. One cell does not extend the full length of the other annular cells, appearing two-rowed. This is similar to the pseudostratification that occurs in animal cell epithelial tissue. The occasional

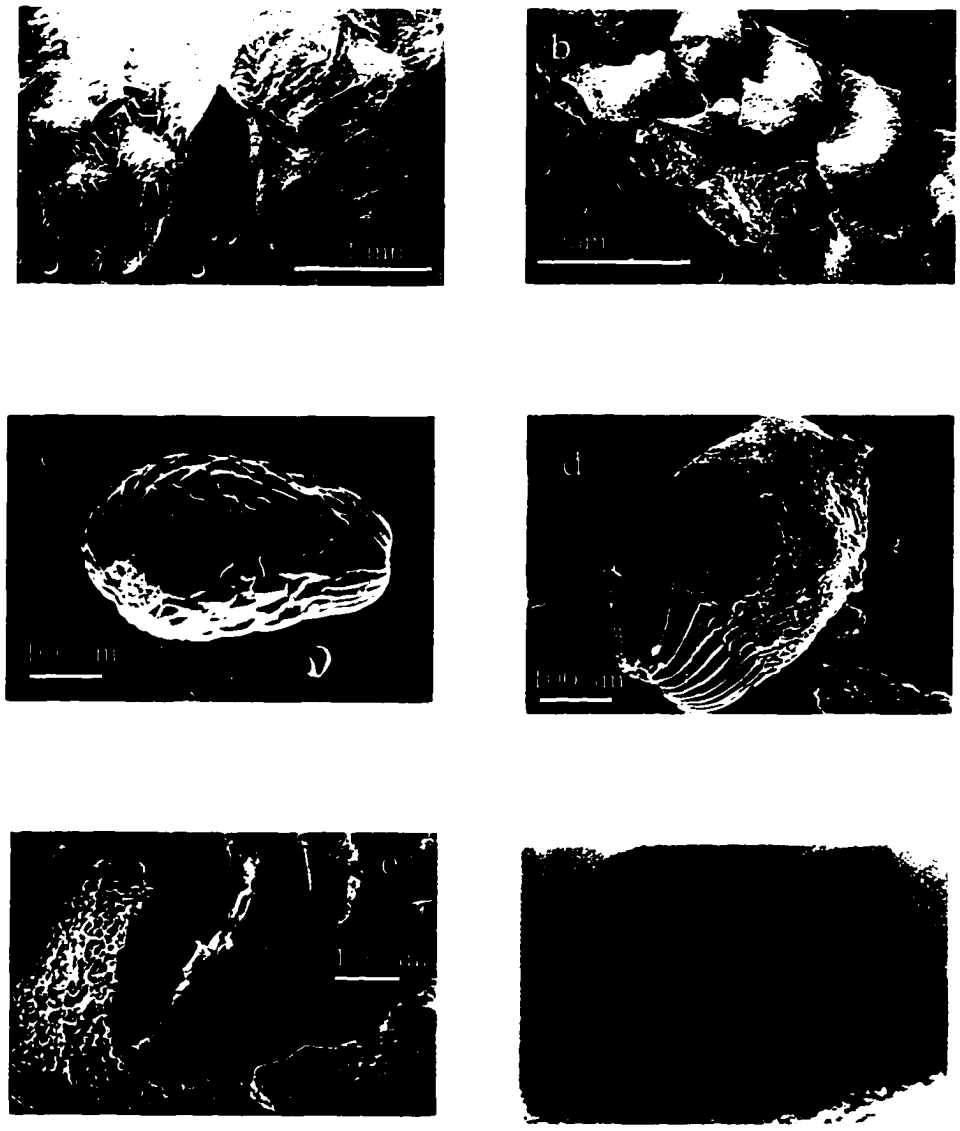


Figure 5.5. Sorophores and sporangia of *Lygodium.* a. *L. polystachyum* sorophore; b. *L. flexuousm* sorophore; c. sporangium *L. palmatum;* d. sporangium *L. volubile ;* e. sporangium with longitudinal dehiscence in *L. flexuosum;* and f. sporangium with attachment stalk (light microscopy at 100x).

two-rowed appearance has provided some support for the ancestral developmental sequence of annulus position in the Schizaeaceae as proposed by Radforth (1938) in species of the fossil, *Senftenbergia.*

The apical plate is comprised of one cell whereas in other members of the Schizaeaceae *s.l,* the apical plate is composed of 1-6 cells. Dehiscence is longitudinal (Fig. 5.5e). In dried specimens of *Lygodium* the sporangia, if mature, can be dehisced and thus, spores are no longer present. In rare instances spores inside sporangia are shrunken and not easily studied. However, in the vast majority of dried specimens seen (> 4000) those with fertile segments had intact sporangia or spores present within dehisced sporangia with mature non-dehydrated spores.

Morphology and Taxonomy

Understanding the taxonomy of *Lygodium* is greatly complicated by the wide variation found in many of the morphologic characters. The intergradations of these variable characters in many taxa engenders more than one approach to its taxonomy. Depending on the perspective of the monographer, species will be either combined or splintered, producing huge pantropical species or narrow, geographically limited taxa.

The characters that exhibit the greatest degree of plasticity are segment shape, size and degree of pubescence. Some degree of segment plasticity is often seen on the same plant as distance from the rhizome increases. The suppression or non-suppression of segment lateral meristems is common in *Lygodium,* often within the same species, creating entire, auricled, lobed or multilobed segments. This presents an immediate problem in descriptions and a degee of overlap in the resultant taxonomy. This variable character is readily observed in *Lygodium flexuosum,* in which asymmetrically lobed segments are the typical form. However, symmetrically lobed segments are

found in many populations. This taxon intergrades with *Lygodium salicifolium* if the lobes become suppressed and with *Lygodium japonicum* if the segments become smaller and the lobes increase in number. It also exhibits variability in the number of segments/pinna-branch, the degree of pinnateness and the presence of an articulation zone. The species has a broad geographic range in the Old World, overlapping those of *Lygodium salicifolium* and *L. japonicum.* Hybridization has been one of the hypothesized explanations for its enormous variability. However, I have seen no specimens with abortive spores to indicate hybridization. Cytologic studies on *Lygodium flexuosum* have shown ploidy levels of 2n, 4n and 6n suggesting either auto- or alloploidy.

Some of the same variation in segment shape and size is noticed in *Lygodium japonicum*, in which collections exhibit one or two overly long, linear central lobes: this prompted Desvaux to segregate this form as a separate taxon, *L. microstachyum,* and, subsequently Tardieu and Christensen to form a variety, *L. japonicum* var. *microstachyum.* This same trend occurs in the New World *L. cubense* in which the segment is either simple linear-lanceolate or bears a small auricle on one side. In one form of this species the segment becomes greatly elongated (twice the size of the typical form) and linear: this form was named by Christ as *Lygodium cubense* var. *stenophyllum* and segregrated by Presl as *Lygodium poeppigianum.*

Often the segment lobes become more incised, thus creating discrete (separate) segments which, in turn, increase the degree of pinnateness. This is the case in some collections of *L. flexuosum, L. venustum, L. kerstenii,* and others. The fertile condition is always accompanied by more segment dissection and greater variability. In all segments with discrete multiple

lobes, the number of lobes varies (e.g., *L. radiatum* with up to 7 lobes, *L. circinnatum* with 2-4 lobes, etc.).

Venation pattern at the base of the segment reflects the segment shape and therefore varies with it. Therefore, in *Lygodium* it is difficult to completely rely on this character to distinguish taxa (as did Prantl, 1881).

Another highly variable character in *Lygodium* is degree of pubescence. Pubescence is often considered a factor directly related to geographic distribution and local habitat characteristics. However, in some taxa of *Lygodium* pubescence appears to be variable in closely situated populations. For example, in *Lygodium venustum*, one of the most variable species in degrees of pubescence, the distribution is not easily explained simply by habitat differences (Fig. 10.16). In *Lygodium palmatum* there is a direct relationship between geographic area and segment pubescence. Those specimens that are glabrous are found usually north of Maryland and those with hairs are found through its southern and western range west to Kentucky, Ohio and Michigan (Fig. 10.3). In *Lygodium flexuosum* pubescence is increased in the Philippines whereas those populations in China are relatively glabrous. Thus, degree of pubescence, in most cases, is not a character useful in delimiting species: kinds of hairs, however, may be useful. For example, few taxa have erect, unicellular hairs on the lamina or multicellular hairs on the margins. *Lygodium polystachyum* possesses both these characteristics. Marginal hairs are present in *Lygodium venustum* and *L. japonicum* and lacking in *L. kerstenii*. In this case, location of the hairs represents an important character as these three taxa are difficult to separate.

Hairs of dormant pinna-buds are present in all taxa to some degree, although a few species are only sparsely puberulent. The presence of swollen, multicellular-based hairs on pinna-buds is another important taxonomic

character. These hairs anatomically express some degree of variation as two-rowed, enlarged basal cells have been observed in *L. flexuosum* and *L. cubense* (an intermediate stage of the stratified multicellular enlarged base).

Another useful, but somewhat variable, character is articulation which is expressed as "jointed" swollen areas at the petiole/segment junction. In strongly articulate taxa there are anatomical differences in the zone of cells that forms the articulation layer so that as the segment abscises, the petiole, with a small crescent-shaped portion of the segment attached, remains on the pinna-branch (Fig. 10.27J). This is obvious in many herbarium collections as pinna-branches bearing intact petioles without segments are observed (e.g., *Lygodium microphyllum, L. articulatum, L. reticulatum, L. lanceolatum, L. salcifolium*). The species that are strongly articulate are usually those with linear-lanceolate to deltoid segments and pinnately branched axes (excluding *L. articulatum* with dichotomously branched axes). There are taxa with swellings at the petiole/segment junction that are not strongly articulate and in which the thin-walled cell layer that causes the segment abscission is absent (Prantl, 1881). This swelling is more apparent abaxially and if covered by hairs may be difficult to identify. The size of the zone is dependent on the age of the segment and in fertile segments it may be lacking entirely. There are many taxa that never have any type of abscission zone (e.g., *L. japonicum, L. kerstenii, L. oligostachyum, L. borneense, L. auriculatum, L circinnatum*). In these species the veins at the segment base divide dichotomously and then may divide again depending on the number of lobes present. In *L. japonicum* and *L. kerstenii* one side vein exits lower than the others. There are also taxa in which some variability exists. For example, *Lygodium venustum* usually has an abscission layer (those forms in which the veins appear to radiate from a central point) but in some West Indian forms it is not present (in these forms

the veins emerge subpalmately). *Lygodium flexuosum*, is almost never articulate, although some collections have a small zone present.

Another area with a thickened zone of cells (pulvini) occurs at the pinna-branch/petiole junction. In some taxa this area is jointed and is a distinguishing character (*L. lanceolatum*). In others this swelling is variable. In those taxa with flexuous pinna-branches a swelling may be present at segment diversions: however, if the pinna-branches are straight, the swelling is less obvious or absent (e.g. *L. cubense*).

Axes, in most cases, are grooved or narrowly winged. The amount of lamina present in winged species is variable within the taxa and is always more exaggerated in fertile pinna-branches and petioles.

The number of segments per pinna-branch varies with position of pinna on the frond and is always increased on fertile branches. The attempt to distinguish *Lygodium micans* from *L. volubile* illustrates this variability and the difficulty in using one or two variable characters to segregate taxa. *Lygodium micans* exhibits 3-4(-5) pairs of alternating segments whereas *L. volubile* has 4-6(-7). This character along with vein angle from the costa was used by Duek (1978) in separating these taxa. Since the characters are variable and no other characters support the segregation they have been synomized in this treatment.

The morphology of *Lygodium* presents the taxonomist with a series of intergradations. Only when these characters can be combined with more stable ones are species easily delineated. The following are considered the stable characters in this genus: pinna stalk size, polycellular based dormant bud hairs, dichotomous vs. pinnate branching, free vs. reticulate venation, strong articulation, margins toothed or entire, with or without a thickened

marginal cell layer, marginal and lamina hairs, and spore ornamentation pattern.

CHAPTER VI

SPORE MORPHOLOGY

Introduction

In his monographic study on *Lygodium,* Prantl (1881) recognized the importance of spore surface structure in resolving taxonomic problems within the genus. Since that time every major systematic treatment or flora has incorporated spore shape and surface structure into the *Lygodium* species descriptions where possible (e.g. Reed, 1946; Copeland, 1947; Holttum, 1959). In recent years the resolution of spore wall ornamentation pattern has been greatly enhanced by the scanning electron microscope (SEM). In conjunction with this a detailed analysis of the structure of the perispore and exospore layers has been investigated with the use of transmission electron microscopy (TEM). The micrographs resulting from both SEM and TEM studies have been included in a survey of fern spores published by Tryon and Lugardon (1991) which has proven to be a valuable resource to pteridologists. These high power photomicrographs have permitted comparision of spore ornamentation and wall layers without the terminological ambiguities often encountered in earlier works that relied on line drawings or a glossary of the descriptors used. One of the problems in comparative spore studies is the lack of a universal terminology: most of the descriptors in use today are derived from earlier publications on pollen (Erdtman, 1957, 1959; Erdtman and Sorsa, 1971). A significant example of ambiguity arises with *Lygodium* in the use of the terms verrucate and tuberculate. What Prantl (1881) calls verrucate, Holttum (1959) and Tryon and Lugardon (1991) regard as tuberculate. Since there are only three basic spore types in *Lygodium,* tuberculate, long-ridged verrucate and reticulate, imprecise terminology can prove problematic. Photomicrographs

preclude the necessity for long descriptions, and terms can be easily clarified when associated with an appropriate photograph. For example, Fig. 6.1f shows tuberculate spores, Fig. 6.4a reticulate spores and Fig. 6.5a verrucate spores.

Recently Lellinger and Taylor (1997) have attempted to address the nomenclatural confusion by defining a terminology for macro-ornamentation and micro-ornamentation sculpturing of fern spores. Macro-ornamentation refers to the spore surface patterns viewed using light microscopy (e.g., Fig. 6.1d). Micro-ornamentation refers to those patterns revealed only using SEM (e.g., Fig. 6.5c). Nonetheless, the most direct and least ambiguous method involves viewing the micrographs themselves.

Spores are often of limited taxonomic value because of their availability and small size. In field identification the low magnification provided by a hand lens or dissecting microscope often limits the use of the spore in determining species even when the character is definitive. Accurate distinction between tubercles and verrucae may be extremely difficult. In addition, collections of *Lygodium* are often sterile as its spores are formed on the uppermost portions of indeterminate fronds which may climb 10 m. When spores are present, herbarium specimens often have immature fertile leaflets or empty sporangia. Juvenile spores may lack a perispore while mature spores may lose the perispore to abrasion presenting the systematist with the same smooth-surfaced end product. However, when adequate microscope equipment and fertile pinnae are available, *Lygodium* spore patterns are invaluable at the species level.

Spore characters most often described are aperture type (monolete vs. trilete), size, shape (e.g. tetrahedral, Fig. 6.1b; globose, Fig. 6.9a), surface pattern (e.g. echinate, rugate, reticulate, tuberculate, etc.) and length of laesura (Fig. 6.1b). Recently the contribution of the exospore and perispore to

the surface architecture, as well as the individual characteristics of the layers comprising the exospore and perispore (Lugardon, 1974; Lugardon and Piquemal, 1992) has added to the potential array of characters spores can provide. Tryon and Lugardon's (1991) atlas on spore morphology reveals that similar ornamentation patterns occur in diverse genera. The large number of fern taxa exhibiting a relatively limited number of surface patterns suggests convergent evolution and parallelism (Wagner, 1974). Even with the limited number of character states that fern spores offer to the systematist, in recent cladistic analyses, spore data have provided useful morphological characters. Stevenson and Loconte (1996) incorporated more than 15 spore character states into their data matrix on fern phylogeny, many of which helped in defining familial relationships. These characters included spore number, shape, aperture type, presence of chlorophyll, perispore characteristics (sculpture, layers, lamellae, untrastructure, rods, cavate), exospore characteristics (type, sculpture, layers), and the presence of a prominent distal and proximal equatorial flange. Pryor *et al.* (1995) incorporated seven spore characters into their phylogeny of ferns based on morphology and rbcL sequences. These included aperture type, chlorophyllous state, equatorial flange, perispore prominance relative to the exospore, persipore surface, exospore structure in layers and exospore surface.

In order to use spore surface characters in the taxonomy of *Lygodium*, an SEM study of spores was undertaken. All potential taxa were observed so that the three basic types of spore surface pattern could be analysed in more detail and used in resolving subgeneric affinities, in distinguishing closely allied species and in reinforcing synonomies or species distinctions.

Materials and Methods

Dried spores (from herbarium specimens or recent collections) were placed either 1) directly on double-sided stick tape on SEM stubs, or 2) first placed in 70% ethanol and then applied to asphalt (roofing asphalt) coated stubs and allowing the alcohol to evaporate under a high intensity lamp. The spores were gold coated in a Technics Hummer II Sputter coater for 2 min. @ 10 milliamps, which deposits approximately 10 nm of metal. Photomicrographs were taken on Lehman College's Hitachi S2700 Scanning Electron Microscope with a Mitsubishi Video Copy Processor at 25 kV with Polaroid 55 Pos.-Neg. 4 X 5 Instant Sheet Film. Initial studies used an Amray 1830 SEM. For general comparisons the magnification was 800X (adjusted only for the largest spores).

In light microscopy observations, measurements were taken after spores were first cleared in 85% lactic acid. Measurements of 10-20 spores from each species were taken at 200 X. The longest axis of the tetrahedral spore was measured to conform to the data presented by Tryon and Lugardon (1991). Spores were measured from several different populations. These measurements were compared with those taken using the SEM. In a number of species, the number of spores per sporangium was counted. The intact sporangium was placed on a hemocytometer slide, broken and the spores counted with the aid of a grid.

Results

Spores of *Lygodium* are trilete, tetrahedral to globose with the longest axis measuring 60-110(-125) μm. The terminology used by Tryon and Lugardon (1991) has been adopted in this study for purposes of comparison. Nomenclatural terms presented by Lellinger and Taylor (1997) have been incorporated into descriptions when clarification is necessary.

There are three basic types of surface spore patterns: tuberculate, long-ridged verrucate, and reticulate (Figs. 6.1-6.7). Three taxa, *L. japonicum, L.*

palmatum and L. kerstenii, have very low surface ornamentation (granulate) resembling suppressed tuberculae or verrucae with no obvious, measurable, projections (Fig. 6.8 a-f) and could be considered a fourth category. The New Caledonian *Lygodium hians,* possesses a range of spore surface patterns, which are all size variations of the same mixed tuberculate-verrucate type (Fig. 6.9a-d).

The greatest number of species are tuberculate and long-ridged verrucate (Figs. 6.1-6.3, 6.5-6.7). Only three species are reticulate, *L. microphyllum, L. reticulatum, and L. versteegii* (Fig. 6.4). *Lygodium versteegii* differs from the other taxa in having a smooth proximal surface with extremely prominent laesurae, the reticulations continuing over the equatorial ridge from the distal surface (Fig. 6.4a,b): this species is also unique morphologically in having suppressed pinna-branches, each branch ending in three entire segments.

The long-ridged verrucate spores exhibit different proximal and distal face ornamentation. The distal face has verrucae that merge into ridges of differing lengths, some of which anastomose. These often have tubercles as micro-ornamentation. An equatorial flange (ridge at or near the equator) may often be observed distally (Fig. 6.7d,e). The proximal face bears a prominent laesura, the ends of which may merge into the exaggerated equatorial ridge. The surface of the proximal face of these spores is tuberculate with widely spaced tubercles. Thus, two different surface patterns exist for the proximal and distal faces. *Lygodium kingii, L. trifurcatum* and *L. volubile* exhibit a prominent proximal equatorial flange (Figs. 6.5f, 6.7a-e).

The mean spore sizes of the taxa of *Lygodium* range from 60-110 μm (Table 6.1). *Lygodium palmatum* (low-tuberculate to granulate), *L. borneense, L. salicifolium* , and *L. microphyllum* have the smallest of all *Lygodium* spores (60, 65, 66, and 66 μm respectively) whereas *L. radiatum* possesses the largest

Table 6.1. Spore morphology of *Lygodium*. The size (μm) and surface ornamentation patterns were observed using SEM and light microscopy. Within a morphological group, species are ranked, by their spore size, from largest to smallest. The mean diameter (μm)of the longest axis is reported, followed by the range in spore diameter.

Tuberculate species	Mean	Range
L. radiatum	110	102-120
L. articulatum	99	85-116
L. venustum	90	83-95
L. lanceolatum	90	85-92
L. flexuosum	89	74-92
L. heterodoxum	88	85-100
L. polystachyum	75	74-78
L. circinnatum	70	54-89
L. salicifolium	66	57-74
L. borneense	65	59-70
Verrucate species		
L. longifolium	95	90-116
L. volubile	95	86-98
L. cubense	94	90-100
L. oligostachyum	94	93-99
L. merrillii	90	89-92
L. kingii	83	67-90
L. smithianum	82	78-89
L. auriculatum	82	72-87
L. trifurcatum	76	67-78
Reticulate species		
L. reticulatum	96	89-100
L. versteegii	91	83-101
L. microphyllum	66	62-73
Low-tuberculate-granulate species to irregular		
L. hians	100	90-125
L. japonicum	76	70-79
L. kerstenii	66	59-80
L. palmatum	60	52-70

spores (mean 110 μm). *Lygodium hians* (irregular in surface pattern) in its largest forms has globose spores > 125 μm. There is no apparent relationship between ornamentation and size.

The laesurae vary from being prominent in some tuberculate taxa (*L. venustum, L. radiatum* and *L. salicifolium;* Figs. 6.1b, 6.3e, 6.1h respectively) to obscured by the tubercules in others (*L. flexuosum, L. polystachyum;* Figs. 6.1g, 6.3d, respectively). The aperture arms range from 1/2 to 3/4 of the radius or extend its entire length. In verrucate spores the laesura are almost always prominent, often the length of the radius and fusing at the equator with the equatorial ridge in a triangular area (e.g., *L. auriculatum* and *L.volubile;* Figs. 6.5b and 6.7d respectively). In the reticulate spores of *L. microphyllum* and *L. reticulatum*, the laesura is difficult to see amidst the reticulations while in *L. versteegii* the proximal face is devoid of reticulations and, therefore, smooth, with a prominent laesura (Figs. 6.4b,f).

In most verrucate spores the verrucae fuse to form long ridges, which often anastomose. This spore type could be the ancestral form of the reticulate spores. A transition can be seen in the spores of *L. kingii* and *L. volubile,* which could lead to the reticuations of *L. microphyllum* (Figs. 6.5f,c and 6.4e, respectively). The verrucate pattern in *L. oligostachyum* is unique in that the ridges are narrow and appear "peaked" (Fig. 6.6d). Many verrucate spores contain a micro-ornamentation in the form of tubercles on or surrounding the verrucae. These tubercles are the sole ornamentation of the proximal face. The verrucate spores are the most diverse group: the equatorial flange, difference between surface structure of the proximal and distal face, presence of secondary tubercles, prominence of laesurae, and confluence of laesura with proximal equatorial ridge all serve as useful characters. In general these spores also exhibit the least variation in size. *Lygodium trifurcatum* has the

smallest spores (76 μm), whereas the remainder are between 82 μm and 95 μm, with the majority greater than 90 μm (Table 6.1).

The high magnifications (2000-3500 X) of the ultrastructure of the tubercles, verrucae or reticulations help distinguish the micro-ornamentation patterns superimposed on the macro-ornamentation architecture. The verrucate spores most frequently contain both low tubercles (not high enough to measure) and 1-2 μm high tubercles on the verrucae and between the verrucae of the distal face and as the primary ornamentation of the proximal face (Fig. 6.5c). The reticulate patterned spores are relatively free of any micro-ornamentation, even on the smooth proximal face of *L. versteegii* (Figs. 6.4b,c). Tubercles vary widely at high magnification. Irregularities in size of tubercles and distances between them are more apparent than at lower magnification (Fig. 6.1b). Between the gemma-like tubercles of *L. articulatum* there are small low tubercles not observed at 800 X (Fig. 6.1e and d respectively).

Many species exhibit spherules, which are structures derived from perisporal material that develop in the sporangium at the same time as the perispore (e.g., *L. polystachyum, L. radiatum, L venustum;* Figs, 6.3c, f and 6.1c, respectively). These bodies are 1.5-3.3 μm in diameter, spherical and may prove to be taxonomically useful.

The spores of *L. japonicum, L. palmatum* and *L. kerstenii* are low-tuberculate, granulate or rugulate, and, in most cases, no measurable protuberances occur on the surface. These spores often lose their perispore and appear smooth (Fig. 6.8a). This is especially true of *L. palmatum* and may be a result of the relatively short viability of the spores (< 6 months) or the harvesting of immature spores. In the New England states the spores of *L. palmatum* are mature in December, spores collected earlier may be immature

and devoid of the perispore. Even when mature, the ornamentation of *L. palmatum* is very limited and difficult to discern unless a SEM is utilized. *Lygodium kerstenii* spores have low tubercles with some resembling those of *L. japonicum*, and often the spores collected from herbarium specimens are smooth due to erosion of the perispore.

Lygodium hians has an irregular spore surface pattern: some globose spores possess tubercles and what appear to be collapsed, irregular verrucae while others have a lower ornamentation pattern with smaller, less prominent tubercles (some of which merge) and collapsed verrucae (Figs. 6.9a-d). This species displays the most diverse spore morphology in size, shape and ornamentation and is also peculiar in its bearing sorophores only at the apex of the segments.

The sporangia of *Lygodium* contain either 128 or 256 spores (Table 6.2). Only two of the taxa counted contained 256 spores/sporangia, *L. microphyllum* and *L. palmatum.* Both species have relatively small spores (66 μm and 60 μm, respectively). Two different collections of *L. palmatum*, both from the same locality, produced numbers of 128 and 256 [Bower (1923) reported 256 for *L. palmatum*]. More populations of this species should be counted to determine how widely this variability occurs.

The following section summarizes observations on the spores using the SEM. The species are discussed in groups defined by spore ornamentation (Table 6.1). Table 6.3 is a summary of the spore characteristics for each taxon (these characters have been incorporated into the cladistic data matrix). All spores are tetrahedral to tetrahedral-globose.

Tuberculate spores

Lygodium articulatum (Fig. 6.1d,e). Distal face: very large tubercles, 8.3 - 12.5 μm in diameter densely packed, discrete, < 0.5 -1.0 μm apart, gemma-like in

Table 6.2. The number of spores in a sporangium in selected species of *Lygodium*. All of the observations were made on freshly collected material.

Species	Collection site	No. Spores per sporangium	Spore size (µm)
L. flexuosum	NYBG Conservatory	128	90
L. heterodoxum	Oaxaca, Mexico	128	88
L. japonicum	NYBG Conservatory	128	76
L. microphyllum	Palm Beach County, Florida	256	66
L. palmatum	Long Pond, Pennsylvania	128, 256	60,54
L. venustum	Santa Cruz, Bolivia	128	90

Table 6.3. Spore characteristics of *Lygodium* species. See footnotes for an explanation of column headings and abbreviations.

Species	1 Shape	2 Pattern	3 Distal Ridge	4 Micro. ornamen.	5 Dimorphic
L. articulatum	tetra	tu	-	-	-
L. borneense	tetra	tu	-	-	-
L. circinnatum	tetra	tu	-	-	-
L. flexuosum	tetra	tu	-	-	-
L. heterodoxum	tetra	tu	-	-	-
L. lanceolatum	tetra	tu	-	-	-
L. polystachyum	tetra	tu	-	-	-
L. radiatum	tetra	tu	-	-	-
L. salicifolium	tetra	tu	-	-	-
L. venustum	tetra	tu	-	-	-
L. japonicum	tetra	lo.tu/gr	-	-	-
L. kerstenii	tetra	gr	-	-	-
L. palmatum	tetra	gr	-	-	-
L. hians	gl;te	tu-v	-	-	-
L. auriculatum	tetra	l-rid.v	+/-	tub	+
L. cubense	te-gl	l-rid.v	-	+	+
L. kingii	tetra	l-rid.v	+/-	lo.tub	+
L. longifolium	tetra	l-rid.v	+	+	+
L. merrillii	tetra	l-rid.v	+	-/miss	+
L. oligostachyum	te-gl	l-rid.v	-	+	+
L. smithianum	tetra	l-rid.v	+/-	+	+
L. trifurcatum	tetra	l-rid.v	+	+	+
L. volubile	te-gl	l-rid.v	+/-	+	+
L. microphyllum	te-gl	retic	-	-	-
L. reticulatum	te-gl	retic	-	-	-
L. versteegii	tetra	retic	-	-	+

1. Spore shape. tetra = tetrahedral; gl = globose; te-gl = tetrahedral-globose
2. Pattern of spore macro-ornamentation. tu = tuberculate; lo.tu/gr = low-tuberculate-granulate; gr = granulate; tu/gr = tuberculate-granulate; l-rid.v = long-ridged verrucate; tu-v = tuberculate and verrucate; retic = reticulate.
3. Distal equatorial ridge. + = present; - = absent; +/- = minimal.
4. Spore micro-ornamentation. + = present; - = absent; -/miss = absent or missing; tub = tubercles; lo.tub = low tubercles.
5. Proximal and distal face of spore dimorphic. + = dimorphic; - = not dimorphic.

Table 6.3. (continued). See footnotes for an explanation of column headings and abbreviations.

Species	6 Prox. Arch	7 Prox. Ridge	8 Las.	9 Las. Size	10 Las. Wide	11 Extra Mat
L. articulatum	na	-	-	66	-	-
L. borneense	na	-	-	100	-	-
L. circinnatum	na	-	+/-	100	-	-
L. flexuosum	na	-	-	75	-	+
L. heterodoxum	na	-	+/-	100	+	-
L. lanceolatum	na	-	+	100	+	+
L. polystachyum	na	-	+/-	100	+	+
L. radiatum	na	-	+/-	100	+	+
L. salicifolium	na	-	+	100	-	-
L. venustum	na	-	+	50	-	+
L. japonicum	na	-	-	75	-	+
L. kerstenii	na	-	+	100	-	-
L. palmatum	na	-	+	66-75	-	+
L. hians	na	-	+	100	-	-
L. auriculatum	lo.tu	+	+	100	+	-
L. cubense	lo.tu	+	+	100	+	-
L. kingii	gr	+	-	100	+	-
L. longifolium	lo.tu	+	-	100	+	-
L. merrillii	gr-psi	+	+	100	+	-
L. oligostachyum	tu	+	+	75	-	+
L. smithianum	tu	+	+	66	-	-
L. trifurcatum	tu	+	+	100	+	+
L. volubile	gr	+	+	100	+	+
L. microphyllum	na	-	-	66	-	-
L. reticulatum	na	-	-	50-70	-	-
L. versteegii	psi	-	+	50	-	-

6. Proximal face architecture if different from distal face.
   na = not applicable (faces similar);
   lo.tu = low-tuberculate; gr = granulate; gr-psi = granulate-psilate;
   tu = tuberculate; psi = psilate.
7. Proximal equatorial ridge. + = prominent (raised); - = obscure.
8. Laesura. + = prominent; - = obscure; +/- = slightly prominent.
9. Laesura length as percent of radius.
10. Laesura ending at the equator in a widened or triangular area.
    + = yes; - = no.
11. Extraperisporal material (e.g. globules or sphericles).
    + = present; - = absent.

appearance, spherical to slightly irregular, and approximately 9.5 μm high. At 3500X very low tubercles occur at the base of the larger gemmae. The tubercles rarely fuse. Proximal face: tubercles as on distal face, laesura not prominent, extending two-thirds the radius, obscured by gemma-like processes.

Lygodium borneense (Fig. 6.2c,d). Distal face: small tubercles, 2.5 μm in diameter, widely spaced, averaging 4-5 μm apart, spherical to slightly irregular in shape, some fused, tubercles low. Proximal face: tubercles as on distal face, laesura not prominent, extending the full length of the radius.

Lygodium circinnatum (Fig. 6.2a,b). Distal face: large tubercles, 4-6 μm in diameter, widely spaced, 2.5-4(8) μm apart, approximately 4 μm high, spherical, some gemma-like as in *L. articulatum*, often varying in size. Proximal face: tubercles as on distal face, laesura not obscure, extending the length of the radius.

Lygodium flexuosum (Fig. 6.1f,g). Distal face: large tubercles, 5-6 μm in diameter, evenly spaced but dense, 1.7-4 μm apart, circular in shape. Proximal face: tubercles as on distal face, laesura with tubercles, not prominent, extending 3/4 the length of the radius.

Lygodium heterodoxum (Fig. 6.2e,f). Distal face: large tubercles, 3-5 μm in diameter, widely spaced, 4-16 μm apart, unevenly distributed, 3-3.5 μm high, small granules present on and between tubercles. Proximal face: tubercles as on distal face, laesura with tubercles, extending the length of the radius and ending in a slightly widened area at the equator.

Lygodium lanceolatum (Fig. 6.3a,b). Distal face: small tubercles, 1.6-2.5 μm in diameter, widely spaced, 4-8.5 μm apart, spherical in shape, spherules present. Proximal face: tubercles as on distal face, laesura with tubercles extending the length of the equator.

Lygodium polystachyum (Fig. 6.3c,d). Distal face; small tubercles, 2-3 μm in diameter, dense, 0.5 μm apart, 2-3 μm high, spherical, some fused, spherules present. Proximal face: tubercles as on distal face, laesura extending the length of the radius, ending in an expanded equatorial area.

Lygodium radiatum (Fig. 6.3e,f). Distal face: small tubercles, 2.2 μm in diameter, widely spaced, at irregular intervals, 2-5 μm apart, spherical, spherules present. Proximal face: tubercles as on distal face, laesura extends length of the radius ending in an expanded area at the equator.

Lygodium salicifolium (Fig. 6.1h). Distal face: low tubercles, 2.5-4 μm in diameter, widely spaced, 2-4 μm apart, spherical. Proximal face: tubercles as on distal face, laesura prominent, with tubercles, extending the length of the radius.

Lygodium venustum. (Fig. 6.1a-c). Distal face: low tubercles, 2-6 μm in diameter, many fused, some contiguous, well-spaced, 5 μm apart, 2-3 μm high, spherules present. Proximal face: tubercles as on distal face, laesura very prominent without tubercles, extending 1/2-2/3 the length of the radius.

Verrucate spores

Lygodium auriculatum (Fig. 6.5a-c). Distal face: verrucae, forming long, low ridges, 25-38 μm long, and 6-8 μm wide, < 10 μm high; well-spaced, low tubercles on or between verrucae; with a minimal equatorial ridge. Proximal face: low, widely spaced, tubercules, no verrucae; laesura prominent, extending the length of the radius and ending in a widened area confluent with the prominent proximal equatorial ridge.

Lygodium cubense (Fig. 6.5d,e). Distal face: verrucae, some fusing to form long ridges, some of the ridges anastomosing, (10)25-30 μm long, 12-16 μm wide, 12 μm high, widely spaced, tubercles scattered on and between verrucae, 3.3 μm in diameter, 3.3 μm high with 4-10 μm between tubercles. Proximal face: tubercles as on distal face, 1.5-2.5 μm in diameter, 2.5-4 μm apart, no verrucae, laesura not prominent, widening into a prominent proximal equatorial ridge.

Lygodium kingii (Fig. 6.5f). Distal face; verrucae forming long ridges, 40 μm long, 7 μm wide, 9-10 μm high, ridges often anastomosing, low tubercles on or between verrucae, (transitional between verrucate and reticulate spore patterns). Proximal face: very low tubercles, height not measurable, (granulate), no verrucae, laesura extending length of the radius, widening into a prominent proximal equatorial ridge.

Lygodium longifolium (Fig. 6.6a). Distal face: verrucae, forming ridges, often anastomosing, 42-89(115) μm long, 15-17 μm wide, equatorial ridge, low tubercles present on and between verrucae. Proximal face: granulate to very low-tuberculate (height of tubercles not measurable), no verrucae, laesura,

not prominent, extending the length of the radius, widening into a prominent proximal equatorial ridge.

Lygodium merrillii (Fig. 6.6b,c). Distal face: verrucae forming ridges, some long, few anastomosing, 42 μm long, 12-14 μm wide, without tubercles (spore may be lacking a perispore), prominent equatorial ridge. Proximal face: smooth (spore may be missing a perispore), no verrucae or tubercles, laesura extending the length of the radius, widening into a prominent proximal equatorial ridge.

Lygodium oligostachyum (Fig. 6.6d,e). Distal face: verrucae forming narrow ridges that appear to form peaks, often anastomosing, 52 μm long, 6 μm wide, 2-3 μm high, large round tubercles scattered on and between verrucae, 2-3 μm in diameter, without a prominent equatorial ridge, spherules present. Proximal face: tubercles as on distal face, scattered, no verrucae, laesura extending 3/4 the length of the radius, with a proximal equatorial ridge.

Lygodium smithianum (Fig. 6.6f). Distal face: verrucae forming ridges, often anastomosing, 17 μm long, 6-7 μm wide, low, round tubercles scattered on and between verrucae, 2-3 μm in diameter, with small equatorial ridge. Proximal face: low-tuberculate to granulate, no verrucae, laesura prominent extending 2/3 the length of th radius, with a proximal equatorial ridge.

Lygodium trifurcatum (Fig. 6.7a,b). Distal face: verrucae forming ridges, often anastomosing, 15-30 μm long, 5 μm wide, tubercles on and between verrucae, equatorial ridge resembles a continuous equatorial verruca. Proximal face: tubercles, < 0.2 μm high and spaced at 2 μm intervals, laesura

prominent ending at equator, widening into a prominent proximal equatorial ridge.

Lygodium volubile (Fig. 6.7c-e). Distal face: verrucae forming narrow ridges often anastomosing, 16-42 μm long, (1)5-8 μm wide, tubercles (2 μm in diameter, 2 μm high) to very low tubercles or granulae over entire surface, without a prominent equatorial ridge, spherules present. Proximal face: granulate to low-tuberculate, no verrucae, laesura prominent, ending at the equator in a prominent proximal equatorial ridge. This species bears the most exaggerated equatorial ridge.

Reticulate Spores

Lygodium microphyllum (Fig. 6.4e,f). Distal face: reticulate, with no micro-ornamentation pattern and no equatorial ridge. Proximal face: reticulate as distal face, laesurae inconspicuous between reticulations, extending 2/3's the length of the radius. Some spores appear transitional between anastomosing verrucate and reticulate.

Lygodium reticulatum (Fig. 6.4d). Globose. Distal face: reticulate, with no micro-ornamentation pattern and no equatorial ridge. Proximal face: reticulate as distal face, laesura not easily visible.

Lygodium versteegii (Fig. 6.4a-c). Distal face: reticulate, at corners where ridges fuse often slightly raised, reticulations 8 μm high, at 2000X no micro-ornamentation pattern observed. Proximal face: reticulations extend around the equator and end in a smooth triangular surface with prominent laesura extending 50% of the radius into the equatorial reticular pattern which is

confluent with the distal face sculpture. There is no micro-ornamentation between the reticulations as can be seen in the high magnification. This is the most distinct proximal face architecture.

Low-tuberculate to granulate to irregular spores

Lygodium japonicum (Fig. 6.8e,f). Distal face: very low-tuberculate, difficult to measure the height of the tubercle, 3.3 μm in diameter, irregularly spaced, averaging 3.3 μm apart. Proximal face: tubercles as on distal face, laesura not prominent with low tubercles, extending 4/5's the radius. One specimen from India (*Gamble 27406* NY) had dense tuberculae resembling that of *L. flexuosum.*

Lygodium kerstenii (Fig. 6.8a,b). Distal face: very low-tuberculate to granulate. Proximal face: very low tubercles as on distal face, laesura not prominent, extending the length of the radius. Spores lose perispore easily making it difficult to assess patterning: some represent a low-tuberculate form most closely resembling *L. japonicum.*

Lygodium palmatum (Fig. 6.8c,d). Distal face: very low-tuberculate to granulate. Proximal face: granulate as on distal face, laesura prominent, extending 2/3-3/4 the length of the radius. Spores often are smooth as a result of the lack of a perispore which is easily cracked and shed or not formed fully if spores are immature.

Lygodium hians (Fig. 6.9). Tetrahederal to globose. Distal face: tuberculate to verrucate (tubercles often fusing), to very exaggeraged verrucae amid tubercles, the verrucae often appearing collapsed. The latter spores are large

and globose to tetrahedral (109-124 μm). Proximal face: the same as the distal either low tubercles or irregular verrucae and tubercles, laesura extends the length of the equator and is covered with the tubercles and verrucae. The surface variation observed from simple tuberculate to an irregular heavy architecture has not been found in any other species. The size and shape differences in spore types often from the same specimen suggest some type of genetic anomaly or possibly an apomictic life cycle, e.g. mitospores.

Abortive spores

Lygodium × *boivinii* (Fig. 6.10a-c). Spores range from small, tetrahedral to large, globose. Most are malformed, cracked or imploded, often with surface globules. There are very large (125-140 μm) and very small spores (< 50 μm). This species is considered a hybrid between *L. lanceolatum* and *L. kerstenii.*

Key

The following key identifies *Lygodium* species based on the data obtained from spore surface observations using the SEM and size ranges obtained from light microscopic studies.

Key to *Lygodium* Spores

1. Spore surface very low-tuberculate to granulate (height of protrusions < 1 μm).
   2. Laesura prominent, extending 1/2-2/3 length of radius.....*L. palmatum.*
   2. Laesura not prominent..... *L. kerstenii.*
1. Spore surface with tubercles, verrucae or reticulations.
   3. Spore surface with round tubercles (base not wider than top), often as high as wide.
      4. Tubercles 1-2 μm high, perispore often peeling or cracked.....
      .....*L. japonicum.*
   4. Tubercles > 2 μm high.
      5. Tubercles 8-12 μm in diameter, gemma-like, densely packed with larger ones discrete, not confluent (< 1-1.5 μm apart).*L. articulatum.*
      5. Tubercles < 8 μm diameter.
         6. Tubercles 4-8 μm in diameter.
            7. Tubercles dense < 0.5-4 μm apart.....*L. flexuosum, L. salicifolium.*
            7. Tubercles well spaced, > 4 μm apart.
               8. Spores 55-85 μm dia. (ave. 70 μm).....*L. circinnatum.*

8. Spores 85-100 μm dia. (ave. 88 μm).....*L. heterodoxum.*
6. Tubercles < 4 μm in diameter.
9. Tubercles widely spaced, 4-8 μm apart.
10. Laesura not widening, ending at equator, not prominent.....*L. borneense.*
10. Laesurae widens, ending at equator.
11. Spores 85-92 μm dia. (ave. 90 μm).....*L. lanceolatum.*
11. Spores 100-120 μm dia. (ave. 110 μm).....*L. radiatum.*
9. Tubercles densely arranged, 0.5-3 μm apart.
12. Laesura prominent, extending 1/2- 2/3 the length of the radius.....*L. venustum.*
12. Laesura not prominent, obscured by tubercles extending length of radius.....*L. polystachyum.*
3. Spore surface with short-long ridged verrucae or reticulations.
13. Spore surfaces with irregular, short- to long-ridged verrucae.
14. Spores with prominent proximal eqatorial ridge.
15. Laesura not prominent.....*L. cubense.*
15. Laesura prominent.
16. Laesura extending length of radius widening into equatorial ridge.
17. Spores < 80 μm diameter.....*L. auriculatum, L. trifurcatum.*
17. Spores > 86 μm diameter.
18. Spherules present.....*L. auriculatum, L. volubile.*
18. Spherules absent .....*L. cubense, L. longifolium.*
16. Laesura extending 2/3-3/4 length of radius.
19. Proximal and distal face with same pattern, spores with larger "collapsed verrucae often globose.....
.....*L. hians.*
19. Proximal and distal face with different pattern.
20. Proximal face with widely spaced round discrete tubercles.....*L. oligostachyum.*
20. Proximal face with low tubercles, < 1-1.5 μm high
.....*L. kingii, L. smithianum.*
14. Spores without a prominent equatorial ridge, laesura prominent.
..... *L. merrillii.*
13. Spore surfaces without verrucae but having reticulations.
21. Both proximal and distal faces with reticulations, spores globose, laesura obscure.
22. Spores > 100 μm in diameter.....*L. reticulatum.*
22. Spores < 70 μm in diameter.....*L. microphyllum.*
21. Proximal and distal faces different, spores tetrahedral, proximal face partially smooth, laesura prominent.....*L. versteegii.*

Discussion

The surface spore patterns in *Lygodium* define four groups that are not strictly compatible with alliances based on gross plant morphology. The taxonomic usefulness of spore surface characters, therefore, appears to be in delineating species, not in producing useful phylogenies. I observed no

difference in spore ornamentation pattern within species. The only exception occurred with *L. salcifolium* and *L. kingii* which appear morphologically conspecific and yet have different spore types (*L. salicifolium* with tuberculate spores and *L. kingii* with verrucate spores; Figs. 6.1h, 6.5f). These two taxa are treated as separate species based on spore data.

In general, the diversity in spore ornamentation patterns in ferns can not compare with the diversity in other morphologic characters. One would expect the small number of spore patterns to have evolved separately in many unrelated taxa. Indeed this observation has been presented by numerous pteridologists (e.g., Wagner, 1974) as an example of parallel evolution.

In *Lygodium* the spore ornamentation is formed by the bilayered exospore or partly by the outer compact layer of a trilayered perispore. This combination of exospore and perispore layering along with the spore aperture type (trilete vs. monolete) has placed *Lygodium* in A. Tryon's "specialized" category of evolutionary spore development (1990). This is in contrast to a "primitive" category for the Ophioglossaceae, Marattiaceae, and Osmundaceae and an "advanced" level for Blechnaceae, Dryopteridaceae, etc. The families in the specialized level have characteristics of both the primitive and advanced levels and contain a phylogenetically diverse group of ferns (Cyatheaceae, Polypodiaceae, Pteridaceae, Dicksoniaceae, Dennstaedtiaceae, Davalliaceae and Gleicheniaceae).

Tryon and Lugardon (1991) describe the contribution of the wall layers to surface ornamentation patterns from transmission electron micrographs. The exospore contours form the reticulations in *L. reticulatum* and *L. microphyllum* whereas a thick perispore partly forms the tubercles in *L. japonicum* and *L. salicifolium* (Tryon & Lugardon, 1991). The patterning in taxa of *Lygodium* is, therefore, not simply an expression of either the exospore

or perispore. Exospore development in ferns was studied extensively by Lugardon (1974). It is initiated by long sheets ("feuillets") formed centrifugally that envelop the tetraspores. The number of sheets initiating the exospore varies from 10-12 in less derived fern genera (Ophioglossaceae) to a single sheet in more derived genera (Blechnaceae). The exospore substructure in *Lygodium* is initiated by a single sheet ("blechnoid type") upon which amorphous sporopollenin is deposited in discrete units which eventually coalesce. These units are covered by granules and fibrils and additional sporopollenin forming a compact outer layer (Lugardon, 1971). The perispore is then deposited over the thicker exospore layer by materials from the plasmodial tapetum. In *Lygodium* the perispore is trilayered: the innermost layer contains scales; the middle layer appears "grid-like"; and the outer layer is compact. According to the TEM studies of the wall layers by Tryon and Lugardon (1991), it is this outer layer that forms the tubercles of *L. salicifolium*. The middle "grid-like" layer is well developed in *L. cubense* and *L. heterodoxum*, less so in *L. volubile*, and almost lacking in *L. microphyllum*. The thickness of this layer apparently has little to do with surface ornamentation patterns. For example, the tuberculate spores of *L. heterodoxum* and the verrucate spores of *L. cubense* both have extensive layering whereas the verrucate spores of *L. volubile* have a reduced middle layer and the reticulate spores of *L. microphyllum* have almost no grid-like layer. That *L. cubense* and *L. heterodoxum* have structurally similar perispores is interesting and presents evidence for an alliance between the pair of taxa that will be evaluated morphologically. Another alliance is suggested between *L. volubile* and *L. microphyllum*, which possess a greatly reduced or absent middle perispore layer and many morphologic affinities. This suggests that further TEM studies should be conducted on all the *Lygodium*

taxa to determine if the structural characteristics of the exospore and perispore can further define species alliances.

The dark masses observed by Tryon and Lugardon (1991) in the TEM's of some taxa (e.g. *L. heterodoxum*) are speculated to be silica deposits as earlier studies detected silica in the spore wall of *L. japonicum* (Edman, 1938). This could provide another useful character if confirmed.

Perispore material is structurally separated from exospore sporopollenin by a fine layer of granules which often disintegrates at spore maturity or after spore release (Lugardon, 1974). The breakdown of this layer of granules with subsequent shedding of the perispore may explain why spores of *L. palmatum, L. japonicum, L. kerstenii, L. borneense* and *L. venustum* often appear smooth under the microscopic.

Globules or spherules (small bodies of superficial perisporal material) present in some taxa of *Lygodium* (e.g., *L. radiatum*) may also prove to be useful taxonomically. *Schizaea* species often have such depositions on their spore surfaces.

Subgeneric groups and spore characters

The macro-ornamentation patterns in *Lygodium* create four groups: tuberculate, verrucate, reticulate, and low-tuberculate-granulate. These patterns may actually represent variations or subsets of the same overall spore morphology. Spherical tubercles that fuse may easily become verrucae and verrucae that fuse to form long ridges that anastomose may become reticulations. The low surface patterns may actually be the more derived form, representing tubercles or anastomosing verrucae that have become suppressed in height. The lack of rigid distinctions in ornamentation adds a plasticity to spore patterns also seen in morphological characters (e.g., segment shape variation in lower outer lobules or central long lobule,

pinna-branch axis variation, straight to flexuous to dichotomous). However, because of secondary spore characteristics, it is readily possible to differentitate between the verruate and tuberculate types. Verrucate spores always exhibit different ornamentation patterns on the proximal and distal faces and equatorial ridges whereas tuberculate spores have neither this face dimorphism or ridges. It is impossible in *Lygodium* to define subgeneric groups based entirely on spore patterns The spore patterns appear to represent homoplasies at the species level and do not support alliances based on morphology. For example, *L. versteegii* is morphologically allied with species that have dichotomously branched pinna-branches (as *L. trifurcatum, L. auriculatum, L. longifolium, L. circinnatum*). Yet *L. versteegii* spores are more like those of pinnate branched species (e.g., *L. microphyllum, L. reticulatum*). The reticulate spore characteristic (*L. versteegii, L. microphyllum*, and *L. reticulatum)* has evolved in this genus at least twice, in dichotomously branched species and pinnately branched species.

Taxonomy and spore characters

Taxonomonically, spore characters are helpful in distinguishing between morphologically similiar species in *Lygodium.* For example, in the Old World, *L. microphyllum* may be mistaken for *L. salicifolium* or *L. smithianum.* The reticulate spores of *L. microphyllum* easily distinguish it from the tuberculate spores of *L. salicifolium* or the long-ridged verrucate spores of *L. smithianum.* Since its introduction into the New World, *L. microphyllum* is often misidentified as *L. volubile.* This again would be easily determined if fertile material were available as *L. volubile* has verrucate spores. Prantl (1881) used spore pattern to separate species within his sections. In "Section Palmata", *L. palmatum, L. circinnatum*, and *L. radiatum* (tuberculate spores) are distinguished from *L. articulatum* and *L. trifurcatum* (the former with dense

large tubercles and the latter with verrucae). With the use of the SEM, all the above species could be separated by spore morphology alone. In Prantl's "Section Volubilia" spores are used to distinguish between *L. micans* (verrucate spores) and *L. volubile* and *L. wrightii* (smooth spores). In the present treatment all these are considered conspecific. Photomicrographs reveal that all these species have the same spore ornamentation pattern and this information combined with the lack of distinct morphogical characters helps support the synonomy.

The smooth surface that Prantl encountered in *L. volubile* and *L.wrightii* spores was probably due to the cracking or eroding of the perispore. This same problem caused Holttum (1959) to recognize spores of *L. borneense* as smooth and contributed to his view that it was a distinct species in Flora Malesiana. Photomicrographs reveal this species has tuberculate spore patterns. The spores of *Lygodium giganteum* Tagawa & Iwatsuki have also been described as smooth. This is most probably the result of a missing perispore. However, since I have not examined the type specimen from which the spores are reported as smooth, this is impossible to determine. *Lygodium giganteum* appears to be a twice pinnate *L. flexuosum*. Only one specimen was seen and this had immature spores.

Large and Braggins (1990) note the difficulty in using herbarium material alone to determine spore pattern: immature and cracked spores, along with those lacking a perispore, are all too common. The few specimens available to early monographers and the lack of high magnification lenses often yielded erroneous data.

Most of the characteristics used in the spore key have been incorporated into the cladistic data matrix. For example, the difference, if any, of the macro-ornamentation pattern on distal and proximal faces, presence or

absence of micro-ornamentation, the length of the laesura (extending the full length of the radius or less than the length of the radius), and the presence of a prominent proximal equatorial ridge (a thickening at the equator in some trilete spores) are variable characters and may prove taxonomically useful when analyzed with other characters.

Fossil spores and spore character polarity

The fossil record of spores similar to those of extant species of *Lygodium* is well documented. Spore morphology data can support hypotheses concerning ancestral taxa. Trilete spores are considered the primitive fern spore type as evidenced by their early presence in the fossil record (Devonian and Carboniferous). Distinct spores were common across Europe and Asia during the Cretaceous. The tuberculate spores of *Lygodium grandis* Bolk. and *L. corrugatus* Bolk. of the lower Cretaceous are very similar to those of the extant species *L. flexuosum* and *L. venustum*. (Bolkhovitina, 1959). Many spores have been described acccording to their ornamentation pattern, but their is no macrofossil evidence to support their placement in the genus. Reticulate spores, resembling those of the extant *L. microphyllum*, are common elements in many middle and late Mesozoic spore assemblages in beds of the Aklavik Range in Northwest Canada (Fensome, 1987). These dispersed spores have been placed by Fensome (1987) in the genus *Ischyosporites* (Couper) Schulz (= *Klukisporites variegatus* Couper) and represent the macrofossil genera *Klukia* and *Stachypteris* which are possible *Lygodium* ancestors. In further studies Fensome placed dispersed tuberculate spores from the same locality into *Concavissimisporites* and *Trilobosporites*.

Bolkovitina (1959) studied the fossil spores of the Schizaeaceae *s.l.* and described eight fossil species assigned to the genus *Lygodium* based on exospore sculpture. The following spore types and fossil spore species were

designated: smooth spores, *L. simplex* Bolk, Lower Cretaceous, USSR (~ extant *L. venustum*); fine confluent tubercles, *L. asper* Bolk., Far East, Aptian (~ extant *L. polystachyum*); coarse sparse tubercles, *L. minutus* Bolk., USSR, Albian (~ extant *L. circinnatum*), *L. gibberulum* K.-M.,USSR, Albian; coarse dense tubercles, *L. magnituberculatum* Bolk, USSR, Lower Cretaceous (~ extant *L. smithianum)*; coarse reticulate, *L. visibilis* Bolk, USSR, Albian (~ extant *L. reticulatum*); and variations on the basic tuberculate ornamentation, *L. splendidum* K.-M., USSR, *L. pseudomirabile* Bolk., and *L. mirabile* Bolk, all of the Lower Cretaceous.

From the studies of Bolkhovitina (1958, 1959) it appears that spores of *Lygodium* appeared in the Jurassic and spread in the Lower Cretaceous. During the Late Cretaceous the number of species declined and their ranges diminished. Disjunct or insular ranges developed during the Paleocene as the Schizaeaceae *s.l.* retreated into the tropics. Due to the cooling and drying of the climate of Northern Eurasia during the Tertiary and Quaternary, the majority of extant taxa are now found in the humid tropical forests of both hemispheres. The greatest distribution and variety of fossil spores attributable to the genus *Lygodium* is seen in the USSR, the Far East, along the whole of Northern Europe to England, and in Australia (Bolkovitina, 1959). In the Aptian and Albian (Middle Cretaceous), the number of species determined by spore type declines and in the Upper Cretaceous, Paleocene, and Oligocene deposits, spores are sporadically found in Europe, Chile and southern Australia. Fensome (1987) suggests that probable Schizaeaceous spores were present in the Triassic but that the morphological characteristics seen in extant species did not occur until the Late Jurassic/Early Cretaceous.

The Jurassic fossil genus *Klukia exilis* (Phillips) Raciborski was extensively studied by van Konijnenberg-van Cittert (1981), who recommended it be

accepted in the Schizaeaceae *s.l.* and theorized that it was most closely allied with *Lygodium.* It has reticulate spores (54-77 μm) with the reticulations continuing from the distal face over the equator to the proximal face only to the ends of the laesurae. The remainder of the proximal face is verrucate. The laesurae are distinct and extend the length of the radius. Pits between the muri on the distal face contain verrucae. These spores, therefore, in some aspects, resemble extant *L. microphyllum/L. reticulatum.* They differ, however, in proximal face ornamentation patterns and also in the micro-ornamentation pattern between reticulations on the distal face. Another allied species of the same geologic era is *Stachypteris spicans* Pomel which has spores with verrucae often fusing into ridges. Its proximal surface is granulate, and granulae are present on the distal verrucae and at the bottom of the pits formed by the fused verrucae. Both *Klukia* and *Stachypteris* have prominent laesurae and thick exospores (van Konijnenberg-van Cittert, 1981). Thus, the entire surface of these fossil spores is covered with granulae or verrucae which on the distal face are superimposed with muri and pits. Van Konijnenberg-van Cittert (1981) theorizes that these spores have characteristics similar to all four types of extant *Lygodium* spores.

Lygodium is represented in the Tertiary (Balme, 1995): *Lygodium skottsbergii* from Chile (Halle, 1940) has granulate spores (85 μm); *L. kaulfussi* has granulate spores (100 μm; possibly lacking a perispore); and *L. poolensis* reticulate spores (65 μm).

The diversity of fossil spore types makes it difficult to determine the ancestral spore architecture. Many of the fossil species assigned by Bolkhovitina (1958,1959) are based solely on spore collections without macrofossil evidence. Manchester and Zaveda (1986), in describing the macrofossil *Lygodium kaulfussi* Heer (Eocene, Wyoming), note that the psilate

spores recovered from the fertile segments are probably the result of the perispore being lost in fossilization or absent prior to fossilization. Since the loss of the perispore is common in extant taxa, it seems plausible to hypothesize that the psilate ancestral spores lacked perispores. Macrofossil evidence (fertile and sterile leaflets), however, suggests that *Lygodium kaulfussi* is an "articulate" relative of extant *Lygodium palmatum* with psilate to granulate spores (Manchester and Zaveda, 1986). This hypothesis is supported by the fact that *Lygodium palmatum* possesses spores with the least ornamentation of extant *Lygodium* species. Thus, by the Eocene, spores of *Lygodium* with minimal ornamentation had evolved.

The above macrofossil and spore data suggest that *Lygodium* ancestors date as far back as the Jurassic (*Klukia* and *Stachypteris*). The spores had reticulate, verrucate or tuberculate architecture (or combinations of all three). This information has led Manchester and Zaveda (1986) to suggest that heavy ornamentation patterns were the primitive spore type for this genus. Structurally, the ancestral spore probably contained a thick exospore and was 50-70 μm with a surface pattern of long-ridged verrucae or reticulations. It had proximal and distal face dimorphism and tubercles or granules present as micro-ornamentation on and between the verrucae and anastomosing ridges (van Konijnenburg-van Cittert, 1991). This type fits no one extant species but resembles more closely those members of the verrucate group. By the Eocene, in some taxa, spore ornamentation appears to be reduced to granulate.

Reticulate spores in ferns have been considered derived by Wagner (1974). However, within any group polarity is determined on evidence from all information sets and, at best, is hypothetical. From the paleobotanical evidence, verrucate and reticulate spores appear to be primitive in *Lygodium* and low-tuberculate, granulate ones possibly the more advanced. However, in

Pyrrosia, for example, verrucate spores are considered more derived than smooth spores (A. Tryon, 1990). This points out the difficulty in generalizing polarity of any character. There can as easily be an increase in ornamentation pattern as a reduction and with relatively few characters in fern spore architecture as compared with the number of genera, it is reasonable to expect that reversals will be common in different groups.

Ecology and spore ornamentation

Reticulate spores in *Asplenium* and *Pyrrosia* are associated with epiphytic species while more fenestrate spores have been found associated with terrestrial habitats (A. Tryon, 1986; Van Uffelen, 1986). Van Uffelen (1986) associates the perispore with dispersal mechanisms and the exospore with protection (primarily from desiccation after shedding). Channels in the exospore connect the inside with the outer environment, and this information may play an important role in germination through the laesura. The perispore is actually a hindrance to germination, but since it is more brittle, it easily breaks. Mitsui (1986) finds that in those ferns with a thin exospore, the perispore assumes a more protective function. Therefore, since *Lygodium* contains a thick exospore, the perispore may serve in dispersal. It has been suggested by Mitsui (1986) that a coarse heavy ornamentation may permit the spores to fall and attach to nearby disturbed areas where successful sporophyte colonization is possible.

Spore number, size, ploidy level and life cycle importance

The sporangium of *Lygodium* contains 128 or 256 spores (Table 6.2). This is characteristic of primitive fern taxa since more advanced species contain 64 spores per sporangium. Wagner (1974) considers 256 spores per sporangium the primitive state and anything less than 128 derived. Spore sizes greater than 70 μm are also considered to be more derived: most *Lygodium* spores are

between 50 and 120 μm. Thus, *Lygodium* falls somewhere between primitive and derived in both number per sporangium and size of spores.

According to Carlquist (1966), large spore size may have an adaptive significance in counteracting wide dispersal in wet high forests where long distance dispersal would place the spores in the lower drier areas. One of the species of *Lygodium* with the largest spores, *L. radiatum*, usually grows in the understory of dense forests and rarely climbs more than 2-3 m. Since the spores will probably not reach the wind column due to the canopy, they will fall close to the ground where the moist undergrowth may provide the necessary habitat for the gametophyte.

Large spore size may also be a feature of increased ploidy levels in ferns. The chromosome numbers of *Lygodium* are n = 28, 29, or 30. There are a number of polyploids in *Lygodium*. However, it was not possible to confirm a correlation between increased spore size and increased ploidy level due to the inaccessibility of vouchered material. Recent chromosome studies on one of the taxa with the largest spores, *L. reticulatum*, indicates it is a simple diploid (n = 30).

The largest potentially viable spore observed is attributed to *Lygodium hians*, a species endemic to New Caledonia, that exhibits a peculiar fertile segment morphology. Some of the spores are very large (125 μm) and the ornamentation pattern is very different from all other species of *Lygodium*. It appears that *L. hians* is some aberrant form of *L. articulatum* of New Zealand in which the fertile portions have become limited to the upper third of the segment with extremely divided sorophores. This type of fertile expression is often seen in hybrids or genetic anomalies. The spores do not appear to be abortive. It is possible that they are mitospores or diploid spores, produced in response to an irregularity in meiosis in which the $2n$ spore produces a diploid

gametophyte, which in turn gives rise directly to a sporophyte without fertilization. The development of the gametophyte and production of a sporophyte is accelerated and often is an adaptation to dry environments (e.g., *Cheilanthes* undergoes this type of apomictic reproduction). It is also possible that the large irregular spores are the result of the same anomalous sorophore gene complex. More collections of this species must be studied cytologically, morphologically and phytochemically. Even though there is variation in the size and shape of the spore, the difference in the surface pattern is only a matter of exaggeration or suppression of the verrucae and/or tubercles.

The sporangia in *Lygodium* taxa that contain 256 spores are usually those with the smaller spores (Table 6.2; *L. palmatum, L. japonicum*).

Spores of the Schizaeaceae *sensu lato*

The spores of the Schizaeaceae *sensu lato (Schizaea, Actinostachys, Lophidium*(?), *Lygodium, Anemia,* and *Mohria)* are as diverse as the general morphlogy of these taxa (Tryon and Lugardon, 1991). *Schizaea* (Schizaeaceae *sensu strictu,* including *Actinostachys*) has bilateral spores [excluding *Schizaea dichotoma* (L.) Sm.] with a bilayered exospore and a thin perispore. The surface often contains spherules. The pattern is rugate, papillate, striate or reticulate. In most cases the size of the spore can be inversely correlated with lamina size. The bilateral spores of *Actinostachys* have striations and tubercles in common with those of both *Anemia* and *Lygodium* respectively.

Anemia and *Mohria* (Anemiaceae s.s.) have trilete spores with a thick bilayered exospore and a thin confluent perispore. *Anemia* subgenus *Coptophyllum* has striate spores (some angular) with a complex perispore, while subgenus *Anemiorrhiza* has striate spores (often less angular) and two species with reticulate spores, and subgenus *Anemia* possesses spores with coarse ridges to narrow ridges with tubercles. The classic parallel ridges or

striations indicative of many *Anemia* spores have made them easily recognized by paleobotanists (e.g. *Cicatricosisporites, Appendicisporites*). *Anemia wrightii* (subgen. *Anemiorrhiza*) has reticulate spores much like those in *Lygodium*. *Mohria* has trilete striate spores with a bilayered exospore and a complex perispore much like spores of *Anemia* subgen. *Coptophyllum*.

Reticulate spore morphology can be seen in *Schizaea, Anemia* and *Lygodium*. Some authors (e.g., Tryon and Lugardon, 1991) have suggested that this is a character that unites the genera in the family. However, it has been seen that reticulate spores within the genus *Lygodium* probably evolved more than once. This type of spore pattern may represent one of the ancestral surface morphologies.

Conclusions

Spore morphology is an extremely valuable character in the taxonomy of *Lygodium*. Some of the basic patterns can be seen with a dissecting scope and are a practical tool in the delineation of species. There appears to be little variation of surface pattern within a species. Only New Caledonian *Lygodium hians* has some degree of spore variation, and this species may represent a genetic variant of *L. articulatum*. Spore morphology has been used in this treatment to separate closely related taxa (e.g., *L. kingii* from *L. salicifolium*, and *L. heterodoxum* from *L. merrillii*). It has also been used to support close "complex" alliances among *L. japonicum, L. venustum* and *L. kerstenii* and among *L. volubile, L. kingii,* and *L. smithianum*.

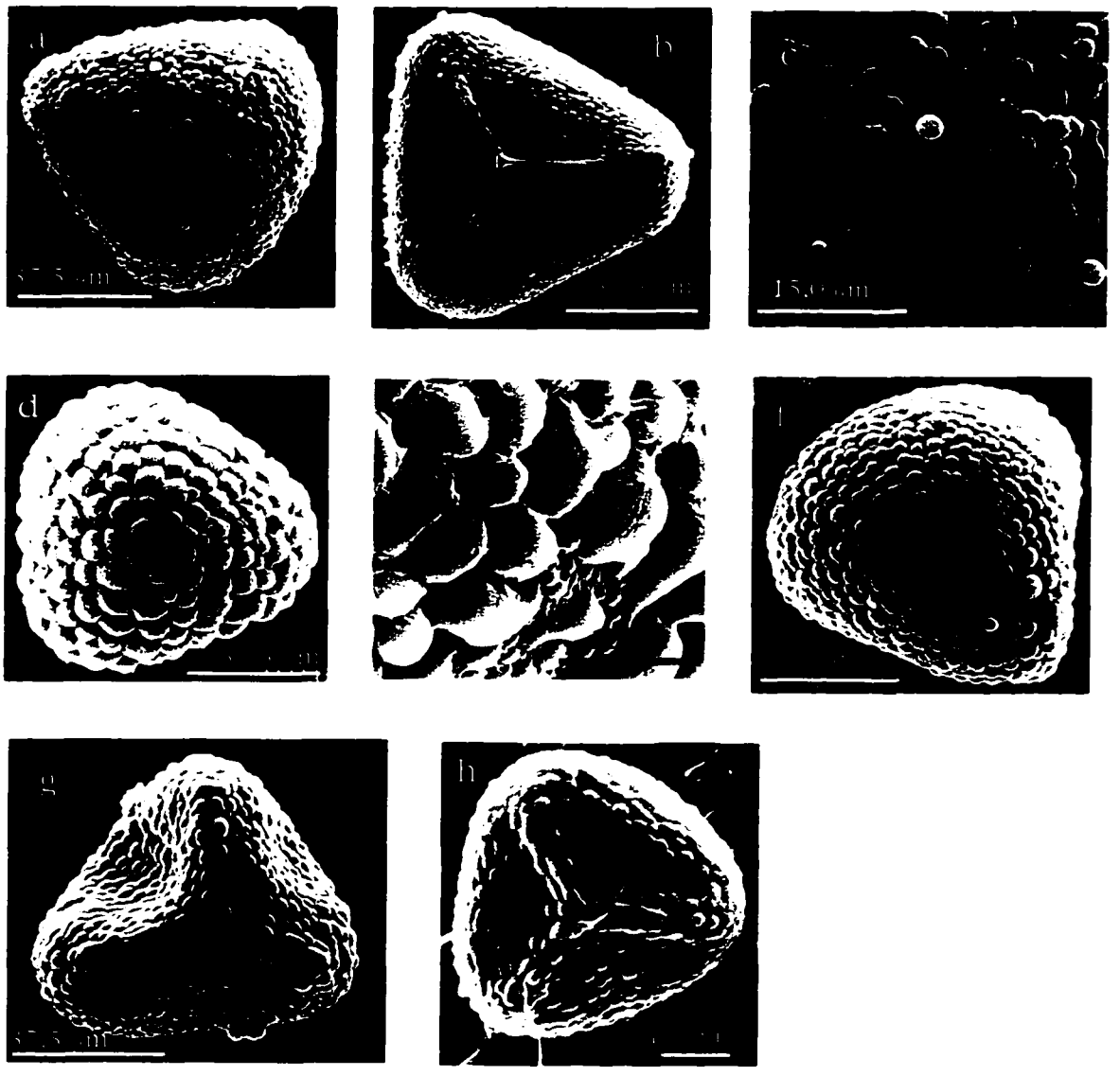


Figure 6.1. Spore morphology of *Lygodium* species with tuberculate spores. (a) *L. venustum* distal view; (b) *L. venustum* proximal view; (c) *L. venustum* detail of surface; (d) *L. articulatum* distal view; (e) *L. articulatum* detail of surface (f) *L. flexuosum* distal view; (g) *L. flexuosum* proximal view; (h) *L. salicifolium* proximal view.

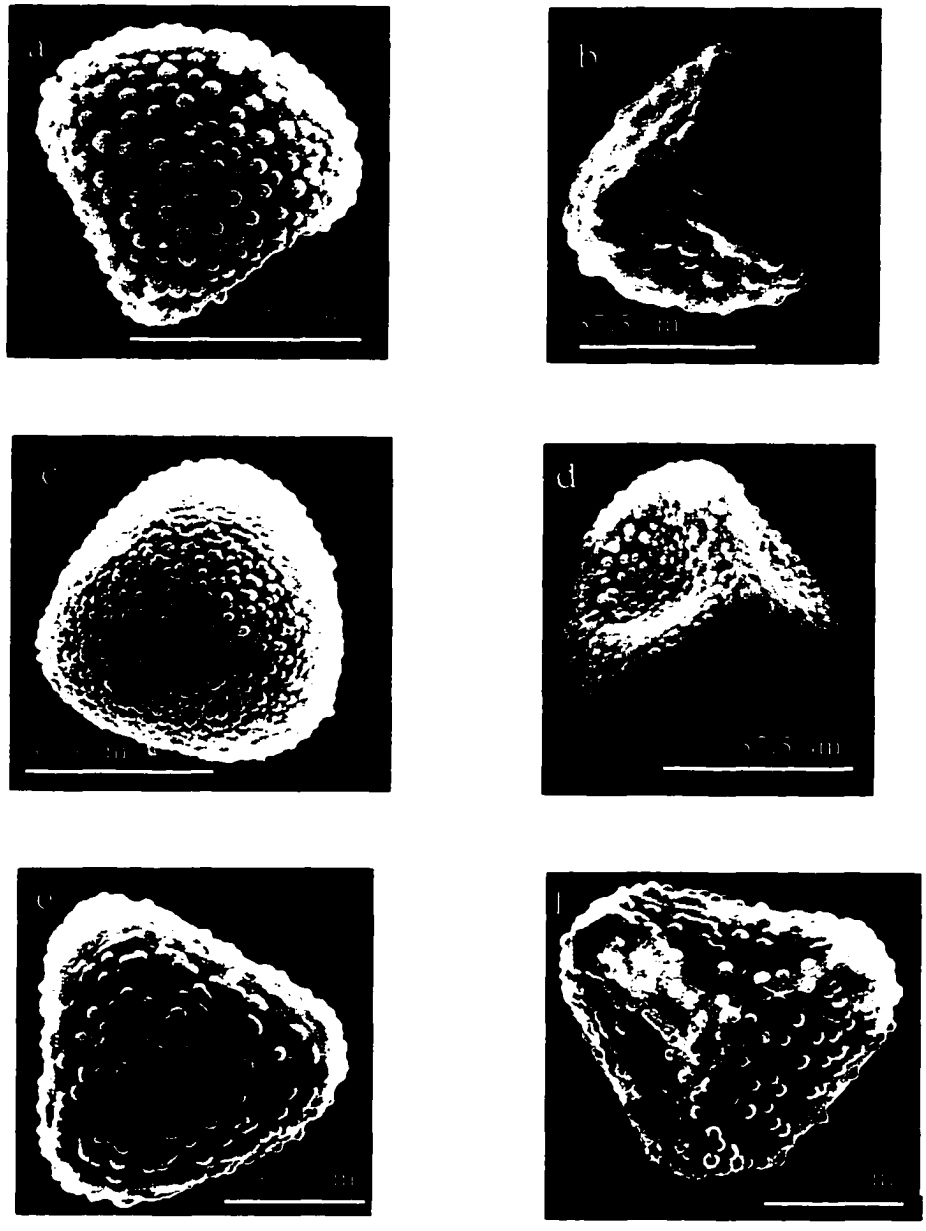


Figure 6.2. Spore morphology of *Lygodium* species with tuberculate spores. (a) *L. circinnatum* distal view; (b) *L. circinnatum* proximal view; (c) *L. borneense* distal view; (d) *L. borneense* proximal view; (e) *L. heterodoxum* distal view; (f) *L. heterodoxum* proximal view.

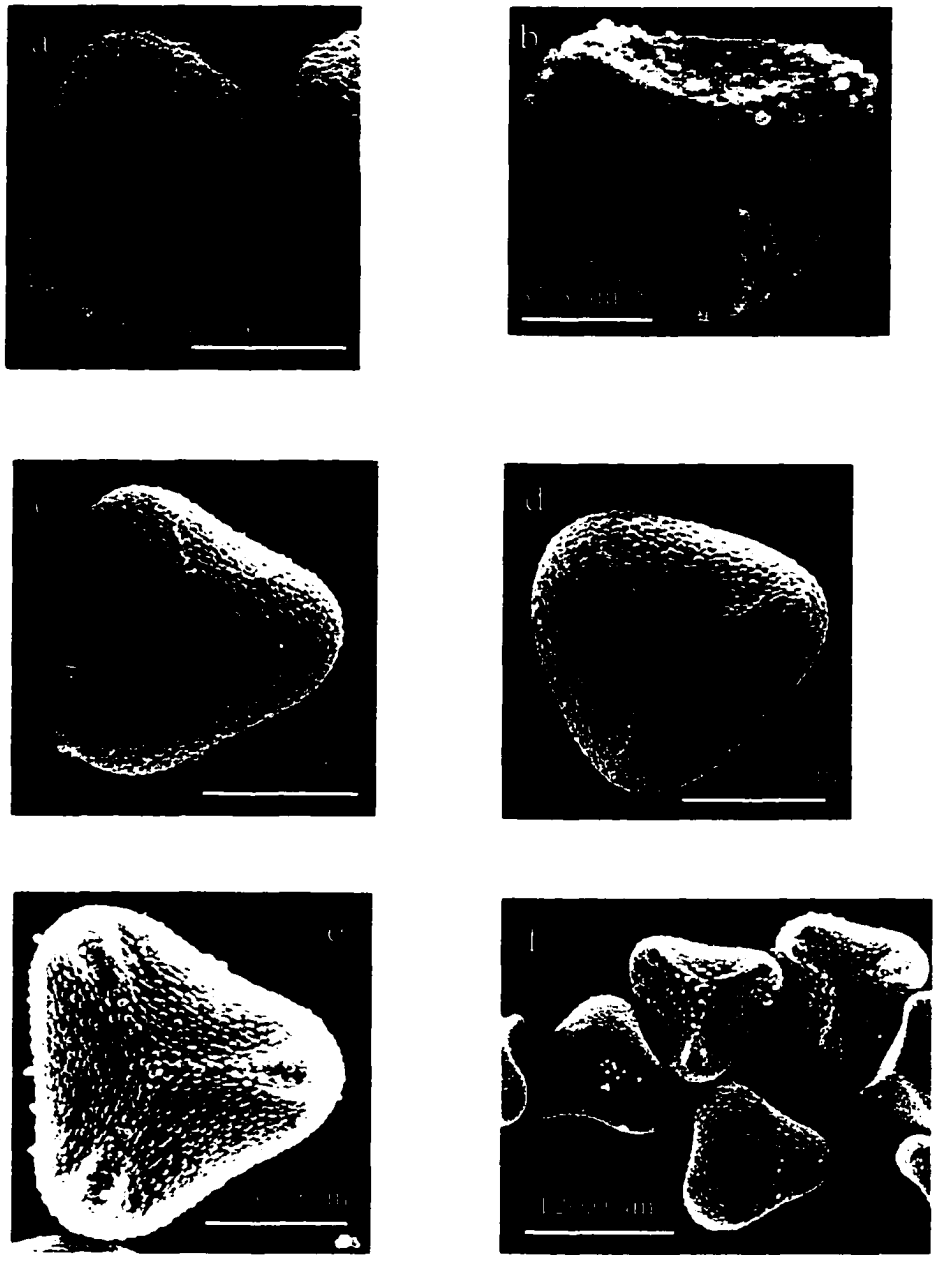


Figure 6.3. Spore morphology of *Lygodium* species with tuberculate spores. (a) *L. lanceolatum* distal view; (b) *L. lanceolatum* proximal view; (c) *L. polystachyum* distal view; (d) *L. polystachyum* proximal view; (e) *L. radiatum* proximal view; (f) *L. radiatum* distal and proximal views.

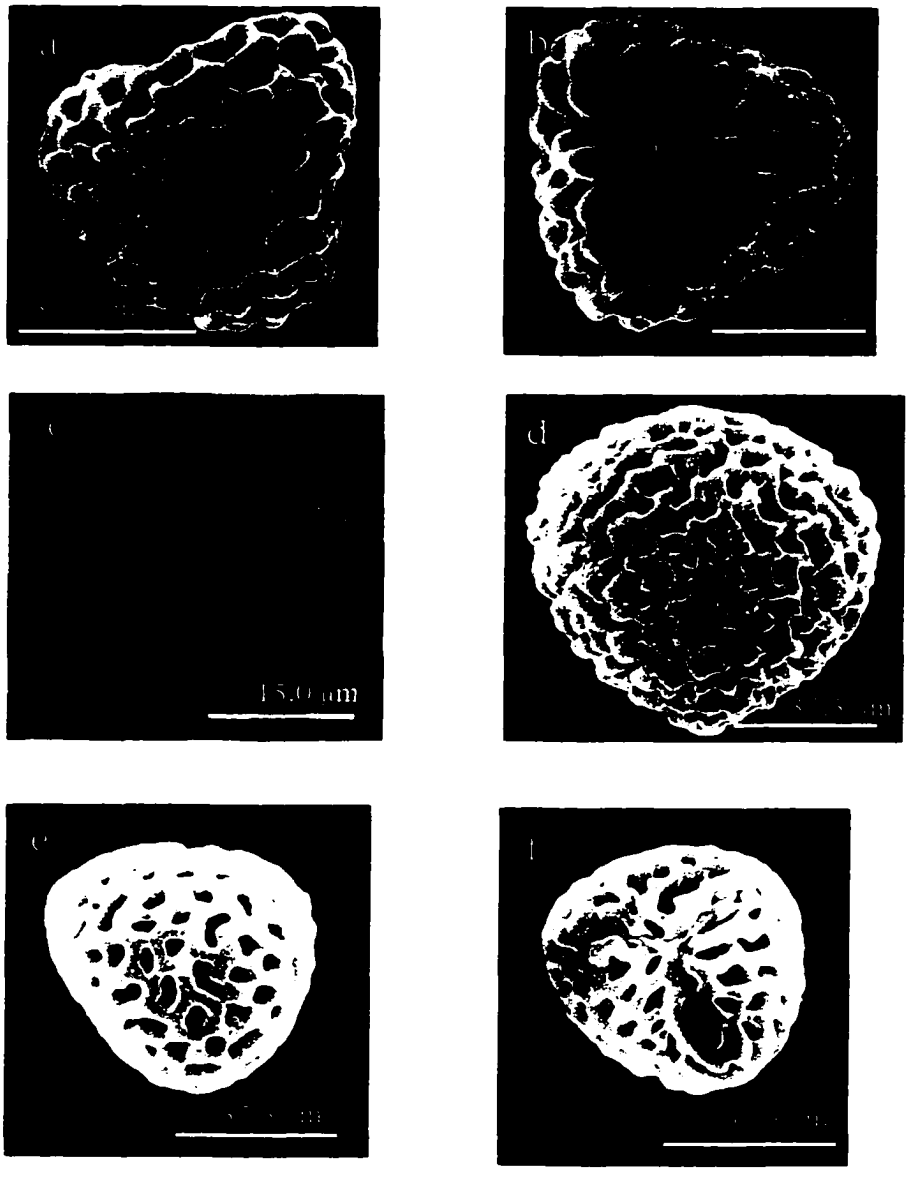


Figure 6.4. Spore morphology of *Lygodium* species with reticulate spores. (a) *L. versteegii* distal view; (b) *L. versteegii* proximal view; (c) *L. versteegii* detail of distal surface; (d) *L. reticulatum* distal view; (e) *L. microphyllum* distal view; (f) *L. microphyllum* proximal view.

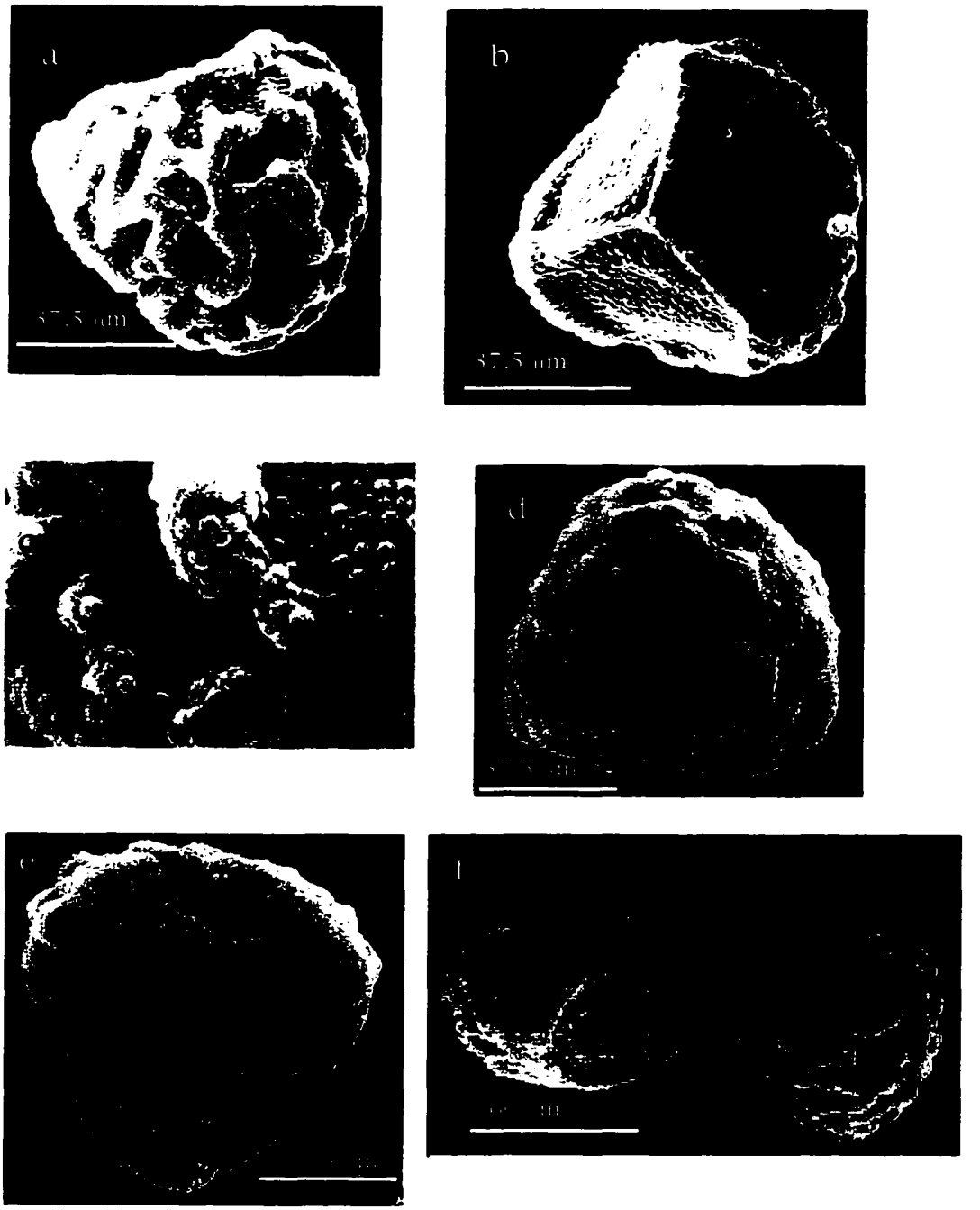


Figure 6.5. Spore morphology of *Lygodium* species with verrucate spores, the verrucae forming ridges. (a) *L. auriculatum* distal view; (b) *L. auriculatum* proximal view; (c) *L. auriculatum* detail of distal surface; (d) *L. cubense* distal view; (e) *L. cubense* proximal view; (f) *L. kingii* distal and proximal views.

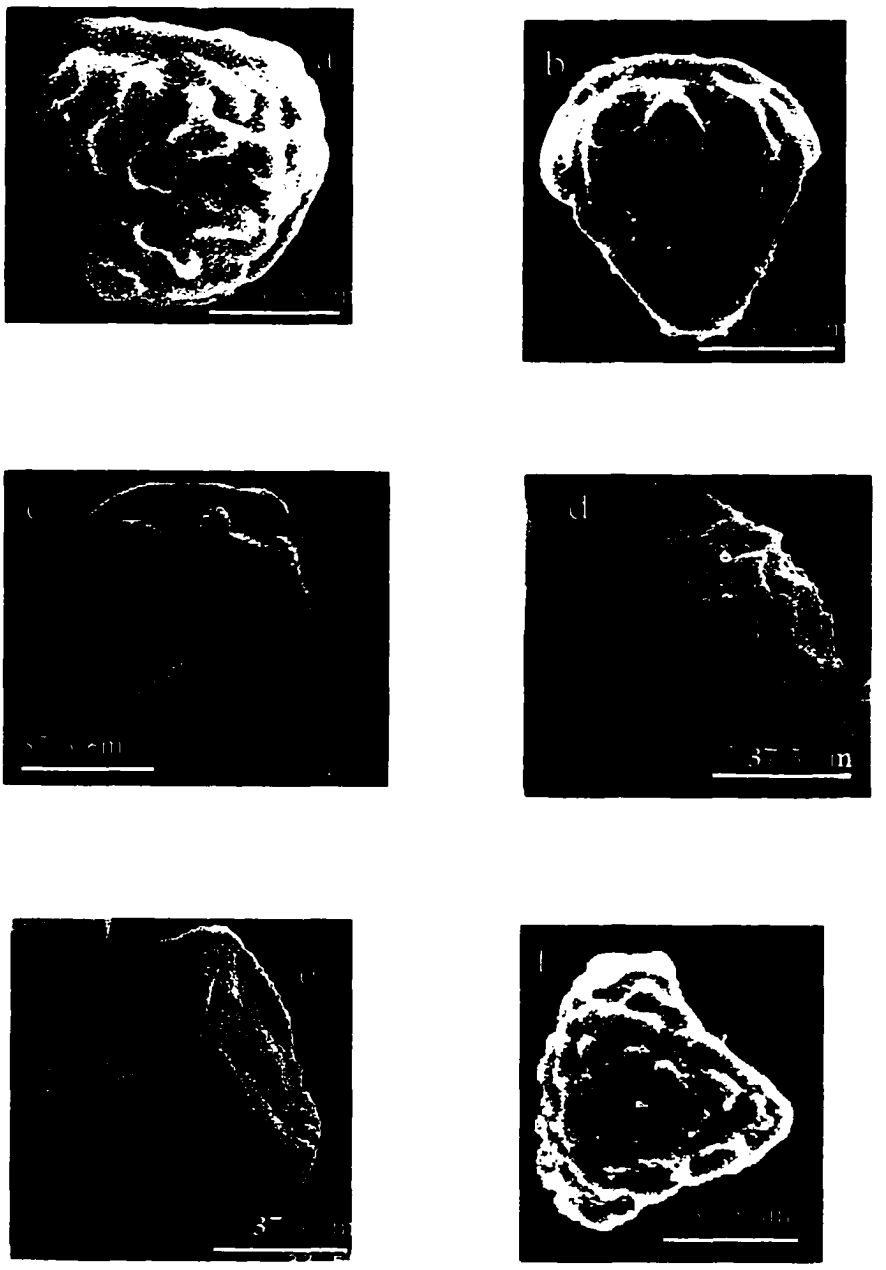


Figure 6.6. Spore morphology of *Lygodium* species with verrucate spores, the verrucae forming ridges. (a) *L. longifolium* distal view; (b) *L. merrillii* distal view; (c) *L. merrillii* proximal view; (d) *L. oligostachyum* distal view; (e) *L. oligostachyum* proximal view; (f) *L. smithianum* distal view.

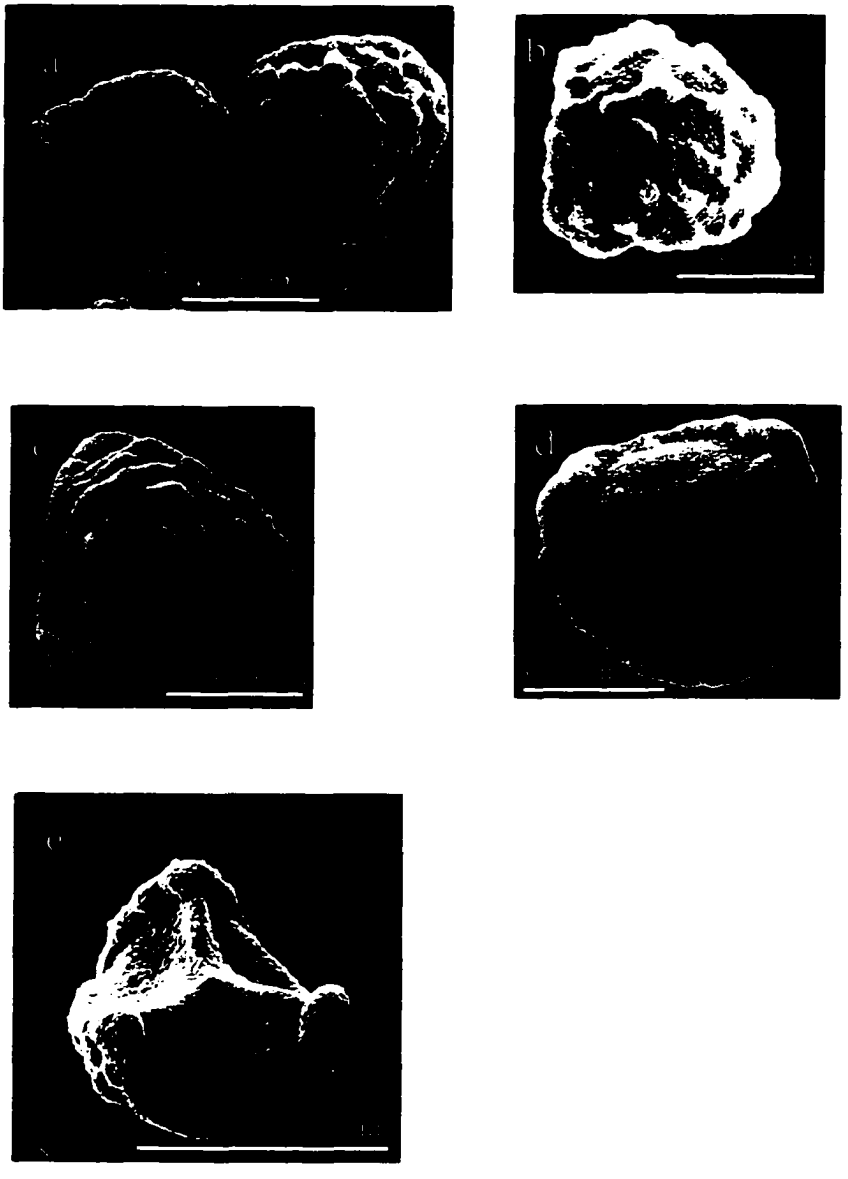


Figure 6.7. Spore morphology of *Lygodium* species with verrucate spores, the verrucae forming ridges. (a) *L. trifurcatum* distal and proximal views; (b) distal view of spore from *L. dimorphum* type specimen (*L. dimorphum* = *L. trifurcatum*); (c) *L. volubile* distal view; (d) *L. volubile* proximal view; (e) *L. volubile* proximal view.

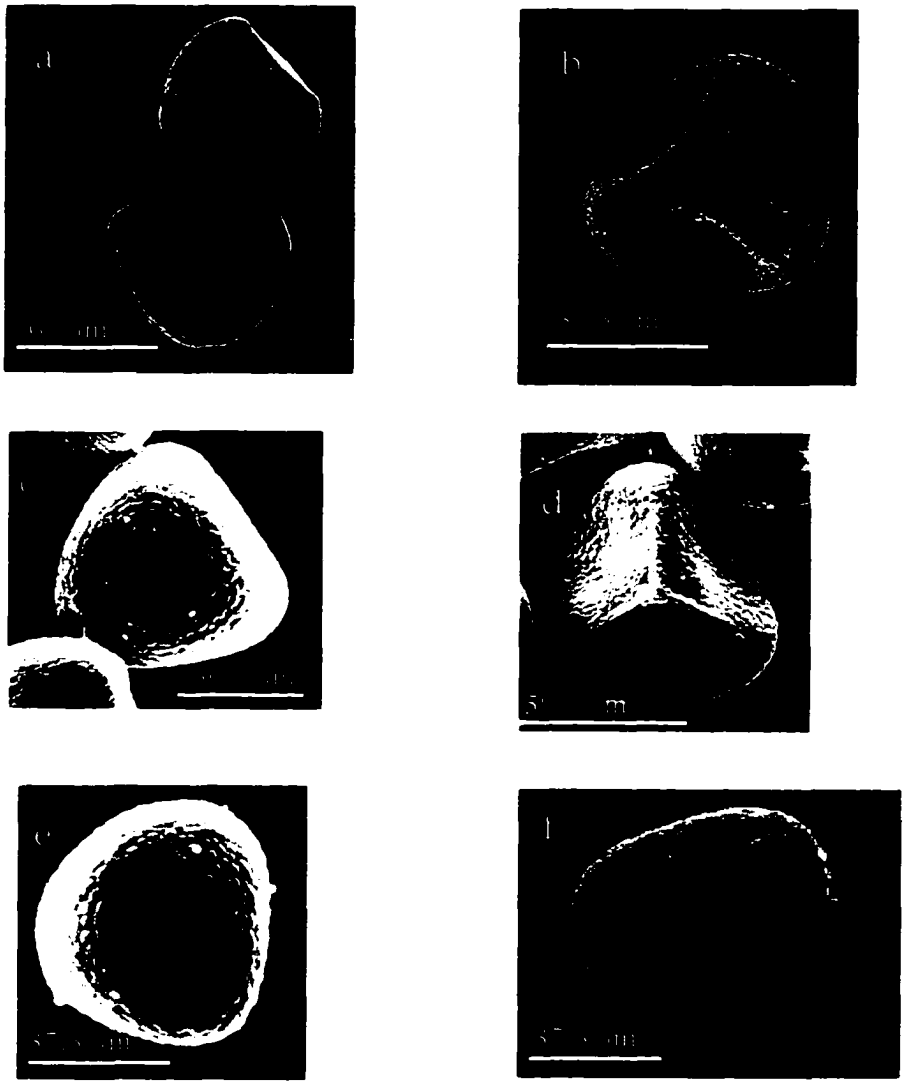


Figure 6.8. Spore morphology of *Lygodium* species with low tuberculate/granulate spore ornamentation. (a) *L. kerstenii* distal view without a perispore; (b) *L. kerstenii* proximal view; (c) *L. palmatum* distal view; (d) *L. palmatum* proximal view; (e) *L. japonicum* distal view; (f) *L. japonicum* proximal view.

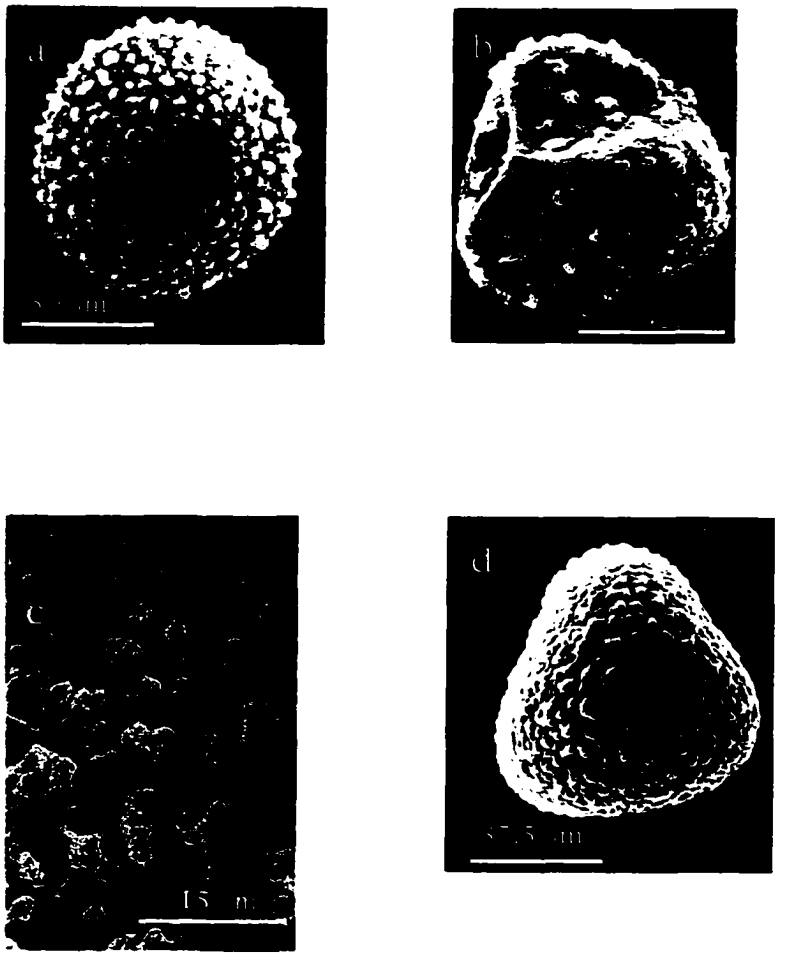


Figure 6.9. Spore morphology of *Lygodium hians*. (a) distal view of globose spore with tubercles and verrucae; (b) proximal view of tetrahedral spore; (c) high magnification view of "collapsed" verrucae and tubercles; (d) distal view of tetrahedral spore with low tubercles and irregular verrucae.

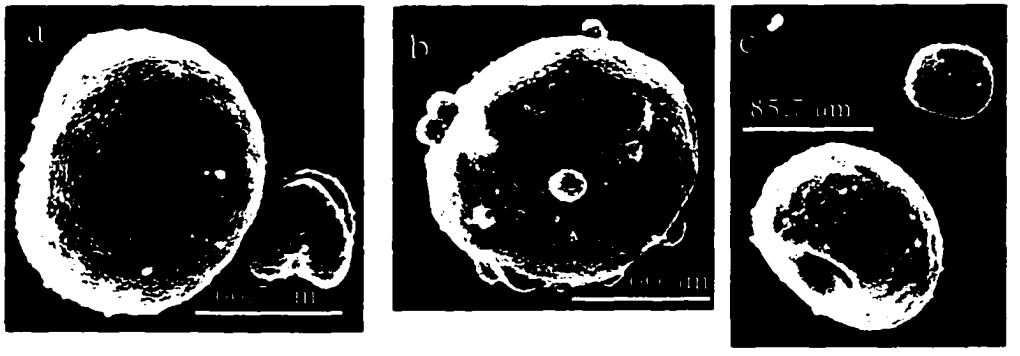


Figure 6.10. Abortive spores of *Lygodium x boivinii*.

CHAPTER VII

GAMETOPHYTE MORPHOLOGY

The spores of several species of *Lygodium* were germinated and grown to observe gametophyte morphology. Nine species of *Lygodium* were grown: *L. articulatum* (Waitakere Range, North Island, New Zealand), *L. circinnatum* (Botanical Garden, China) *L. flexuosum* (Conservatory, NYBG), *L. japonicum* (Conservatory, NYBG), *L. lanceolatum* (Fern Society, Spore Exchange, Madagascar), *L. microphyllum* (Gemini Botanical Garden, Florida), *L. palmatum* (Long Pond, Pennsylvania), *L. venustum* (Santa Cruz, Bolivia) and *L. volubile* (Bahia, Brazil). The spores were sown on a variety of growth media, including 0.5% Noble Agar, sterilized pot chards, sterilized glass beads, and peat pots which were rehydrated with boiling water. In some cases the sporangia were immersed in 70% ethanol to remove superficial contaminants such as fungi and bacteria, washed with sterilized water, and then opened over the growth media. The gametophytes were watered with steam-distilled or deionized water until sporophyte production. The plants were kept under fluorescent lights on a 12 hr light/12 hr dark cycle.

The spores of both *Lygodium articulatum* (New Zealand) and *L. palmatum* (Pennsylvania) had a very short viability. This inverse relationship between percent germination and storage time may be a result of temperate habitats. In the northeastern United States *L. palmatum* spores are mature in late winter (December-February). Even when mature spores were sown soon after collection there was only approximately 50% germination: one of the problems with these spores is that the perispore often cracks prematurely rendering the spore nonviable. Spores kept for six months to one year had less than 5% germination. This is probably a direct consequence of the

temperate climate in which it grows. As the climbing fronds are semi-evergreen and remain twined around their support structure, spores drop from sporangia in late winter to germinate that spring. If germination does not occur in the spring most spores lose their viability with few overwintering in the soil bank. The spores of *Lygodium articulatum*, which grows in sub-tropical areas in New Zealand, were collected (Waitakere Range), sent air-mail and sown within seven days and yet germination was less than 2%. A horticulturist of the Auckland Botanical Society (S. Jones, pers. comm.) in New Zealand reports that spore viability is very short, and when germination occurs,it is very difficult to obtain sporophytes. The tropical taxa, on the other hand, had high germination percentages (e.g., *Lygodium microphyllum, L. venustum, L. japonicum*) and long storage times (1-2 years for *Lygodium japonicum*). However, *Lygodium circinnatum* spores collected in China and sown within 1 month of collection had poor germination.

The fastest germination time for filament production was 5-7 days in *Lygodium japonicum* whereas the longest time occurred with *L. lanceolatum and L. articulatum* spores(2-4 months). *Lygodium* spores are considered photoblastic: germination is mediated by the phytochrome system (Raghavan and Huckaby, 1980; Nakamura and Maeda, 1995). The presence of a phytochrome pigment was found in spores of *Lygodium japonicum*, which germinated in red light (Tomizawa *et al.*, 1982). The presence of a light requirement for germination is an evolutionary prerequisite for persistent "spore banks" as germination must not occur in the soil layer. *Lygodium microphyllum* has been shown to form spore banks (Wee, 1974). This species in Florida has become an insidious alien in cypress swamps and saw grass communities and appears to be spreading rapidly. The fact that its spores are present in spore banks only exacerbates the problem.

The light requirement for germination in photoblastic spores can be substituted with antheridiogens, gibberellins, or often high temperatures (Naf, 1958). *Lygodium* produces antheridiogens and as a consequence ungerminated spores in the soil or leaf litter adjacent to the gametophyte (producing the antheridiogen) will be triggered to germinate and produce antheridia.

In general, it took 21 days after sowing to produce cordate gametophytes and a total of 40 days (average of all species grown) to have antheridia and archegonia (Fig. 7.1a-c). In the case of *Lygodium japonicum* and *L. venustum* sporophytes were present in 60 days while it took 90 days for *L. microphyllum* and 120 days for *L. palmatum.* Twiss (1910) reported that in *Lygodium circinnatum* it took 10-12 days for an apical cell to begin dividing to produce the thallus. Antheridia were present within 3 weeks and archegonia in 6 weeks. The antheridia contained 128 sperm. The sperm of *L. japonicum* are illustrated in Fig. 7.1d. The archegonial neck consisted of two cells. Mueller (1982a) reported sporophytes in 8-10 weeks for *Lygodium japonicum* and it took 5-6 months for the plants to begin climbing.

The initial stages of gametophyte development in *Lygodium*, post-germination, have been studied by Nayer and Kaur (1971), Stokey (1951) and Twiss (1910), who found little variation among the taxa.

Gametophytes of *Lygodium* are symmetrically cordate, with a thickened apical notch and a large number of rhizoids. The prothallus initally may be asymmetrically cordate or often half-cordate as in *Anemia.* However, as it matures, the wings become equal. Antheridia and archegonia are produced on the under-surface of the prothallus: archegonia along the cushion flanked by antheridia on wings or among the rhizoids (Fig. 7.1 a-c). The neck of the archegonium is directed away from the notch. The rhizoids can become so

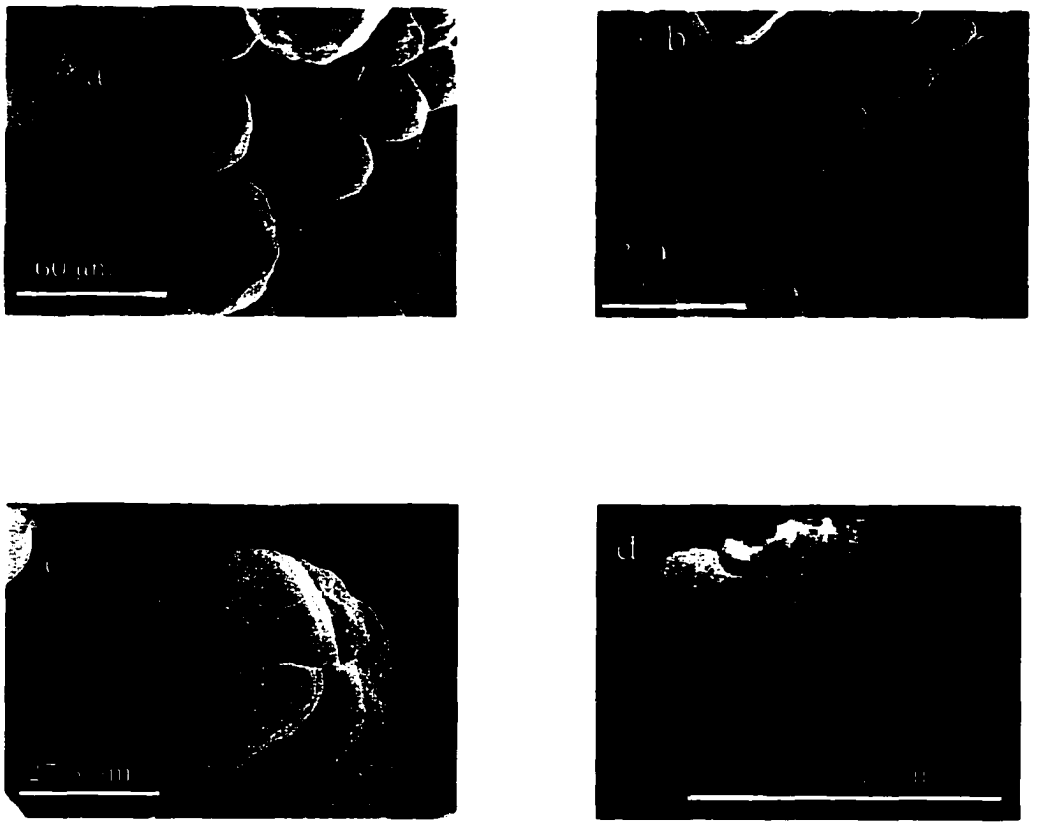


Figure 7.1. Antheridia (a and b) and archegonia (c) from *Lygodium venustum* and sperm (d) from *Lygodium japonicum*.

profuse as to push the entire gametophyte up from the soil surface. Trichomes were not observed. However, there are reports of a few hairs on the cushion-portion of the thallus in some taxa (Chu and Wee, 1989; Rogers, 1923).

Antheridiogens have been isolated from *L. flexuosum, L. circinnatum, L. heterodoxum* and *L. japonicum* (Voeller and Weinberg, 1969; Näf, 1979; Furber *et al.*, 1995; Yamamuchi *et al.*, 1996; Yamane *et al.*, 1979). These chemicals are produced by an archegoniate thallus (usually larger than other thalli present and with a well-developed meristem; Rashid, 1970). Antheridiogens diffuse into the surrounding soil and cause adjacent gametophytes to produce antheridia. Often these "male" gametophytes are very much smaller. If fertilization does not occur, the archegoniate thallus produces antheridia and self-fertilization can occur. Most often the first thalli produced are male, with subsequent female and bisexual thalli produced later. Sensitivity to antheridiogens has been reported to last 1-2 weeks (Voeller and Weinberg, 1969). Rashid (1970) reports that in *Lygodium flexuosum* the majority of initial gametophytes were male (84%) and that the introduction of auxins causes archegonia to form first compared to the usual initial production of antheridia. Tryon and Vittale (1977) have reported an antheridiogen system in a natural population of *Lygodium heterodoxum* in Mexico.

Prothallial regeneration may also occur. Small cordate structures are produced along the lateral edge of one of the lobes, which often bear antheridia. Atkinson (1990, pers. comm.) reports that this regeneration is common in *Osmunda, Plagiogyria* and *Elaphoglossum* and it has been illustrated by Rogers (1924). Miller (1990, pers. comm.) termed the same structures prothallial colonies. It appears that age may be a trigger for this extra growth. These "colonies" can remain alive for at least 6 months. This

regeneration was observed in old unfertilized thalli in all species grown in this study.

The gametophytes of all nine species studied were remarkably similar morphologically. Some species produced more small male gametophytes than others but this could simply be a product of differences in spore germination, spore numbers present, and anteridiogen production. There were no discrepancies between the work of previous authors and the results obtained here.

The gametophytes of the Schizaeaceae *s.l.* are diverse. *Schizaea s.s.* has branched, uniseriate, filamentous gametophytes devoid of hairs. They are subterranean or grow on the surface. Atkinson (1965) reported that these gametophytes can live more than 13 years. The rhizoids are mycorrhizal, antheridia are borne on short lateral branches, while archegonia are produced in clusters on filaments or lateral cushions, entirely exposed. The archegonial neck consists of three cells (Nayar and Kaur, 1971). *Schizaea* splinters, *Actinostachys* and *Lophidium*, have gametophytes that are subterranean, radially symmetrical and fleshy. In *Lophidium* the gametophyte is branched with endophytic fungi while in *Actinostachys*, it is unbranched, tuberous and mycorrhizal. The archegonial ventor is embedded in the gametophyte tissue, borne on lateral cells and the antheridia, also on the sides, has a stalked basal cell. *Anemia* and *Mohria* have cordate, symmetrical or asymmetrical gametophytes. Club-shaped glandular hairs are present in both. *Anemia* produces antheridiogens: these are effective in stimulating antheridia in *Lygodium* at very high concentrations (10-20 x what is effective in *Anemia*), while *Lygodium* antheridiogens do not affect *Anemia*. Therefore, it appears that antheridiogens are structurally similar but usually genus-specific. The structure of the antheridia seem to be the same in all

genera, except that in *Lygodium* the cap cell may be divided (*L. palmatum, L. volubile, L. kerstenii*, Fig 7.1a). Thus, *Lygodium* shares the asymmetrical cordate charactersitic in its juvenile stages of gametophyte development with *Anemia* and *Mohria.*

Taxonomically fern gametophytes often have useful morphological characters at the genus level but not at the species level.

CHAPTER VIII
PHYTOCHEMICAL ANALYSIS

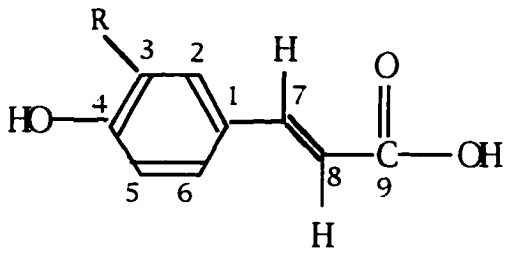
Introduction

A variety of phytochemicals have proven useful in plant systematics (Harborne and Turner, 1984). Prior to the current advances in DNA technology, biochemical studies focused on micromolecular secondary compounds (compounds that are not part of the primary metabolic pathways of the plant and are smaller in size than the macromoleclues). These chemicals were surveyed in plants and the presence or absence of a particular compound and the biosynthetic pathway responsible for its production were used to establish taxonomic and phylogenetic relationships. The restrictive distribution of many of these phytochemicals in ferns and fern allies has helped in the resolution of some taxonomic problems (Richardson, 1984, 1989; Wallace, 1989). The phytochemical diversity of ferns was last summarized by Soeder (1985) and Gottlieb and coworkers (1990). Many chemicals have been isolated, identified, and surveyed from a wide array of species (e.g., Imperato 1979, 1982, 1991; Veit *et al.*, 1992; Widén *et al.*, 1983). In general, the taxonomic usefulness of the micromolecular chemical data has been disappointing (Pryor *et al.*, 1995).

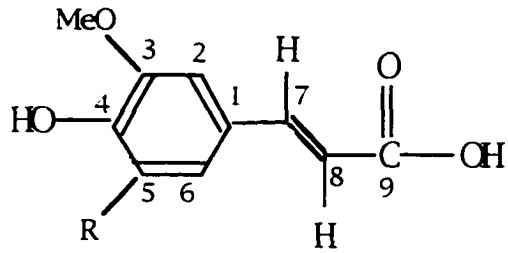
Micromolecular secondary compounds include phenolics (derivatives of or polymers of a C6 ring structure), terpenes (derivatives of isoprene units), organic acids, lipids and nitrogen compounds (alkaloids, indoles, aromatic amines, etc.). Of these the phenolic compounds have been most widely studied. These include simple phenols and phenolic acids, phenlypropanoids, flavonoids, anthocyanins, tannins and quinones. This study investigates

phenlypropanoids which are naturally occurring phenolics containing an aromatic ring attached to a 3-carbon side chain (C6-C3) derived biosynthetically from phenylalanine and which may contain more than one C6-C3 residue (Harborne, 1984). The phenylpropanoids isolated in this investigation are derivatives of hydroxycinnamic acids (HCA's).

Four hydroxycinnamic acids are commonly found in plants: *p*-coumaric acid, caffeic acid, ferulic acid, and sinapic acid (Fig. 8.1). These HCA's enter several important biosynthetic pathways involving the following reactions and products: a) reduction reactions leading to *p*-coumaryl alcohol, coniferyl alcohol (ferulic acid as the precursor) and sinapyl alcohol, involved in the synthesis of lignin; b) condensation reactions involved in the synthesis of flavonoids; c) degradation reactions leading to the hydroxybenzoic acids; and d) conjugation reactions leading to esters, amides or glycosides (Walker, 1975; Strack & Mock, 1993). These compounds are, therefore, important in the structure, pigmentation, growth regulation, disease resistance and anti-herbivorous mechanisms of plants. Free hydroxycinnamic acids are rarely found in plants and are most often conjugated with sugars (glycosides). These conjugates increase the solubility of the HCA in the cell resulting in its decreased toxicity to the cell and preventing its subsequent oxidation by diphenol oxidases (Harborne, 1979; Walker, 1975). The parent HCA, trans-cinnamic acid, is conjugated with a sugar before any hydroxylation steps to *p*-coumaric or caffeic acid derivatives. Since such glycosylation occurs frequently in these and other secondary compounds (e.g. flavonoids), the diversity and number of sugars present becomes as important as the parent molecule and increases the potential usefulness of the compound in systematics.



If R = H, p-coumaric acid
If R = OH, caffeic acid



If R = H, ferulic acid
If R = OMe, sinapic acid

Figure 8.1. Structures of hydroxycinnamic acids.

Bate-Smith (1962) pioneered the study of plant phenolics using simple paper chromatography. HCA's fluoresce under ultraviolet light (UV) with characteristic color changes after exposure to ammonia fumes. These chemical properties permit relatively rapid and accurate screening. The simplicity of this technique, and the lack of information on plant chemicals at the time, caused an explosion of research in the field of phytochemistry and biochemical systematics (e.g., Harborne, 1964, 1968; Harborne and Williams, 1975; Cooper-Driver & Swain, 1977; Cooper-Driver, 1980). With the advent of more sensitive chromatographic techniques [high performance liquid chromatography (HPLC) in conjunction with UV/Vis spectroscopy] smaller quantities of plant material could be analyzed, an important asset in the investigation of ferns. Once a compound is isolated, the complete elucidation of its structure is then possible using nuclear magnetic resonance spectroscopy (NMR) in conjunction with mass spectroscopy (MS). Veit and coworkers (1995) successfully used reversed-phase HPLC analysis as a sensitive and selective method to analyze plant phenolics both quantitatively and qualitatively. Their study of HCA conjugates (e.g., caffeoyl meso-tartaric acid) in *Equisetum arvense* employed the use of these current methodologies in the structural identification of the compound and in surveying its presence in related plant groups in order to determine related taxa among the fern allies and to substantiate parentage in *Equisetum* hybrids.

Fern phytochemistry was pioneered by Harada and Saiki (1955) and the study of phenolics in ferns by Bohm in a series of investigations in the late 1960's. Bohm identified compounds by using acid and alkaline hydrolyses or enzymatic degradation, followed by thin layer or paper chromatography. The products were then identified under ultraviolet light by their characteristic fluroescence. Glass and Bohm (1969b) surveyed 92 species of ferns for

phenolic acids and found 87% contained *p*-coumaric acid, 87% caffeic acid, 74% ferulic acid, 5% sinapic acid and 2% *o*-coumaric acid. These compounds were also found present in *Lygodium japonicum, L. palmatum, L. flexuosum* and *L. circinnatum* with sinapic acid present only in the latter species (Bohm & Tryon, 1967).

The fairly ubiquitous presence of some of the HCA's in plants has caused most systematists to assume that their use in clarifying phylogenies would probably be negligible. In order to determine the validity of this premise concentrated methanolic extracts of various taxa of *Lygodium* were assessed using HPLC. A large number of compounds was observed and prompted this study. In a chromatogram the number of peaks is indicative of the number of different compounds present: no two compounds will elute at the same retention time with the same absorption spectra (Fig. 8.2). The availability of an HPLC in the Harding Laboratories of the New York Botanical Garden (NYBG) and a mass spectrometer at Lehman College of CUNY, as well as the expertise of Dr. Barbara Meurer-Grimes in the use of these analytical tools in the isolation, purification, and identification of phenylpropanoids, resulted in the following phytochemical study.

This study attempts to identify and structurally elucidate HCA conjugates from *Lygodium japonicum* and related species. The major compounds might be diverse enough in structure and content to have a selective distribution that could be utilized to study species affinities. This work departs from previous studies in that it isolates and purifies the intact, unhydrolysed chemical conjugates so that they can be identified structurally. One of the primary goals of this investigation was to develop a methodology to purify these chemicals from ferns. The isolated compounds can then be used as standards

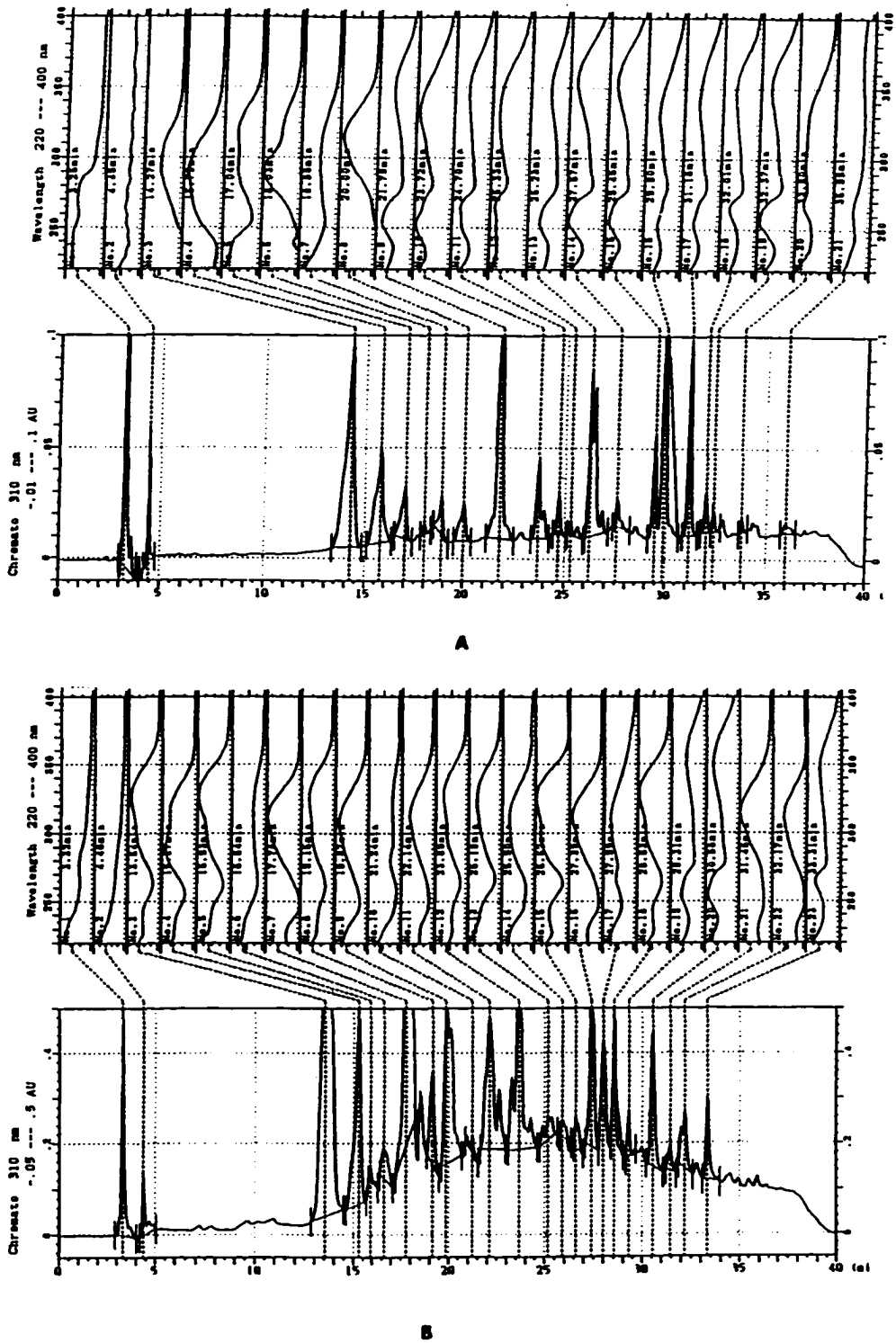


Figure 8.2. HPLC chromatograms and UV/Vis absorption spectra of methanolic extracts of *Astrolepis sinuata* (A) and *Lygodium palmatum* (B).

to test their occurrence in other taxa using co-chromatography. The structural elucidations have added to our knowledge of plant chemistry in general and the methodologies employed can be repeated in other fern groups.

Materials and Methods

Methodologies used in the extraction, isolation, purification and identification phases of this study were newly modified for the *Lygodium* HCA's. *Lygodium japonicum* was chosen for the initial chemical analysis due to the overwhelming number of studies on this species in various areas of pteridology (i.e., morphology, gametophyte structure, antheridiogen production, patterns of twining, spore morphology, phytochemistry, etc.; Bohm & Tryon, 1967; Mueller, 1982a,b; Nayer & Kaur, 1968, 1971; Vaudois & Laurent, 1976).

Plant Collection

Samples for initial HPLC surveys of various fern taxa were collected from the Propagation Range and the Enid Haupt Conservatory at the New York Botanical Garden, from herbarium specimens and from field collections. The locality of the collection, collector and collection number, type of collection (freshly dried or herbarium specimen) and reproductive state of the plant material extracted in the fern species surveyed are summarized in Table 8.1. Those specimens collected at the Propagation Range and the Conservatory of the New York Botanical Garden were dried for 1 week prior to extraction. All extracts from herbarium specimens were taken from sheets containing extra material in packets from the Fern Herbarium (NY).

Species of *Lygodium* collected for bulk samples for the isolation and purification of chemical compounds were obtained from the Conservatory. A major renovation of the Enid Haupt Conservatory was in progress and all three

TABLE 8.1. Fern species surveyed for phytochemical components.

Fern Species	Locality	Condition+	Quantity (mg)
Adiantum tenerum	Propagation Range	dried, fertile	1010
Adiantum raddianum	Propagation Range	dried, fertile	640
Angiopteris evecta	Conservatory	dried, sterile	1000
Anemia colimensis	Mickel 1692	dried, sterile	720
Anemia hirta	Brazil, Amorim 832	dried, s/f	260
Anemia presliana	Brazil, Carvalho 3768	dried, sterile	480
Anemia phyllitidis	Propagation Range	dried, sterile	410
Anemia tomentosa	Conservatory	dried, sterile	280
Athyrium filix femina	Propagation Range	dried, fertile	750
Asplenium bulbiferum	Propagation Range	dried, sterile	840
Azolla sp.	Conservatory	fresh, sterile	220
Botrychium decompositum	Conservatory	dried, sterile	150
Ceratopteris thalictroides	Texas, Correll 35295	herb., sterile	80
Cheilanthes alabamensis	Propagation Range	dried, fertile	1110
Astrolepis (Chelianthes)sinuata	Propagation Range	dried, fertile	1010
Cystopteris fragilis	New York (Garrison)*	dried, sterile	910
Dennstaedtia globulifera	Propagation Range	dried, sterile	960
Dicksonia fibrosa	Conservatory	dried, sterile	1020
Dicranopteris pectinata	Panama, Nee & Smith 11104	herb., sterile	250
Dryopteris affinis	Propagation Range	dried, sterile	690
Elaphoglossum ciliata	Propagation Range	dried, fertile	1020
Gleichenia bancroftii	Mexico, Hallberg 1527	herb, sterile	420
Llavea cordifolia (A)	Propagation Range	dried, sterile	460
Llavea cordifolia (B)	Propagation Range	dried, fertile	710
Lygodium circinnatum	Conservatory	dried, sterile	300
Lygodium flexuosum	Conservatory	dried, sterile	380
Lygodium heterodoxum	Costa Rica, Jimenez 3895	herb., +/- fertile	360
Lygodium heterodoxum	Panama, Liesner1973	herb., fertile	350
Lygodium heterodoxum	Mexico, Hallberg 1262	herb., fertile	470
Lygodium japonicum	Conservatory	dried, +/- fertile	300
Lygodium microphyllum	Conservatory	dried, sterile	230

Table 8.1 (continued).

Fern species	Locality	Condition+	Quantity
Lygodium microphyllum	Florida, (Moyroud)*	dried, fertile	1010
Lygodium palmatum (A)	Pennsylvania (Garrison)*	dried, sterile	1020
Lygodium palmatum (B)	Pennsylvania (Garrison)*	dried, fertile	1010
Lygodium radiatum	Panama, Welch 19828	herb., sterile	660
Lygodium reticulatum	Fr. Polynesia, Wagner,	herb. sterile	700
Lygodium salicifolium	Caroline Isl., Canfield 512	herb., fertile	130
Lygodium smithianum	Africa, Congo, Lewalle 3126	herb., fertile	230
Lygodium venustum	Bolivia, Nee 39670	dried, fertile	1030
Lygodium volubile	Venezuela, Beitel 85041	herb., sterile	1000
Lygodium volubile	Brazil, Pirani 2426	herb., fertile	450
Marattia laxa	Conservatory	dried, sterile	1010
Marsilea sp.	Propagation Range	fresh, sterile	1000
Ophioglossum reticulatum	Conservatory	dried, sterile	410
Osmunda cinnamomea	New York (Garrison)*	dried, sterile	550
Osmunda claytoniana	New York (Garrison)	dried, sterile	660
Osmunda regalis	New York (Garrison)	dried, sterile	630
Pellaea falcata	Propagation Range	dried, sterile	930
Phanerophlebia nobilis	Conservatory	dried, sterile	680
Pteris semipinnata	Propagation Range	dried, fertile	640
Polypodium asplenioides	Propagation Range	dried, +/- fertile	810
Salvinia sp.	Propagation Range	fresh, sterile	950
Schizaea elegans (A)	Brazil, Thomas 9254	dried, fertile	1000
Schizaea elegans (B)	Brazil, Thomas 9254	dried, fertile	1000
Schizaea pennula (A)	Brazil, Sant'Ana 130	dried, fertile	920
Schizaea pennula (B)	Brazil, Sant'Ana 130	dried, sterile	1030
Thelypteris decussata	Propagation Range	dried, sterile	650
Todea barbara	Conservatory	dried, sterile	1020
Vittaria lineata	Propagation Range	dried, sterile	820
Woodwardia radicans	Propagation Range	dried, sterile	830

+ Freshly collected specimens for this study were air-dried for up to 1 week prior to extraction. The reproductive state is indicated as sterile, fertile, s/f indicating both sterile and fertile fronds were used in the extraction, and +/- fertile, indicating some sporangia present but not mature.
* Specimens freshly collected for the purpose of extraction-not for the primary purpose of herbarium collections have not been assigned collection numbers.

species of *Lygodium* growing in the Fern House were completely cut down. This presented a fortuitous opportunity.

Extraction of Samples for Chemosystematic Survey

Samples were air dried (1 gm dried approx. 1 week), weighed (minimum quantity of 0.2-0.5 g), cut into small pieces, placed in plastic centrifuge tubes and ground in 10 ml of 80% methanol using a Polytron tissue homogenizer. The samples were then stirred for 1 hour, centrifuged for 5 min. (table top centrifuge, low speed), and after retaining the supernatant, an additional 5 ml methanol was added and the process repeated. The new extracts were combined and stored in a freezer (- 20°C) to prevent degradation and isomerization.

Extraction of Bulk Samples of *Lygodium*

Three species of *Lygodium* were collected for chemical analysis. A voucher specimen was prepared for each taxon (NY). After removal of the rachis and some of the primary pinna-branches, 287.4 g of *L. circinnatum*, 286.0 g of *L. japonicum*, and 97.2 g of *L. flexuosum* were separately placed in 10 liter extraction jars. The plants were extracted with 80% methanol on rotating machines for 16 hours, filtered, additional methanol added and rotated for an additional 6 hours. The final extracts were combined and evaporated on a rotary evaporator. They were stored at -20°C until further purification.

Purification of HCA's from *Lygodium japonicum*

Column Chromatography - Polyamide

The 5-10 L extracts were evaporated to approx. 500 ml, and a 200 ml aliquot was separated on Polyamide (MN Polyamide SC 6, Polycaprolactam, Grain Size < 0.07 mm, Alltech). The Polyamide was layered onto a Buchner funnel and eluted using vacuum filtration with 1 liter each of distilled water, 20% aq. methanol, 40% aq. methanol, 60% aq. methanol, 80% aq. methanol, 100%

methanol, and finally 0.05% ammonium hydroxide in methanol. The extracts were evaporated on a rotary evaporator to reduce volume and analysed by TLC and analytical HPLC.

Column Chromatography - Sephadex LH 20

The next phase of separation involved the use of Sephadex LH 20 (Lipophilic, Sephadex LH 20, Sigma) column chromatography. Approximately 50 gm of LH 20 was hydrated in steam distilled water and poured into a column (J.T. Baker, 52 cm. column). The reservoir contained either 20% methanol or distilled water depending on the fraction. Initially the 20% methanol polyamide fraction was applied to the column and 100 ml fractions were collected for 12 hours, a large fraction overnight and subsequent 100 ml fractions the second day. Later in the study the water fractions from the polyamide separation were separated using the same technique. In one instance when further purification of an isolated compound (D4) was required a small LH 20 column (10 ml) was used and 1 ml fractions collected. All samples were evaporated on a rotary evaporator and analyzed by TLC and HPLC.

Acid and Alkaline Hydrolyses

Alkaline hydrolysis was performed to separate the HCA's from their conjugates (Markham, 1982). 1 M NaOH was mixed with the sample (1:1) compound so that the final concentration was 0.5 M. The mixture was incubated for 30 min at room temperature and then acidified with 1 M HCl. Then 2.0 ml ethyl acetate was added and mixed (Vortex mixer) for 15 sec. The ethyl acetate phase (top layer) was collected in a round bottom flask and the ethyl acetate extraction was repeated. The combined ethyl acetate phases were evaporated to dryness and reconstituted with 100-200 µl methanol. The hydrolysate was then analyzed using TLC. In order to prevent caffeic acid from

decomposing, a small scoop of sodium borohydrate was added to the hydrolysis assay. Samples were stored at -20°C until further investigation.

Acid hydrolysis was performed to separate the sugars from the HCA's. 1 M HCl was mixed with the sample (1:1) so that the final concentration was 0.5 M. The solution was boiled in a water bath (tubes tightly sealed) for 30 min. The hydrolysis was stopped by immersing the tube in an ice bath and the solution was neutralized with 1 M NaOH. As with alkaline hydrolysis, the hydrolysis assay was extracted with ethyl acetate, however the ethyl acetate was discarded. The evaporated precipitate (salt-like) was reconstituted with 100-200 µl methanol (Mabry *et al.*, 1970). Samples were stored at -20°C until further investigation.

Thin Layer Chromatography

Detection of HCA Conjugates

Cellulose and silica plates were utilized for one-dimensional thin layer chromatography (TLC). Cellulose plates were prepared at Harding Laboratories from Cellulose powder (microcrystalline, Avicel) as no purchased plates provided the same degree of separation and color intensity with ultraviolet light. Silica plates were purchased (Uniplate, GHLF, 250u, Analtech, P.O. Box 7558, Newark, Delaware 19714). The solvent system for the cellulose plates was butanol:acetic acid:water (4:2:1; v:v:v; BAW). The results were viewed using long wavelength (366 nm) ultraviolet (UV) light and UV light before and after treatment with NH_4OH fumes. This method was used to monitor compound purification. To test for the presence of primary amino groups, the plates were sprayed with ninhydrin (0.200 mg ninhydrin/100 ml acetone), heated in an oven at 100°C for 5 min and observed for purple spots.

Detection of Sugars

Silica gel plates were buffered by spraying with 0.5 M prim. Na_2HPO_4 (6.9 g Na_2HPO_4 in 100 ml dist. H_2O + 33 ml methanol) and heated for 1 hr at 100°C. The solvent system was 2-propanol:acetone:0.1M lactic acid in water (4:4:2). After plate development, a spray reagent was used to visualize the sugars: 4 ml aniline, 4 gm diphenylamine, 200 ml acetone, and 30 ml *o*-phosphoric acid (88%). This method was modified from that of Hansen (1975). The following sugar standard solutions (100 mM) were used for identification by comparison with authentic substances (5 µl applied to prepared silica plate): L-arabinose, fructose, galactose, glucose, D-glucuronic acid (Na salt), maltose, L-rhamnose, sucrose, and L-xylose (Sigma Chemical Co., ST. Louis, MO 68178-9936).

Detection of HCA's

Silica gel plates (as above) were used with the solvent system toluene:acetic acid (water saturated; 2:1). The following HCA standard solutions (1 mg/2 ml methanol) were prepared and tested: caffeic acid, chlorogenic acid, *o*-coumaric acid, *p*-coumaric acid, trans-cinnamic acid, ferulic acid, and sinapic acid (Sigma Chemical Co., ST. Louis, MO 68178-9936 and Carl Roth, dist. by Atomergic Chem. Corp.).

Analytical and Preparative HPLC

Extracts and column fractions were chromatographically analyzed via high performance liquid chromatography (HPLC) combined with UV/Vis spectroscopy. Analytical HPLC is used to survey the types of compounds present in a plant extract and preparative HPLC is used, on a larger scale, to collect compounds after separation on the column. This results in the isolation of a pure compound. The separation methodology was developed for this project. The following HPLC equipment was used: Waters 990 Series Photodiode Array Detector, Waters 600E Multisolvent Delivery System and Waters 700 Sample Injector. For analytical HPLC a Nucleosil column (250 x 4.6 mm, 5µ) was

used, and for preparative HPLC, a Nucleosil column with the dimensions 250 x 21.20 mm, 10 μ, was used (Phenomenex, 2320 W. 205th St., Torrance, CA 90501). The gradient profile is given for both analytical and preparative chromatography.

Analytical

Solvent A: 2% phosphoric acid in water

Solvent B: 2% phosphoric acid, 48% water, 50% acetonitrile

Time (min)	%A	%B	Sparge (ml/min)
Initial	100	0	10
45	35	65	10
49	35	65	10
50	100	0	100
60	100	0	10

Preparative

Solvent A: water

Solvent B: 50% water, 50% methanol

Time (min)	%A	%B	Sparge (ml/min)
Initial	90	10	10
30	40	60	10
34	40	60	10
35	90	10	100
45	90	10	10

Elution of the compounds was detected by using a Photodiode Array Detector and scanning the area of maximum peak absorbancy from 700 to 400 nm. All HCA's have diagnostic UV absorption properties that can be used for the identification of the aglycon component.

HPLC Co-Chromatography

The chemicals isolated by the procedures outlined above were then tested with various species of *Lygodium* to detect their presence or absence. A small quantity of the isolated compound is added to the extract of the species to be tested. The HPLC traces are compared before and after the addition of the

authentic material. If the compound is present, the peak in question should be higher than before the addition of authentic material. This procedure is more accurate than merely identifying compounds by their retention time and absorption characteristics.

In Vial I, 10 µl of the isolated compound was added to approx. 50 µl of the fern extract. Vial II contained only 50 µl of the fern extract. Both vials were filled with methanol/water to a total volume of 500 µl. The following species of *Lygodium* were tested (species chosen by availability): *L. circinnatum, L. flexuosum, L. microphyllum, L. palmatum, L. radiatum, L. reticulatum, L. salicifolium, L. smithianum, L. venustum,* and *L. volubile.* In the case of one of the compounds purified from *Lygodium japonicum*, compound C1, various fern taxa in genera other than *Lygodium* were also tested using the same technique.

NMR-Spectroscopy

All NMR experiments were conducted at the CUNY Applied Biomedicine NMR Center, College of Staten Island (CSI) under the direction of Dr. Ruth Stark by Dr. Cherryl Tihal. High-resolution two-dimensional NMR experiments were performed at CSI using a Varian Unity*plus* 600 MHz spectrometer equipped for sensitive detection of ^{13}C through the ^{1}H nuclei and with pulsed field gradients that remove artifacts and improve the efficiency of spectral acquisition (Hurd & John, 1991). After purity of the compound was confirmed using one-dimensional spectra, ^{1}H chemical shifts, ^{1}H scalar couplings, and ^{13}C chemical shifts were analyzed in conjunction with suitable databases to tentatively identify the sugar and phenylpropanoid functional groups (Varian 200, 500 MHz spectrometers). Two-dimensional NMR experiments were used to elucidate the detailed molecular structure. Double-quantum-filtered correlated spectroscopy (DQF-COSY) data were analyzed to

delineate through-bond interactions between proton pairs on the separate sugar and aromatic moieties (Nagayama *et al.*, 1980). Total correlation spectroscopy (TOCSY) was used to identify larger scalar-coupled proton networks within each molecule (Braunschweiler & Ernst, 1982). This information was augmented by heterocorrelated multiple-quantum correlation (HMQC) determinations of bonded proton-carbon pairs (Muller, 1979). Finally, the covalent connections between functional units and the long-range proton-carbon bonded interactions was established with heterocorrelated multiple bond correlation experiments (HMBC; Bax & Summers, 1986)

Mass Spectroscopy

All mass spectroscopy (MS) experiments were performed at Lehman College, Phytochemistry Laboratory, by Dr. Barbara Meurer-Grimes on a Finnigan Mat LCQ system. Using only μg quantities this system can provide an accurate molecular weight and yield complex fragmentation patterns that are often characteristic of the particular compound being tested (an important asset in the identification of new natural compounds). The compounds were analyzed via direct infusion wih a syringe pump at a flow rate of 10 μl/min, using the electrospray ionization source (ESI). The capillary temperature was set to 250^{o}C and the capillary voltage to 37V. The samples were examined on the negative and positive ion mode, using full mass scans, MS/MS as well as MS/MS/MS experiments.

Results

Phytochemical Profile on Ferns

In order to determine the phytochemical profile of *Lygodium* and to assess the plausibility of a detailed HCA study, a preliminary survey of 12 species of *Lygodium* and 42 representatives from a phylogenetically wide variety of fern

families was conducted on methanolic extracts using reversed-phase HPLC. These results are summarized in Table 8.2. The compounds easily distinguishable by HPLC in conjunction with UV/Vis spectroscopy are the HCA's and the flavonoids which have characteristic absorption spectra. Analytical HPLC results from *Lygodium japonicum* and *Astrolepis sinuata* are compared in Fig. 8.2. The extract of *Astrolepis sinuata* yielded six HCA derivatives (compounds No. 3-8) and eleven flavonoids (compounds No. 9-19). The results of the phytochemical profile of *L. japonicum* indicated compounds No. 3-18, 21 and 22 were HCA conjugates and only Compounds No. 19, 20 and 23 were flavonoids. Those compounds in which the spectra and maximum absorbance were not readily assignable as either HCA or flavonoid were rated as unknown (e.g., compound No. 20, 21 of *Astrolepis sinuata*). No attempt was made to distinguish among compounds based on quantitative differences: compounds, analyzed at 310 nm, were assigned as HCA derivatives or flavonoids.

The number of HCA's present in *Lygodium* was unusually high compared with most of the other fern taxa surveyed. Fifty-five percent of the compounds showing absorption maxima at or around 310 nm were HCA's in 75% of the species of *Lygodium* whereas only 33% of the other fern taxa surveyed contained that percentage of HCA's. In seven species of *Lygodium* HCA's made up more than 70% of the total phytochemical profile (*L. heterodoxum, L. japonicum, L. microphyllum, L. radiatum, L. reticulatum, L. salicifolium, and L. smithianum*). Only the following fern taxa other than species of *Lygodium*, contained this many HCA's: three species of *Osmunda* (*O. regalis, O. cinnamomea* and O. *claytoniana*), *Dennstaedtia globulifera*, *Ceratopteris thalictroides*, *Llavea cordifolia*, and the water ferns, *Salvinia* sp.

TABLE 8.2. Phytochemical profile of fern species as determined by high performance liquid chromatography (HPLC).

FERN SPECIES	Total Number Compounds Eluted	Percentage of Total by Type HCA's (%)	Flavonoids (%)	Unknown Compounds (%)
Adiantum tenerum	22	18	28	54
Adiantum raddianum	16	38	44	18
Angiopteris evecta	13	54	31	15
Anemia colimensis	23	52	30	18
Anemia hirta	5	20	60	20
Anemia phyllitidis	6	83	17	0
Anemia tomentosa	26	50	23	27
Athyrium filix femina	6	50	17	33
Asplenium bulbiferum	16	31	50	19
Azolla sp.	6	100	0	0
Botrychium decomposita	14	43	21	36
Ceratopteris thalictroides	8	75	25	0
Cheilanthes alabamensis	5	60	0	40
Astrolepis (Cheilanthes) sinuata	10	30	70	0
Cystopteris fragilis	24	38	16	46
Dennstaedtia globulifera	26	76	4	20
Dicksonia fibrosa	15	67	0	33
Dicronopteria pectinata	24	33	33	33
Dryopteris affinis	9	45	0	55
Elaphoglossum ciliatum	18	17	22	61
Gleichenia bancroftii	15	67	27	6
Llavea cordifolia	9	78	0	22
Lygodium circinnatum	25	42	16	42
Lygodium flexuosum	15	67	0	33
Lygodium heterodoxum	20	75	20	5
Lygodium japonicum	16	78	22	0
Lygodium microphyllum	15	87	7	6
Lygodium palmatum	24	42	29	29
Lygodium radiatum	19	79	21	0
Lygodium reticulatum	11	73	27	0
Lygodium salicifolium	12	83	17	0
Lygodium smithianum	7	85	15	0
Lygodium venustum	26	42	42	16
Lygodium volubile	18	56	28	16
Marattia laxa	14	22	0	78
Marsilea sp.	12	58	17	25
Ophioglossum reticulatum	10	0	10	90
Osmunda cinnamomea	20	80	15	5
Osmunda claytoniana	21	71	24	5
Osmunda regalis	7	50	25	25

Table 8.2. (continued).

FERN SPECIES	Total Number Compounds Eluted	Percentage of Total by Type HCA's (%)	Flavonoids (%)	Unknown Compounds (%)
Pellaea falcata	13	69	0	31
Phanerophlebia nobilis	12	66	0	34
Platyzoma microphylla	14	50	21	29
Polypodium asplenioides	8	38	0	62
Pteris semipinnata	21	33	43	24
Salvinia sp.	12	84	8	8
Schizaea elegans	19	37	21	42
Schizaea pennula	24	54	13	33
Thelypteris decussata	14	43	36	21
Todea barbara	8	50	25	25
Vittaria lineata	16	37	25	38
Woodwardia radicans	12	34	8	58

and *Azolla* sp. A few of the fern taxa analyzed had unknown compounds comprising at least 70% of the chemical constituents being eluted: *Marattia laxa* and *Ophioglossum reticulatum* are both in unique families and probably contain equally unique chemicals.

This survey was conducted to compare the diversity of UV detectable compounds in *Lygodium* with other fern taxa. The results are useful only on a comparative basis. To draw any phytochemical or phylogenetic conclusions would require more samples of the same and additional taxa. For example, the number of compounds eluting in *Anemia hirta, Athyrium filix femina, Azolla* sp., and *Cheilanthes alabamensis* were too few to allow for any conclusions as to their phenolic profile. Although a survey of this type is beyond the scope of this work, we can draw the following conclusions:

1) species of *Lygodium* contain large numbers of HCA's compared with flavonoids;

2) it might be useful for taxonomic investigations to compare the HCA conjugates present among species of *Lygodium* to discern any restricted distributions; and

3) it might be informative for taxonomic investigations to compare fern taxa with an abundance of HCA's with those with a prevalance of flavonoids.

In order to investigate these questions further, it was decided to isolate and identify some of the phenylpropanoids in *Lygodium* and trace their occurrence in other taxa. It was after the analysis of these initial data that the process of isolation and characterization of the HCA's of *Lygodium* was initiated.

Stability of Extracts Under Experimental Conditions

A major concern of this study was the availability of material and the

condition of the plant material at the time of extraction. Since the genus is pantropical many taxa were not readily available fresh or recently collected. The intended study was to compare plant material obtained from a number of different sources and stored under different conditions. It needed to be established that these storage conditions did not affect the phenolic profile of the extracts. Therefore, fresh vs. dried (1-2 weeks) material, fertile vs. sterile material, and herbarium vs. freshly dried material were compared using HPLC. Towards the end of the study, refrigerated extracts were also compared with freshly extracted fractions.

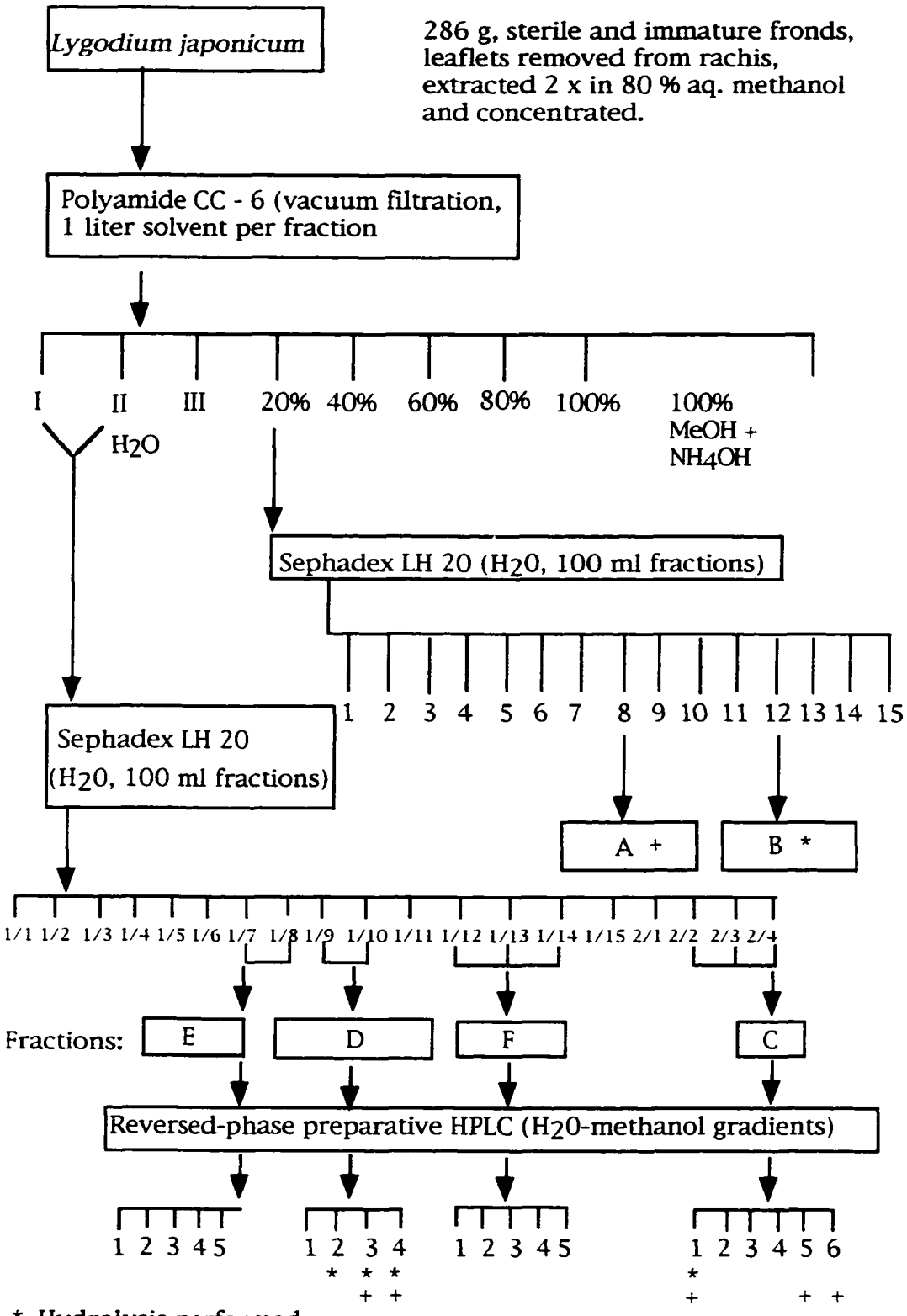
There was no difference in the chemical complement in the fresh vs. recently dried material. There was little difference in sterile vs. fertile with the exception of *L. palmatum* and *L. microphyllum.* In these species the fertile plants contained 1 or 2 additional compounds and a few HCA's were greatly reduced in concentration. In view of these results the fertile and sterile extracts were combined for the co-chromatography. Herbarium material was in general consistent with freshly dried material. However, a major problem was that the small amount of material available (usually less than 500 mg) made the extracts very dilute. This would influence the chemical diversity eluted chromatographically. Of the 12 taxa of *Lygodium* used in the phytochemical analysis, three were extracted from herbarium material: *L. radiatum, L. salicifolium and L. smithianum.* These taxa were important members of generic subgroups and difficult to obtain fresh. In each case the chromatograms of these taxa displayed chemical diversity as well as concentrations almost comparable to freshly dried specimens. The other 14 taxa treated in this revision were not tested phytochemically because material was unavailable (e.g., herbarium material only and often too old, dried with alcohol, or lacking sufficient material).

Purification of HCA's from *Lygodium japonicum*

Twelve phenlypropanoids were initially isolated from *Lygodium japonicum* using open-column chromatography on polyamide and Sepahdex LH20, as well as preparative HPLC. The purification sequence is outlined in Fig. 8.3. The purity of each fraction was analyzed using one-dimensional, non-overlapping TLC and analytical HPLC. Similar fractions were combined before attempting final purification. The fractions were given simple alphabet designations (A-F), whereas the compounds isolated from those fractions were labelled with numbers (1-6). HPLC chromatograms of the combined fractions for Compounds C and D are shown in Figs 8.4 and 8.5 respectively indicating the compounds collected from each using preparative HPLC. The overall procedure resulted in the isolation of twelve pure compounds, several of which were selected for further characterization. Ten additional compounds were isolated and need either further purification or have not been studied as yet. Table 8.3 summarizes the fractions isolated and the experiments conducted on each. All 22 compounds are summarized in Table 8.4 indicating their characteristic analytical HPLC retention times and UV absorption spectra. All the steps in the process of isolation and elucidation of compound fractions were confirmed with TLC and HPLC.

Hydrolysis

In order to learn as much as possible about the compounds before further structural elucidation by NMR and MS, portions of the most concentrated compounds were hydrolyzed and the hydrolysis products identified. Results were obtained from six samples and are listed in Tables 8.5 and 8.6.
Acid hydrolysis ruptures the glycosidic linkages releasing the component sugar(s). The sugar standards were chosen as those monosaccharides or



* Hydrolysis performed

\+ Compounds structurally elucidated by NMR and MS

Figure 8.3. Chromatographic purification of phenylpropanoids isolated from *Lygodium japonicum*.

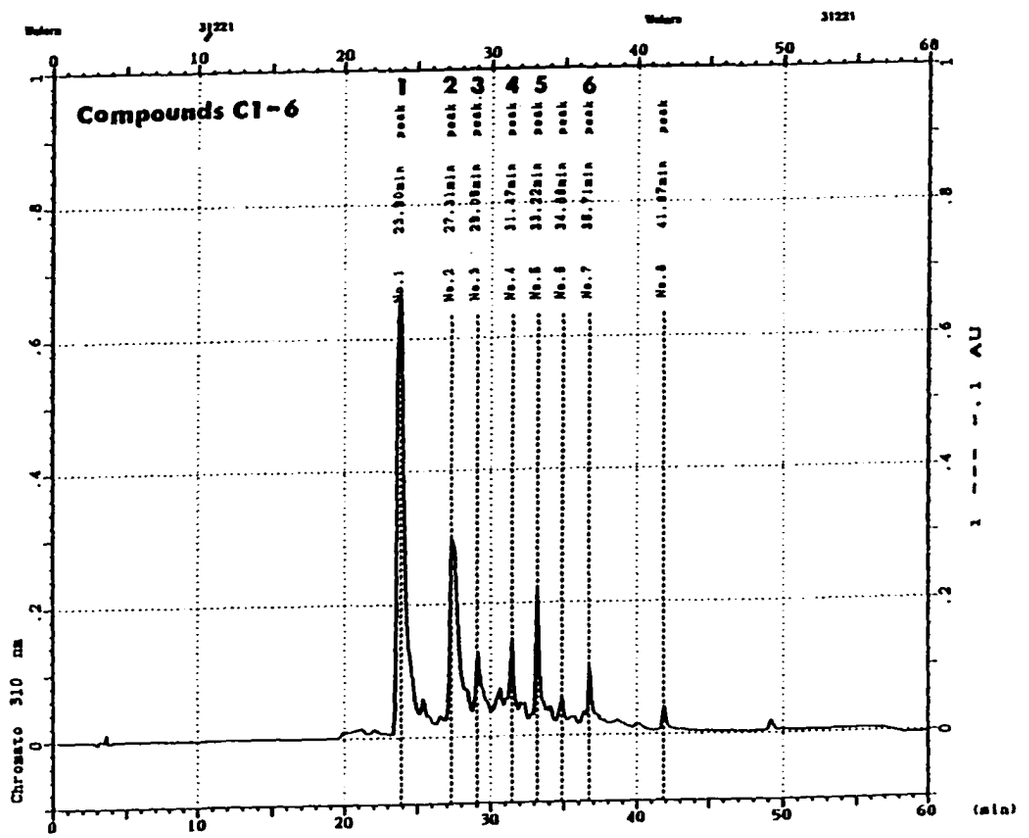


Figure 8.4. The HPLC chromatogram of the combined column fractions, 2/2, 2/3 and 2/4 from which Compounds C1-C6 were collected by preparative HPLC.

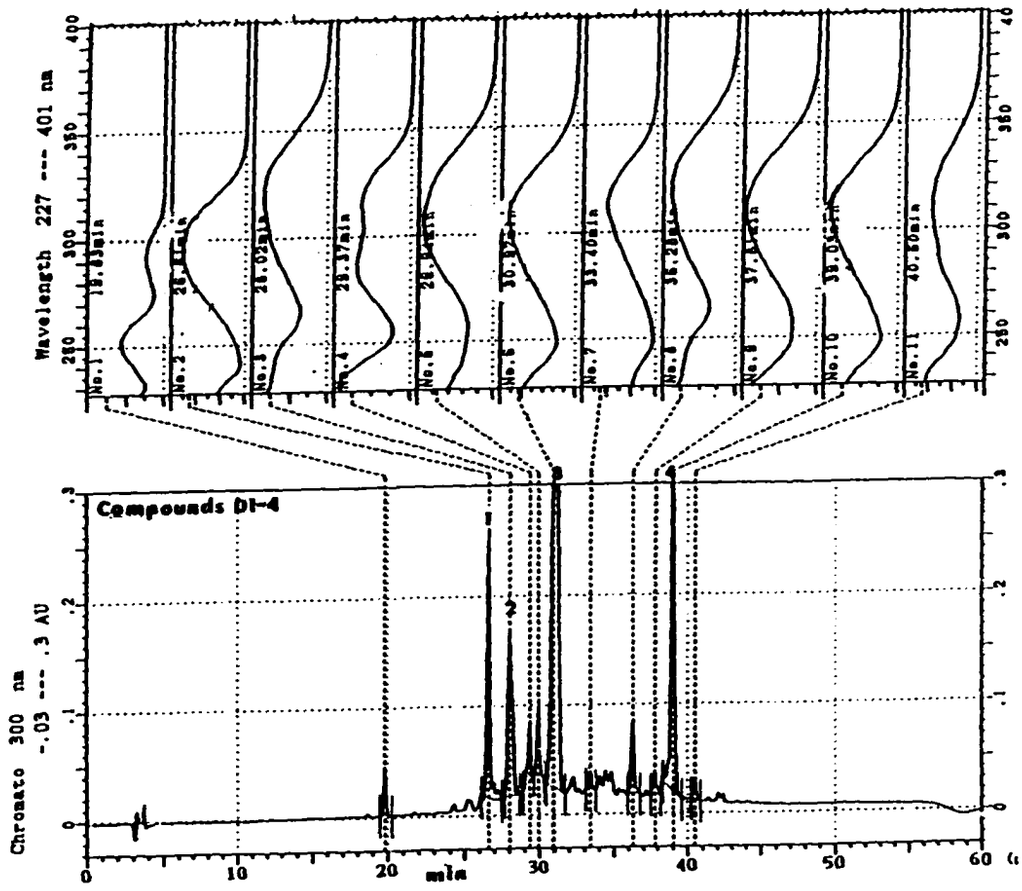


Figure 8.5. The HPLC chromatogram and UV/Vis absorption spectra of the combined column fractions 1/9 and 1/10 from which Compounds D1-D4 were collected by preparative HPLC.

Table 8.3. Experiments conducted on fractions isolated from *Lygodium japonicum* (indicated by an "X").

Compound	Hydrolysis		NMR	MS	Co-chrom.
	Acid	Alkaline			
A	-	-	X	X	-
B	X	X	-	-	-
C1	X	X	X	X	X
C2	X*	X*	-	-	X
C3	-	-	-	-	X
C4	-	-	-	-	-
C5	X*	X*	X	X	-
C6	-	-	X	X	X
D1	-	-	-	-	-
D2	X	X	-	-	X
D3	X	X	X	X	X
D4	X	X	X	X	X
E1	-	-	-	-	X
E2	-	-	-	-	-
E3	-	-	-	-	X
E4	-	-	-	-	-
E5	-	-	-	-	-
F1	-	-	-	-	X
F2	-	-	-	-	-
F3	-	-	-	-	-
F4	-	-	-	-	-
F5	-	-	-	-	-

* Compounds hydrolyzed with weak results

Table 8.4. Chromatographic properties (HPLC) of phenylpropanoids isolated from *Lygodium japonicum*. Compounds F 2 and F 3 require further purification: two compounds are present in each.

Compound	Retention Time(min.)	λmax (nm)
A	26.01	235, 290, 310 sh
B	30.10	240, 300 sh, 330
C 1	25.32	240, 300 sh, 330
2	28.04	315
3	30.31	245, 300 sh, 330
4	32.24	240, 300 sh, 335
5	34.18	240, 300 sh, 330
6	41.63	315
D 1	19.27	250, 290
2	26.49	295
3	30.06	310
4	38.47	310
E 1	26.56	250, 288, 340
2	30.72	305
3	30.98	310
4	29.56	310
5	34.88	305
F 1	26.90	235, 295, 315
2	28.81/31.14	315/310
3	33.98/35.81	310/310
4	37.62	315
5	41.95	315

λmax = largest peak; sh = shoulder

Table 8.5. Acid hydrolysis products of phenylpropanoids isolated from *Lygodium japonicum* identified by thin layer chromatography* (including standards).

Compound	Rf	Color	Sugar Fraction
B	0.37	navy-black	maltose or glucose
	0.96	olive green	rhamnose-like
C1	0.40	faint dark blue	maltose or glucose
D2	0.93	green-brown	rhamnose-like
D3	0.91	green-brown	rhamnose-like
D4	0.27	navy-black	maltose
	0.91	faint olive green	rhamnose-like
Standards			
arabinose	0.44	blue-green	
fructose	0.42	brown-blue	
galactose	0.31	light blue	
glucose	0.40	navy	
glucuronic acid	0.06	gray-black	
maltose	0.36	navy	
rhamnose	0.78	olive	
sucrose	0.49	brown-black	
xylose	0.60	brown-black	

* Silica plates (see Materials and Methods section, Chapter 8).

Table 8.6. Alkaline hydrolysis products of phenylpropanoids isolated from *Lygodium japonicum* identified by thin layer chromatography* (including standards).

Compound	Rf	Color		Ret. Time	λ max	HCA
		UV(350nm)	UV(350nm) +NH_3	(min.)	366nm	Identification
B	0.22	white-blue	green-blue	30.10	240,290sh,320	caffeic
	0.67	dark purple	dark purple	35.84	310	p-coumaric
C1	0.25	white-blue	white	30.77	240,290sh,325	caffeic
	0.59	bright blue	no change	-	-	decomposition product
D2	0.18	white-blue	bright green	30.77	240,290sh,325	caffeic
D3	0.13	white-blue	blue	-	-	unknown
	0.44	dark purple	dark purple	34.17/35.87	300/310	p-coumaric(cis/trans)
D4	0.45	dark purple	dark purple	34.53/36.31	295/300	p-coumaric(cis/trans)
	0.61	blue(faint)	yellow-blue	-	-	decomp. product ?
	0.95	brown(faint)	brown-black	35.80	300sh, 315	unknown
Standards						
caffeic	0.34	white-blue	bright blue green	30.35	240,295sh,330	
chlorogenic	0.09	dark purple	no change	29.90	240,300sh,330	
trans-cinnamic	0.93	light gray	yellow	50.03	280	
p-coumaric	0.67	dark purple	darker purple	35.73	310	
o-coumaric	0.73	white	yellow-green	43.04	270,325	
ferulic	0.81	bright blue	yellowish	37.79	240,295sh,320	
sinapic	0.73	yellow green	bright aqua	39.17	240,325	

* cellulose plates

disaccharides commonly conjugated with phenols (Harborne, 1979). Some of the results proved difficult to interpret as the hydrolysate products did not exactly match any of the standard reference sugars tested using TLC (Table 8.5). However, all had R_f values and appropriate color changes characteristically similar to glucose or maltose. In compounds B, D2, D3, and D4 rhamnose-like hydrolysis products were also indicated. When D3 and D4 were structurally elucidated by NMR and MS, only glucose was present, no methyl groups indicative of rhamnose were found. Therefore, the additional spots on the TLC plate may have been decomposition products.

The results of the alkaline hydrolyses indicated the presence of either *p*-coumaric acid or caffeic acid as the HCA aglycon present in the isolated compounds (Table 8.6). In compounds D3 and D4, two spots occured on TLC as well as two peaks on HPLC. The results are consistent with a cis/trans isomer (one being a decomposition product) of *p*-coumaric acid.

In compound B both *p*-coumaric acid and caffeic acid were indicated - this was later explained as the isolate was not fully purified and contained two different chemicals. In D4 ferulic acid and an unknown isolate were potentially present along with *p*-coumaric acid. In the subsequent structural elucidation phases using NMR and MS this compound proved to be the most difficult to analyze. It is known that the relatively harsh hydrolysis conditions may give rise to degradation products, which were probably detected here.

In order to rule out the possibility of moieties other than sugars and HCA aglycones in the *Lygodium* HCA's, TLC was performed to test for the presence of any primary amino groups. The TLC plates were sprayd with ninhydrin, and none of the isolates showed a positive response. This indicated that the *Lygodium* HCA's are not substituted with amino acids or amines.

Nuclear Magnetic Resonance and Mass Spectroscopy

Twelve compounds were given to Drs. Ruth Stark and Cherryl Tihal at the College of Staten Island (CSI) for NMR analysis. One-dimensional NMR-spectra were used to confirm the purity of each compound. At this time, some compounds were returned for further purification or because there was insufficient material for further experimentation. A summary of the NMR experiments conducted is presented in Appendix I.

Mass spectra of the isolates were obtained using a newly acquired LC/MS/MS system at Lehman College by Dr. Barbara Meurer-Grimes. A summary of the MS experiments in also provided in Appendix I.

The structures of four of the twelve isolates have been elucidated and appear in Figs. 8.6-8.9. These compounds are β-1-O-caffeoylglucose (isolate A), 4-O-(E)-caffeoylglucose (isolate C1), 4-O-*p*-coumaroyl glucose (isolate D3) and an unnamed compound (isolate D4). A fifth isolate (C5) has been partially identified as a caffeoyl-unknown-β glucoside. All compounds are glucosides of either *p*-coumaric acid or caffeic acid.

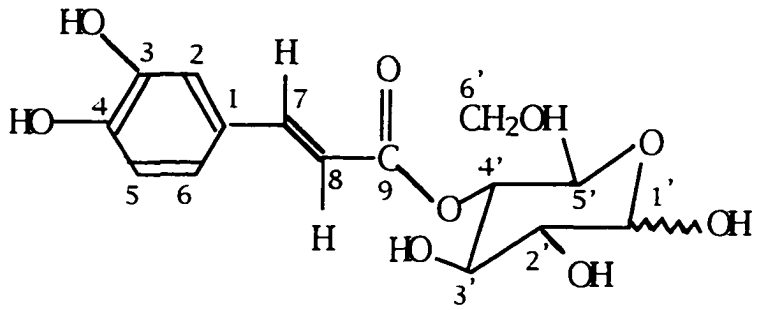
Summary of Isolated Fractions

The following is a summary of the chemical characteristics of the isolates from *Lygodium japonicum.*

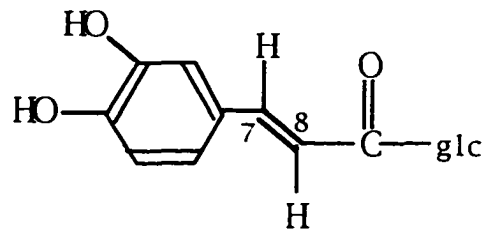
Compound A. β-1-O-caffeoylglucose (Fig. 8.7). This was one of the first compounds isolated from the *L. japonicum* extract, using the 20% methanol fraction of the polyamide fractionation and subsequent purification on Sephadex LH20. Using analytical conditions, it had a HPLC-retention time of 26.0 mins and UV/Vis absorption maxima of 240, 290 and 320 nm. The structure was elucidated primarily by NMR from ^{1}H spectra, and COSY, TOCSY, HMBC, and HMQC experiments (see Appendix I).

Compound B. This compound was isolated from the 20% methanol fraction of the polyamide fractionation and subsequent purification of Sephadex LH20. Under analytical conditions, it had a HPLC retention time of 30.1 min and a UV/Vis absorpion maxima of 240, 300, and 330 nm. There were two products from acid hydrolysis as analyzed by TLC: one was a maltose or glucose compound and the other a rhamnose-like compound. Alkaline hydrolysis yielded two fractions: caffeic and p-coumaric acid. This compound proved too dilute to continue identification as further purification was required as revealed by 2-dimensional NMR. Compounds C1-C6 represent isolates from the water fraction of the polyamide-treated *Lygodium* extract, which was purified by chromatography on Sephadex LH20. Fractions 2/2 to 2/4 were combined and further purified by preparative HPLC (refer to Fig. 8.3).

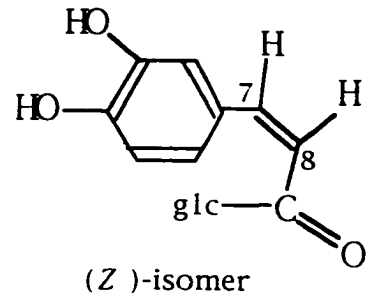
Compound C1. 4-O-(*E*)-caffeoylglucose. C1 was one of the major compounds in the water fraction of the polyamide-treated *Lygodium* extract. Compound C1 showed a retention time of 35.5 mins (preparative HPLC) and UV-absorption maxima of 240, 300sh, and 330 nm. The purity was checked using the analytical HPLC method, and the compound showed a retention time of 24.2 mins. The UV-data and the retention time indicated that this isolate may be similar to Compound A, but not identical, e.g., also a conjugate of caffeic acid. On TLC plates the compound gave a bright blue-green fluorescence turning blue-white when treated with ammonia vapours. Upon mild alkaline hydrolysis caffeic acid was identified as the hydrolysis product. These features indicate that the substance is indeed a caffeic acid ester. The structure of the isolate was fully elucidated by NMR and MS. It is evident from



4-O-(*E*)-caffeoylglucose



(*E*)-isomer



(*Z*)-isomer

Figure 8.6. Proposed structure of Compound C1, 4-O-(*E*)-caffeoylglucose including (*E*)-isomer and (*Z*)-isomer.

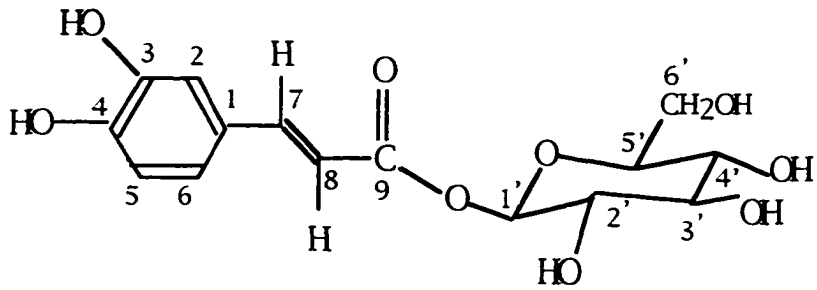


Figure 8.7. Proposed structure of Compound A, β-1-O-caffeoylglucose.

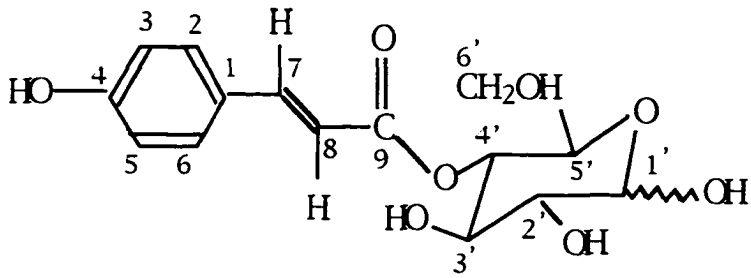


Figure. 8.8. Proposed structure of Compound D3, (*E*)-isomer of 4-0-*p*-coumaroyl glucose.

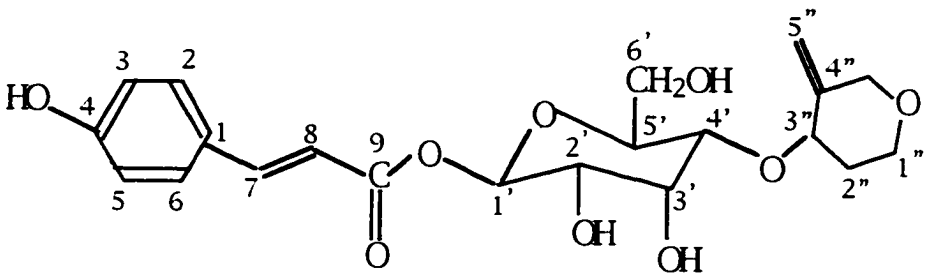


Figure 8.9. Structure proposal for Compound D4 from *Lygodium japonicum*.

the NMR data that a mixture of four isomers was present. The NMR assignments are listed in Appendix I, and the structures are shown in Fig. 8.6.

Compound C2. Under analytical conditions the retention time of C2 was 28.0 min with a UV/Vis absorption maxima of 315 nm. This compound was too dilute to perform NMR or MS experiments. The results were very difficult to discern in alkaline hydrolysis, but it appeared that *p*-coumaric was present as part of the compound. The compound was used in the co-chromatography experiments and found to have an interesting pattern of distribution in the taxa of *Lygodium* tested (Table 8.7).

Compound C3. This isolate had a 30.3 min HPLC retention time with a UV/Vis absorption maxima of 245, 300, 330 nm. Initial TLC results indicated a caffeic acid derivative. This compound has not been structurally elucidated with NMR or MS as yet. It was used in the distribution studies and was not found in any other species of *Lygodium* tested except for trace amounts in *L. salicifolium.*

Compound C4. This fraction had an analytical HPLC retention time of 32.24 min and an absorption maxima of 240, 300, and 335 nm. The sample was too dilute to perform NMR experiments and further isolation of the compound would be necessary.

Compound C5. C5 has a 34.2 min HPLC retention time and 240,300, and 330 nm absorption maxima. Initial TLC of the compound indicated a caffeic acid conjugate. The structure of this compound has not been completely elucidated but analysis of the NMR and MS experiments indicates two fractions present: one a caffeoyl–1-O-glucose-4-Unknown mixed with an isomer of the caffeic acid and also with isomers of the unknown moiety. This compound is the caffeoyl version of Compound D4 and is new to natural product chemistry.

Compound C6. The analytical HPLC retention of C6 is 41.6 min wih a UV/Vis absorption spectra of 315 min. The data of NMR and MS experiments are being

analyzed. Initial TLC results indicated a *p*-coumaric conjugate. This fraction is also more complex and contains a HCA linked to an unknown moiety linked to glucose. The distribution of this compound in other taxa of *Lygodium* is sporadic.

Compounds D1-D4 are isolates of the water fraction post-polyamide fractionation, followed by purification on Sephadex LH 20 (Fig. 8.3). Fractions 1/9 and 1/10 were combined and preparative HPLC performed to yield the 4 purified compounds (D1-D4).

Compound D1. This fraction had an HPLC retention time of 19.3 min and an absorption spectra of 250 and 290 nm. The peak characteristics are interesting and unique. Initial TLC produced yellow spots. However, this compound proved to dilute for NMR experimentation and further isolation would be needed to concentrate the sample.

Compound D2. This fraction had an HPLC retention time of 26.5 min with an absorption spectra of 295 nm. Hydrolysis yielded caffeic acid as the aglycon component and a rhamnose-like sugar. This compound has been used for distribution studies even though no NMR studies have been performed as yet. It has a sporadic presence in taxa of *Lygodium* that do not appear to show any phyletic pattern.

Compound D3. 4-O-*p*-coumaroyl glucose (Fig. 8.8). D3 was another major compound isolated from the water fraction after chromatography on Sephadex LH20 (fraction numbers 1/9 and 1/10). The compound was purified from the combined LH20 fractions by the same preparative HPLC method. The substance eluted at Rt = 51.4 and 51.5 min, giving each a peak for the (*E*) and (*Z*) isomers of the compound. Retention time in the analytical HPLC system was 30.0 and 30.9 mins for the two isomers. The UV-absorption maxima were determined at 309 and 310 nm. Upon mild alkaline hydrolysis, *p*-coumaric acid was released

from the isolate and identified by HPLC and comparison with an authentic sample. The acid hydrolysis did not yield any results. It was therefore assumed that NM3 is an ester of p-coumaric acid. The ^{1}H NMR spectra and the ^{13}C NMR spectra revealed some similarities between C1 and D3. The signal assignments are shown in Appendix I.

Compound D4. This compound has a 38.5 min retention time with HPLC and a UV/Vis absorption spectra of 310 nm. Upon alkaline hydrolysis, p-coumaric acid was released and acid hydrolysis yielded maltose or glucose and a rhamnose-like sugar. This isolate has been tentatively identified as a *p*-coumaroyl-1-O-glucose-4-moiety and its structure elucidated in Fig. 8.9. It consists of isomers of the *p*-coumaric fraction and also the unknown moiety fraction. This isomerization has made the NMR and MS data interpretations difficult. This compound is new to natural product chemistry. An analysis of the NMR and MS data is contained in Appendix I. In co-chromatography experiments this compound had a restrictive distribution being present only in *Lygodium venustum* and in trace amounts in *L. circinnatum, L. heterodoxum, L. salicifolium,* and *L. smithianum.* A distribution pattern of *L. venustum* and *L. japonicum* is important because these are related and may represent an example of allopatric speciation.

Compounds E1-E5 and F1-F5 represent the products of the water fraction of the polyamide fractionation followed by purification on Sepahdex LH 20. Compounds E1-E5 are the combination of fractions 1/7 and 1/8 with further purification by preparative HPLC. Compounds F1-F5 represent the combination of fractions 1/12-1/14 and final purification by preparative HPLC. No structural elucidation has begun on these isolates. Some of the compounds require further purification. However, some are interesting and initial HPLC and TLC analysis indicate the possibility of *o*-coumaric or sinapic

acid derivatives: both of these having very restrictive occurrence in plants in general. The compounds that were pure on HPLC analysis and had strong peaks were used in the co-chromatography (E1, E3 and F1): only those compounds will be described below.

Compound E1. This isolate had an analytical HPLC retention time of 26.6 min with a UV/Vis absorption spectra of 250, 288 and 340 nm. This peak was unique. The initial TLC data showed the presence of a bright white-purple spot that turned blue-green with ammonia fumes with an Rf of 0.76 (BAW, 4:2:1). This could represent a caffeic, ferulic or sinapic acid conjugate (Harborne, 1984). This compound was found to be present in a variety of the *Lygodium* taxa tested.

Compound E3. This isolate had an HPLC retention time of 30.9 min with a UV/Vis absorption spectra of 310 nm. TLC data of the conjugate revealed dark purple spots after ammonia fumes indicative of a *p*-coumaric derivative and also bright yellow spots that might be suggestive of an *o*-coumaric acid conjugate. This compound was used in the co-chromatography experiments (only the largest peak was compared on the chromatograms, e.g., the *p*-coumaric acid conjugate) and showed a sporadic occurrence in the taxa of *Lygodium* tested.

Compound FI. This compound's HPLC retention time was 26.9 min with a UV/Vis absorpion spectra of 235, 295, and 315 nm. This compound revealed reddish-purple spots on TLC, which was difficult to assign as one of the standard HCA conjugates discussed thus far. It also had a sporadic occurrence in the other taxa of *Lygodium* tested.

Distribution of HCA's in Other Species of *Lygodium*

The final phase of this phytochemical study involves the co-chromatography of isolated compounds with 11 taxa of *Lygodium*. The number

of compounds tested was limited by the amount of the isolated chemical remaining after structural elucidation. The number of taxa of *Lygodium* tested was limited by the availability of plant material.

The results are summarized in Table 8.7. Caffeoyl 4-glucose (compound C1) was found to be present in all species of *Lygodium* tested. The methodology of analyzing results is illustrated in Fig. 8.10 in which two HPLC chromatograms are presented for *L. palmatum*, one in which Compound C1 was present (+ C1) and the second in which C1 was absent (- C1). This compound, therefore, could prove to be a synapomorphy for the genus. In order to determine its presence in other fern taxa, it was tested with 20 other fern genera and found to be present in 2 of the 3 species of *Osmunda* tested (*O. cinnamomea* and *O. claytoniana*), *Todea barbata* and *Dennstaedtia globulifera* (Table 8.8) but in none of the members of the Schizaeaceae *s.l.* Thus, it would appear that the accumulation of caffeoyl 4-glucose occurs too sporadically in ferns to be of importance in resolving any phylogenetic relationships at the familial level but may prove important at the genus level. Caffeoyl 4-glucose is also a very common natural product known to occur widely in many species of angiosperms. In order to determine its occurrence in ferns, a much wider survey should be undertaken. Compounds C2, *p*-coumaroyl 4-glucose (D3) and E3 were found in 8 of the 11 taxa tested. Thus, the *p*-coumaroyl 4-glucose conjugate (D3) appears much more restricted in *Lygodium* than the caffeoyl 4-glucose conjugate.

Compound C3 was found only in *L. japonicum* and *L. salicifolium.* Morphologically, these two taxa are very distinct. This compound would have to be tested in the other 14 species of *Lygodium* treated in this revision to decide if this is a restrictive character and an example of parallel evolution of

TABLE 8.7. Occurrence of HCA conjugates in eleven species of *Lygodium*: (-) indicates the absence of the compound in the taxa, (+) indicates its presence, and trace (tr) indicates an amount too small to ascertain.

LYGODIUM SPECIES	COMPOUNDS									
	C 1	C 2	C 3	C 6	D 2	D 3	D 4	E 1	E 3	F 1
L. circinnatum	+	-	-	+	-	+	tr	tr	+	+
L. flexuosum	-	-	-	-	+	-	-	-	tr	+
L. heterodoxum	+	tr	-	+	tr	+	tr	tr	+	tr
L. microphyllum	+	-	-	-	-	+	-	+	tr	?
L. palmatum	+	+	-	-	+	+	-	-	+	-
L. radiatum	tr	+	-	-	+	+	-	+	+	-
L. reticulatum	+	+	-	+	+	+	-	-	-	+
L. salicifolium	+	+	tr	+	tr	+	tr	tr	tr	-
L. smithianum	+	+	-	-	+	-	tr	+	-	+
L. venustum	+	+	-	+	-	-	+	-	-	+
L. volubile	+	+	-	+	-	+	-	-	+	-

Table 8.8. The occurrence of caffeoyl 4-glucose (Compound C1) in select fern taxa.

Fern Taxa	Compound C 1*
Adiatum tenerum	-
Anemia colimensis	-
A. hirta	-
A. phyllitidis	-
A. presliana	-
A. tomentosa	-
Angiopteris evecta	-
Ceratopteris thalicatroides	-
Dennstaedtia globulifera	+
Dicksonia fibrosa	-
Dicranopteris pectinata	-
Gleichenia bancroftii	-
Marsilea sp.	-
Osmunda cinnamomea	+
O. claytoniana	+
O. regalis	-
Platyzoma microphylla	-
Schizaea elegans	-
S. pennula	-
Todea barbara	tr

* + = presence of compound
- = absence of compound

tr=amount too small to positively ascertain

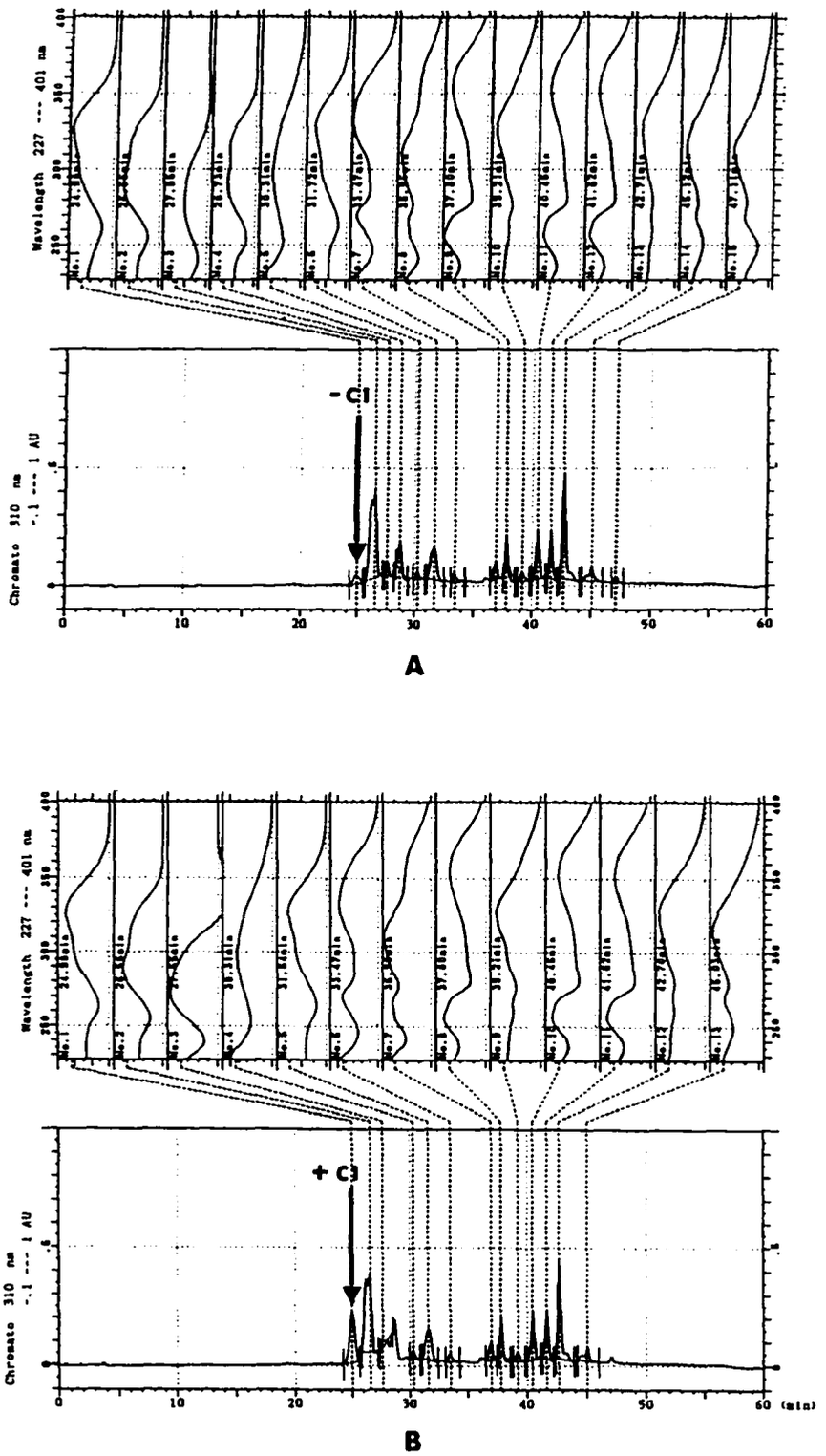


Figure 8.10. The HPLC analysis of the extract of *Lygodium palmatum* with Compound C1 absent (A) and present (B).

biosynthetic pathways or actually more common in its distribution. In order to do this many Old World taxa would need to be collected with enough material for chemical extractions (e.g., *Lygodium hians*, New Caledonia; *L. kingii*, New Guinea; *L. auriculatum*, Malaysia; etc.).

Lygodium flexuosum had the least number of chemicals in common with *L. japonicum*. This is interesting since morphologically *L. flexuosum* intergrades with both *L. japonicum* and *L. salicifolium* . *Lygodium salicifolium* had almost all compounds in common with *L. japonicum*, yet these taxa are morphologically distinct.

The chemical profile in the New World species (*L. heterodoxum, L. palmatum, L. radiatum, L. venustum and L. volubile*) does not appear to be distinctly different from the Old World species (*L. circinnatum, L. flexuosum, L. japonicum, L. microphyllum, L. reticulatum, L. salicifolium*, and *L. smithianum*). There is no significant similarity between species with reticulate spores (*L. microphyllum and L. reticulatum*) and those with verrucate spores (*L. smithianum and L. volubile*). *Lygodium palmatum*, the only temperate species has a chemical profile similar to that of *L. radiatum* (Central America to Venezuela) - these two species are morphologically related by having palmately arranged leaflets.

All the phytochemical compounds and their occurrence in the various taxa tested have been included in a cladistic analysis and will be discussed later.

Discussion

In the most recent analyses of fern evolution both morphological and molecular data have been analyzed (Stevenson & Loconte, 1996, Hasebe *et at.*, 1995 ; Pryer *et al.*, 1995). Biochemical data (micromolecular) were not present in the latter two of these analyses (Hasebe *et al.*, 1995 and Pryor *et al.*, 1995) while Stevenson and Loconte (1996) used data on the occurrence of 3-

deoxyanthocyanidin. This is due in part to the lack of chemical surveys in ferns and to the sporadic occurrence of some chemicals within the families that have been studied (Pryer, 1995). In this study the presence of Compound C1, present in all species tested, has not proven to be informative at the species level. However, Compounds C6, D3, D4 and E1 are included in the data matrix and have proven useful characters in the cladistic analysis.

The usefulness of fern chemistry in discerning parentage in hybridizations has been shown in species of *Asplenium* (Harborne *et al.*, 1973) and *Equisetum* (Veit *et al.*, 1995). The analysis of flavonoids and cinnamic acids has provided useful systematic characters in *Adiantum* (Cooper-Driver & Swain, 1977). Flavonoid chemistry has also been useful at the generic level (Giannasi & Mickel, 1979) and in discerning species affinities (summarized by Wallace, 1989).

The simplest compounds isolated in this study are *p*-coumaroyl 1-glucose (compound A), *p*-coumaroyl 4-glucose (compound D3), and caffeoyl 4-glucose (C1) (Fig. 8.6-8.8). Two additional, more complex, compounds have been partially identified as *p*-coumaroyl-glucose conjugated with an unknown moiety (compound C6) and *p*-coumaroyl 1-glucose conjugated with the same unnamed moiety (Compound D4; Fig. 8.9). All are glucose conjugates of common HCA's. As indicated in the Introduction, these chemicals are the precursors for many important biochemical pathways. The double bond in the side chain of HCA's (refer to Fig. 8.1) causes them to exist as cis/trans isomers. These isomers show different biological properties and different retention times when analyzed using HPLC and TLC and have proven to be a major problem in NMR and MS experiments over time (see Appendix I).

It is quite interesting chemically, ecologically and phylogenetically that one species of *Lygodium* (*japonicum*) yielded 6 different purified compounds

that consist of monomers of glucose and a simple HCA. Two of the other 10 isolates on initial study may contain either o-coumaric or sinapic acid (Compound E1 and F1), both very restrictive in occurrence. However, the former was proven informative in the cladistic analysis. Compound D4 is new to natural product chemistry and was found to be present in *L. venustum* and in trace amounts in *L. circinnatum, L. heterodoxum, L. salicifolium,* and *L. smithianum,* also a compound useful in resolving the phylogeny of *Lygodium.*

Most of the research to date on glycosylated phenolics has studied the structural complexity of flavonoid glycosides. Harborne (1979) reports flavonols and flavones associated with multiple sugar groups in which 2 or more phenolic groups are associated with more than 10 disaccharides and 7 trisaccharides. In the case of the flavones, linkages of the sugar to the carbon skeleton is a common feature. Flavonoids with 3 or more sugars have also been described in a monocot (Morita *et. al.,* 1976) and a moss (Osterdahl and Lindberg, 1977). Imperato (1979) reported 1-caffeyllaminaribiose, a new hydroxycinnamic acid-sugar derivative from *Asplenium adiantum-nigrum* L. This compound was new to natural product chemistry.

Bohm (1968) reported the simplest compound (caffeolyglucose) present in 4 of 5 species of *Cyathea* tested and none of the other 36 fern species surveyed (no species of *Lygodium* was tested). These results seem consistent with the data for compound C1. *Cyathea* species should be studied co-chromatographically with our isolated caffeoyl 4-glucose. He reports that due to "phenolic smearing" a detailed reexamination should be undertaken as the compound may be present in some other ferns tested but undetected. Veit and co-workers (1995) found caffeoyl-tartaric acids present in *Equisetum arvense* and *E. scirpoides* and in none of the 21 fern genera tested . One caffeoyl-tartaric acid, di-*E*-caffeoyl-(2S-3S)-(-)tartaric acid (chicoric acid) had been

reported in *Onchyium japonicum* as well as *L. circinnatum* in a very early study (Hasegawa and Taneyama, 1973), however, Veit and his co-workers did not find it present in any of the ferns surveyed and we did not find it either.

Sulfate esters of 1-caffeolyglucose and 1-*p*-coumarylglucose were first reported by Cooper-Driver and Swain (1975) in *Pteridium aquilinum* and 10 species of *Adiantum*. Subsequently Imperato (1981, 1982, 1990) has found caffeoylgalactose sulfates in *Ceterach officinarum* and *Adiantum capillus-veneris* as well as *p*-coumarylglucose sulfates in the latter and recently 1-caffeylglucose 6-sulfate and 3-sulfate in *Cystopteris fragilis*. Harborne (1979) reports sulfation as an alternative to glycosylation in plants in providing water and sap solubility and decreased phenolic toxicity. Neither the tartaric acid derivatives nor the sulfate derivative are present in the compounds isolated from *L. japonicum* that have been subjected to NMR and MS.

The distribution of the *Lygodium* HCA's in other species does not allow for many conclusions at the taxonomic level. Caffeoyl 4-glucose (C1) present in all species of *Lygodium* tested had a sporadic occurrence in the other fern taxa surveyed. It appears that this compound will not prove systematically useful for broad phylogenetic resolution: however, more ferns must be surveyed utilizing a greater quantity of plant material for extraction.

Compound C3 may be a species-specific chemical as it is present only in *L. japonicum* and in only trace amounts in *L. salicifolium* (indicating that plant extractions should be made from other geographic localities) and would have to be tested against all the other species of *Lygodium* before any further conclusions could be drawn. However, if it were specific to *L. japonicum*, it is important in supporting the independent status of the three sister species, *L. venustum*, *L. kerstenii* and *L. japonicum*.

Hybrids are not common in *Lygodium* but do exist - probably more exist than are recognized. Considering the ploidy levels found in *Lygodium*, any method to help clarify hybridization events would be important and compounds as C3, with very restrictive distributions, are important as species markers. Flavonoids have been successfully used in the study of hybridizations as the chemical complement appears to be additive in the hybrids (Connant & Cooper-Driver, 1980; Harborne *et al.* 1973). C-glycosylxanthones have been used to distinguish between the hybrid, *Asplenium adiantum-nigrum* L. and one of its parents, *A. cuneifolium* Viv. (Richardson and Lorenz-Liburnau, 1982).

No trends were noted in chemical composition between Old World species compared with New World species. Studies with 58 species of *Adiantum* (Cooper-Driver & Swain, 1977) have shown that Old World species contained a greater proportion of proanthocyanidins while New World species contained more acylated flavonol glycosides and sulfated cinnamic acid derivatives. From these data Cooper-Driver and Swain (1977) suggested that the Old World species are the more primitive. Neither the species of *Lygodium* tested with reticulate spores (*L.microphyllum and L. reticulatum*) nor those with large verrucae and exaggerated equatorial ridges (*L. smithianum and L.volubile*) showed any significant similarities in their chemical profile. Flavonoid chemistry together with spore morphology has proven useful in evaluating relationships between the two closely related fern genera, *Hemionitis* and *Gymnopteris* (Giannasi & Mickel, 1979). Those species with the same spore morphology in each genus produced the same chemical complement of flavonoids suggesting that the taxa should be placed within the same genus. In *Bommeria*, species with crested spores produced chemicals that species with

reticulate spores did not. These data helped define species alliances within the genera (Haufler & Giannasi, 1982).

The phytochemical similarities between two New World species with characteristic palmate segments, *L. radiatum* and *L. palmatum*, are interesting. The chemical profiles of these two species are exactly alike except for one of the chemicals (E1). In Prantl's treatment (1881) these species are placed in the same subgroup based on morphologic characteristics and this phytochemical study would tend to support this alliance. The new compound, D4, was present only in *L. japonicum* and *L. venustum.* This would support a strong alliance between the two and adds some evidence for a possible synonomy.

Our knowledge of the diversity of HCA conjugates is increasing with the use of HPLC, NMR spectroscopy and MS spectrometry. The potential questions arising from this initial study far exceed any answers. Are *Lygodium venustum* and *Lygodium japonicum* the same pantropical species, even though the phytochemical profiles differ in the 10 chemicals tested? How would the profile of *Lygodium kerstenii* compare? Is the African species *L. kerstenii* conspecific with *L. japonicum* or *L. venustum*? How closely allied are the African species *L. smithianum* and the New World *L. volubile*? Is *L. kingii* more closely allied with its geographic neighbor, *L. salcifolium* or with the New World, *L. volubile*? There are many alliances that chemical data could help resolve with more collections, extractions, and chemical resolution. The methodology is now in place for these studies.

CHAPTER IX

PHYLOGENETIC ANALYSIS

Introduction

The prior treatments of *Lygodium* (Presl, 1845; Hooker and Baker, 1874; Prantl, 1881; Reed, 1946; and Duek, 1978) all separate the genus into subgeneric units based on combinations of the same two characters: branching pattern (major axes or veins at base of segment) and segment shape. These two characters are also used to support hypothetical "lineages" as presented by Prantl (1881) and Reed (1946).

Presl (1845) divides the genus into four subgenera: *Gisopteris* (palmate venation at segment base); *Ugena* (dichotomous venation at segment base); *Arthrolygodes* (pinnate venation; *L. articulatum* only); and *Eulygodium* (pinnate veins) containing two sections, *Articulata* (articulate species) and *Continua* (species without articulation). Hooker and Baker (1874) consider two subgenera: *Eulygodium* (free veins) which is further divided into palmate and pinnate forms; and *Hydroglossum* (net-veined). Prantl's treatment (1881) returns the delineation among sections to venation pattern at the base of the segment: *Volubilia* (pinnate; segments rarely laciniate); *Flexuosa* (pinnate; segments laciniate or pinnate); and *Palmata* (costa repeatedly dichotomous). Prantl did not divide the genus into subgenera. Reed (1946) organizes the genus into three subgenera: *Gisopteris* (repeatedly dichotomous) with Sections *Eugisopteris* (sterile segments with pedate costa, fertile segments with pinnate costa), and *Arthrolygodes* (sterile and fertile segments dichotomous); *Eulygodium* (pinnate); and *Odontopteris* (pinnate without basal cutting). Finally, Duek (1978) follows the subgroupings of Prantl for the New World species of *Lygodium*. In both Prantl's and Reed's treatments, spore characters are used to distinguish species within the subgroups. Recognizing the

ambiguity of subgeneric categories in *Lygodium*, Copeland states in Genera Filicum (1947, p. 24) "...the genus is natural and so homogeneous that there has been no agreement as to its division into natural groups."

Both Prantl (1881) and Reed (1946) present "ancestral lineages" in order to place their subgeneric systems into a phyletic sequence. Prantl (1881) suggests two independent radiations: one from an original *palmatum*-like ancestor (*L. articulatum*) and the second from a *japonicum*-like ancestor with parallel development proceeding throughout the world. In this heirarchy, the simple segment taxa (e.g., *L. volubile, L. microphyllum, L. salicfolium*) are the most derived, evolving from a *venustum* -like ancestor in the New World and a *flexuosum*-like ancestor in the Old World. The ancestral *L. articulatum* gave rise to *L. radiatum* and *L. palmatum* in the New World and to *L. circinnatum* in the Old World. *Lygodium circinnatum* is then purported to be the ancestor of *L. flexuosum*. Prantl did not indicate any connection between the *L. articulatum* ancestor and the *L. japonicum* ancestor. It is probable that Prantl was not suggesting that *Lygodium* was polyphyletic, but that a common ancestral taxon was not easily inferred.

It is interesting that paleobotanists hypothesize a *L. palmatum*-like ancestor for the genus that had highly dissected fertile blades and palmate segments (Jennings and Eggert, 1977) with the pinnately branched, linear-lanceolate segments more derived (e.g., *L. microphyllum*). My analysis implies that the basal *Lygodium* was highly dimorphic and dichotomously branching.

Reed (1946) suggests that the ancestors of *Lygodium* had pinnate segments with large verrucate spores whose verrucae often fused to form reticulations. The taxa with palmate segments and scabrate to granulate spores evolved later. Thus, Prantl's and Reed's ideas are somewhat in opposition to each other.

Table 9.1. A comparison of the subgeneric classification of *Lygodium* by Prantl (1881), Reed* (1946), Presl (1845) and Hooker and Baker (1874).

	Prantl	**Reed**
Section I: Palmata	*L. palmatum*	Subgenus Gisopteris
	L. articulatum	Section 1. Eugisopteris
	L. circinatum	*L. palmatum*
	L. digitatum	Section 2. Arthrolygodes
	L. radiatum	*L. articulatum*
	L. trifurcatum	*L. trifurcatum*
		L. circinnatum
		L. versteegii
		L. digitatum
		L. merrillii
		L. matthewii
		L. semihastatum
		L. moskowskii
		L. dimorphum
		L. derivatum
		L. basilanicum
		L. hians
		L. mearnsii
		L. borneense
		L. teysmannii
		L. radiatum
Section II: Flexuosa	*L. japonicum*	Subgenus Eulygodium
	L. subalatum	*L. flexuosum*
	L. mexicanum	*L. colaniae*
	L. venustum	*L. japonicum*
	L. flexuosum	*L. subareolatum*
	L. cubense	*L. conforme*
	L. heterodoxum	*L. brycei*
		L. kerstenii
		L. mexicanum
		L. venustum
		L. cubense
		L. heterodoxum
		L. polymorphum
		L. oligostachyum
		L. pedicellatum

Table 9.1 (continued).

	Prantl (continued)	**Reed** (continued)
Section III: Volubilia	*L. volubile*	Subgenus Odontopteris
	L. wrightii	*L. volubile*
	L. micans	*L. micans*
	L. salicifolium	*L. salicifolium*
	L. smithianum	*L. smithianum*
	L. lanceolatum	*L. boivini*
	L. scandens	*L. lanceolatum*
	L. reticulatum	*L. kingii*
	L. pinnitifidum	*L. scandens*
		L. reticulatum

Hooker and Baker	**Presl**
Subgenus Eulygodium	Subgenus Gisopteris
L. palmatum	*L. palmatum*
L. dichotomum	
L. digitatum	Subgenus Ugena
L. semihastatum	*L. circinnatum*
L. trifurcatum	*L. dichotomum*
L. scandens	*L. heterophyllum*
L. volubile	*L. flexuosum*
L. pinnatifidum	*L. semihastatum*
L. polystachyum	
L. subalatum	Subgenus Arthrolygodes
L. japonicum	*L. articulatum*
Subgenus Hydroglossum	Subgenus Eulygodium
L. heterodoxum	Section Articulata
L. lanceolatum	*L. scandens*
L. reticulatum	*L. salicifolium*
	L. semibipinnatum
	L. poeppigianum
	L. volubile
	L. hastatum
	L. venustum
	L. cubense
	L. pohlianum
	Section Continua
	L. meyenianum
	L. pinnatifidum
	L. maculatum
	L. serrulatum
	L. rottlerianum
	L. tenue
	L. japonicum
	L. mexicanum
	L. schiedeanum
	L. commutatum

* the fossil types of Reed's classification have been omitted.

Along with the above phyletic hypotheses, it is also possible to envision the basal *Lygodium* as having pinnately arranged segments and sporangia borne abaxially on the margins or close to the margins (as in *L. polystachyum*). This form then diverged into two lines: one in which the lamina surrounding the veins diminished (as in *L. palmatum*) and the other in which sporangia were produced on outgrowths of the margin (sorophores, as in *L. volubile).* It could also be hypothesized that the basal*Lygodium* was similar to the pinnately branched *L. flexuosum* or *L. japonicum*, in which a suppression of the segment's basal lobes resulted in an entire segment group whereas an increase in size of the basal lobes gave rise to the palmate group.

Conventional assignment of character polarity is problematic: many morphological characters considered derived in ferns by classical phylogenetic hypotheses (e.g., Wagner, 1973) could be pleisiomorphic in *Lygodium* (e.g., articulation, reticulate spores, reticulate veins). A preliminary analysis of the phytochemical data did not produce informative species alliances except to suggest a close relationship between *L. palmatum* and *L. radiatum,* and between *L. japonicum* and *L. venustum.* Spore morphology data are difficult to interpret from a phyletic standpoint as heavy ornamenation patterns may be the more basal form in *Lygodium*, but whether overall pattern (reticulate, tuberculate, long-ridged verrucate) is the most basal is speculative.

In order to analyze all the data objectively, a cladistic study was performed with *Anemia* chosen as the outgroup. The choice of *Anemia* as the sister-group to *Lygodium* is well-documented in the literature (refer to Introduction). Recent morphological cladistic analyses by Stevenson and Loconte (1996) consider the Schizaeaceae a monophyletic group in the order

Pilulariales. *Lygodium*, as the first branch, is segregated in the subfamily Lygodioideae, while the *Anemia-Mohria* clade is placed in the subfamily Schizaeoideae, tribe Anemineae. Hasebe and co-workers (1994, 1995) in an rbcL gene analysis of fern genera also recognize the *Lygodium, Anemia, Actinostachys* group as monophyletic. However, they found that the differences in the estimated numbers of synonymous nucleotide substitutions per nucleotide site (Ks) among the genera of the Schizaeaceae were greater than the average intergeneric Ks value and higher than the average interfamilial Ks value for ferns. Therefore, these authors concluded that the members of the Schizaeaceae *s.l.* probably diverged from each other at an earlier time than did most families and genera of ferns. This hypothesis is supported by the long evolutionary history and the morphological divergence among the genera. As a result, the authors suggest the genera be treated as separate families as has been accepted by some botanists (e.g., Reed, 1946; Pichi Sermolli, 1959; Naumann, 1993). In their combined morphological and molecular analysis, Pryor and co-workers (1995) found strong support for the clade *Anemia, Actinostachys and Lygodium.* Whether *Lygodium, Anemia/Mohria*, and *Schizaea/Actinostachys* are raised to family status or instead treated as diverse members of the Schizaeaceae *s.l.*, the choice of *Anemia* as the outgroup in this cladistic analysis is well-supported.

Methods

The 26 species of *Lygodium* were scored for 42 characters (Table 9.2) including 111 character-states (Table 9.3). Seventeen characters had more than 2 states: these characters were designated as unordered (Maddison and Maddison, 1992, p. 79). The scorings were based on the morphological observations and phytochemical data obtained in this study and the direct observations of Dr. John Mickel for the outgroup, *Anemia.* Matrices were run

Table 9.2. Taxa in the cladistic data matrix and their character state assignments. Characters coded with letters are polymorphisms[1]. A question mark represents missing data; a dash non-applicable data.

	Character State Values				
	10	20	30	40	
Taxon	|	|	|	|	
1. *Anemia*	0011000-21	0a020b01a0	2c0d01a000	a0????3110	00
2. *L. articulatum*	1100122010	1212100001	2321110000	10?????000	10
3. *L. auriculatum*	1100012211	0122011000	0122001110	11?????020	10
4. *L. borneense*	1100011210	0122000011	0121100000	00?????010	10
5. *L. circinnatum*	1100012011	0122020002	e121200000	0011111021	00
6. *L. cubense*	1100111111	1212101000	0222211110	11?????000	10
7. *L. flexuosum*	1100011021	0121001110	0e11110001	1000000110	01
8. *L. heterodoxum*	1100012010	0111020011	0121200000	111111?010	00
9. *L. hians*	1100122010	1212100001	2125010010	10?????000	10
10. *L. japonicum*	1100021021	0221030120	1314010001	00????1100	01
11. *L. kerstenii*	1100021021	0211030020	0214010000	00?????101	10
12. *L. kingii*	1100011221	1101100010	0112001110	11?????020	00
13. *L. lanceolatum*	1100?11221	1121100002	0121200001	10?????010	00
14. *L. longifolium*	1100011211	0122020010	1122201110	11????0010	00
15. *L. merrillii*	1100?11021	0111020010	0122001170	11?????011	10
16. *L. microphyllum*	1100121020	0110100000	0123110000	0001012011	00
17. *L. oligostachyum*	1100011011	1322031020	1312011111	10?????010	00
18. *L. palmatum*	1100122001	0002020101	2314010001	0001002000	10
19. *L. polystachyum*	1100011021	0101140120	a111000001	01?????110	00
20. *L. radiatum*	1100012100	0002020011	0021000001	110101?021	01
21. *L. reticulatum*	1100021221	1110100010	0123110000	1011002010	10
22. *L. salicifolium*	1100011221	1110100000	0121000000	0011110010	10
23. *L. smithianum*	1100011021	1111000011	0122011110	100011?021	00
24. *L. trifurcatum*	1100012211	0122010011	2322001111	01????0020	00
25. *L. venustum*	1100021021	0111030120	0211010001	1010101100	01
26. *L. versteegii*	1100?11010	0122000101	2113011000	10?????000	00
27. *L. volubile*	1100011121	1111100111	0122001111	11110011e0	00

[1]Character states for polymorphisms: a = 0,1; b = 0,4; c = 2,3; d = 0,3; e = 1,2.

Table 9.3. Characters and character states used in the cladistic analysis of *Lygodium.*

Character	Character States	
1. Rachis growth	0: determinate	1: indeterminate
2. Indusium	0: absent	1: present
3. Stele type	0: medullate protostele	1: siphonostele
4. Gametophyte hairs	0: absent	1: present
5. Rhizome	0: short creeping	1: long creeping
6. Pinna-stalk length(mm)	0: absent 2: over 2.5	1: 0.5 to 2.5
7. Pinna-bud	0: absent 2: recessed	1: prominent
8. Pinna-bud hairs	0: polycellular absent 2: polycellular present	1: bilayered
9. Branching pattern of primary pinna branches	0: not branched 2: pinnate branched	1: dichotomous branched
10. Hairs on primary pinnna branches	0: glabrous (absent)	1: pubescent (present)
11. Pulvinus at pinna-branch and petiole junction	0: absent	1: present (swollen)
12. Sterile pinna-branches times pinnate	0: simple 2: twice pinnate	1: once pinnate 3: 3 or more pinnate
13. Axis of segments	0: terete to angular 2: narrowly winged	1: grooved
14. Segment petiole length	0: length same proximal-distal 1: length decreases proximal -distal 2: not applicable	
15. Segment articulation	0: absent	1: articulate

Table 9.3. continued.

Character	Character States	
16. Sterile segment shape	0: simple 2: palmate 4: pinnatifid	1: bifid 3: subpalmate
17. Segment base symmetry	0: symmetrical	1: asymmetrical
18. Lamina indument	0: absent	1: present
19. Segment margin	0: entire to crenate 2: lobed (some toothed)	1: serrate to dentate
20. Segment margin layers	0: 1-2 rows 2: over 5 rows	1: 3-4 rows
21. Fertile/Sterile segments	0: monomorphic 2: dimorphic	1: semimorphic
22. Fertile pinna-branches times pinnate	0: absent 2: twice	1: once 3: three or more
23. Indusia indument	0: not applicable 2: glabrous	1: pubescent
24. Spore macro-ornamentation pattern	0: striate 2: verrucate 4: granulate	1: tuberculate 3: reticulate 5: mixed
25. Laesura prominent or obscure	0: prominent 2: visible not raised	1: obscure
26. Laesura length	0: full length	1: less than radius
27. Macro-ornamentation on spore faces	0: distal=proximal	1: distal not=proximal
28. Proximal equatorial ridge	0: absent	1: present
29. Micro-ornamtation pattern	0: absent	1: present

Table 9.3 (continued).

Character	Character States	
30. Extra perisporal globules or sphericles	0: absent	1: present
31. Mean spore size (μm)	0: 80 or less	1: more than 80
32. Laesura ending in a widened triangular area	0: absent	1: present
33. Compound C6	0: absent	1: present
34. Compound D3	0: absent	1: present
35. Compound D4	0: absent	1: present
36. Compound E1	0: absent	1: present
37. Base chromosome number	0: x=28 2: x=30	1: x=29 3: x=38
38. Indument on segment veins	0: glabrous to slightly pubescent	1: pubescent
39. Vein angle from costa	0: less 30 degrees 2: over 50	1: 31-50 degrees
40. Clavate hairs on lamina	0: sparse	1: abundant
41. Veins ending	0: at margin	1: before margin
42. Margin prominently toothed	0: absent	1: present

both with and without phytochemical data. The final matrix included 27 sporophytic characters, 9 spore characters, 4 phytochemical characters, 1 gametophytic character and 1 cytological character (Table 9.4). Parsimony analyses were conducted on a Macintosh Power PC 6500 300 Mhz with compatible versions of MacClade 3.07 (Maddison and Maddison, 1992) and PAUP 3.1.1 (Swofford, 1993). Multistate characters were nonadditive and equally weighted at 1. Heuristic searches were conducted using TBR branch swapping with MULTIPARS (random addition sequence; 1000 replicates). Due to the large number of potential trees, exhaustive searches are likely to be impractical for data sets with more than 11 taxa (Forey *et. al.*, 1992). Branch and bound methods can be applied to data sets of up to about 25 taxa (Forey, *et. al.*, 1992). Since the data set used for *Lygodium* contained 27 taxa (including the outgroup, *Anemia*) and 42 characters, all searches for minimal trees were heuristic. While these searches may not find the optimal tree, they are the most practical methods given a large data set. Both stepwise addition sequences and branch swapping improve the utility of heuristic methods and were applied here.

Results and Discussion

Parsimony analysis resulted in a set of 4 trees at 217 steps [Consistency Index (CI) = 0.355, Retention Index (RI) = 0.525; Figs. 9.1-9.2)]. Polytomies occurred in a small part of each of two of the three major clades. The most basal node for *Lygodium* results in a dichotomy: one branch leading to the clade containing *L. flexuosum, L. japonicum, L. kerstenii and L. venustum* and the other branch leading to a further dichotomy. Here one branch leads to the terminal taxon, *L. polystachyum* and the other to a node which dichotomizes to produce two clades: one containing *L. articulatum-L. lanceolatum* and the second *L. auriculatum-L. volubile* (Fig 9.3). Thus, *Lygodium* is divided into

Table 9.4. Character status information for the strict consensus tree. All characters were unordered and had an equal weight of one. CI=Consistency index, RI=Retention index, and RC=Rescaled consistency index (Maddison and Maddison, 1992). See Table 9.3 for complete character names.

Character		States	CI	RI	RC
1.	rachis growth	2	1.00	0.0	0.0
2.	indusium	2	1.00	0.0	0.0
3.	stele type	2	1.00	0.0	0.0
4.	gametophyte hairs	2	1.00	0.0	0.0
5.	rhizome	2	0.50	0.75	0.38
6.	pinna-stalk length	3	0.50	0.71	0.36
7.	pinna-bud	3	0.40	0.57	0.23
8.	pinna-bud hairs	3	0.29	0.44	0.13
9.	branch patt.pri.pin.br	3	0.50	0.82	0.41
10.	indument pri.pin.br.	2	0.25	0.50	0.13
11.	pulvinus pin.br.pet.ju.	2	0.25	0.67	0.17
12.	ster.pin.br.times.pin.	4	0.57	0.40	0.23
13.	axis segments	3	0.17	0.23	0.04
14.	segment pet.length	3	0.40	0.75	0.30
15.	segment articulation	2	0.25	0.67	0.17
16.	sterile segment shape	5	0.63	0.67	0.42
17.	segment base symet.	2	0.33	0.33	0.11
18.	lamina indument	2	0.25	0.57	0.14
19.	segment margin	3	0.33	0.54	0.18
20.	segment margin layers	3	0.29	0.50	0.14
21.	fert/ster segments	3	0.44	0.38	0.17
22.	fert.sec.pin.br.pin.	4	0.50	0.29	0.14
23.	indusia indument	3	0.40	0.63	0.25
24.	spore macro orn.	6	0.56	0.69	0.38
25.	laesura prom/ob	3	0.25	0.25	0.06
26.	laesura length	2	0.33	0.83	0.28
27.	macro-orn. faces	2	0.50	0.78	0.39
28.	prox.equat.ridge	2	0.50	0.88	0.44
29.	micro-orn.pattern	2	0.33	0.75	0.25
30.	ex.perisp.globs/sph.	2	0.13	0.22	0.03
31.	mean spore size	2	0.22	0.13	0.03
32.	laesura ends wide/tri.	2	0.17	0.44	0.07
33.	Compound C6	2	0.25	0.25	0.06
34.	Compound D3	2	0.50	0.50	0.25
35.	Compound D4	2	0.25	0.25	0.06
36.	Compound E1	2	0.33	0.50	0.17
37.	base chrom. number	4	0.60	0.60	0.36
38.	indum. on seg. veins	2	0.50	0.83	0.42
39.	vein angle from costa	3	0.38	0.58	0.22
40.	clavate hairs lamina	2	0.25	0.40	0.10
41.	vein ending	2	0.13	0.22	0.03
42.	papillate margins	2	0.33	0.33	0.11

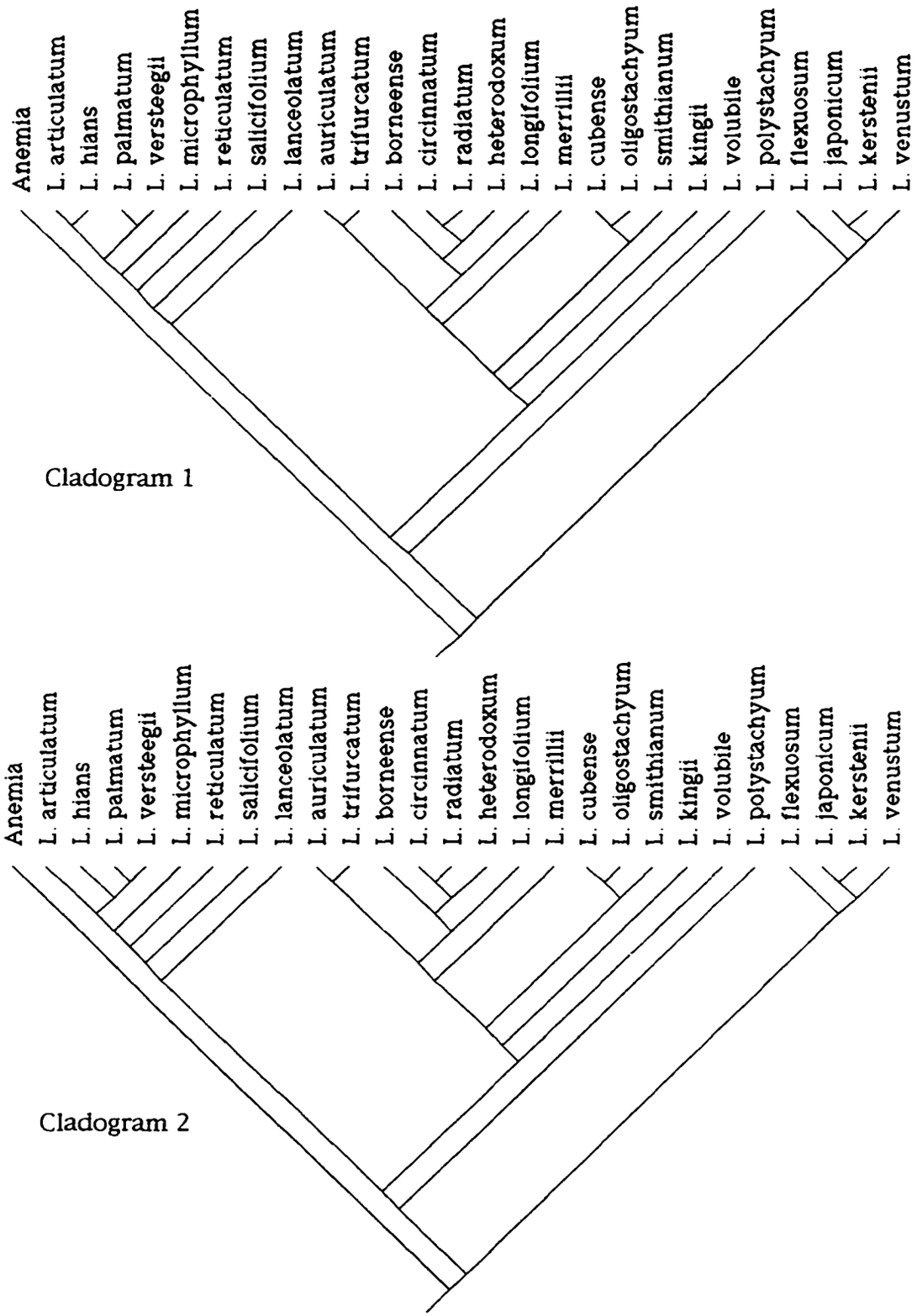


Figure 9.1. Cladograms one and two from a cladistic analysis of *Lygodium*.

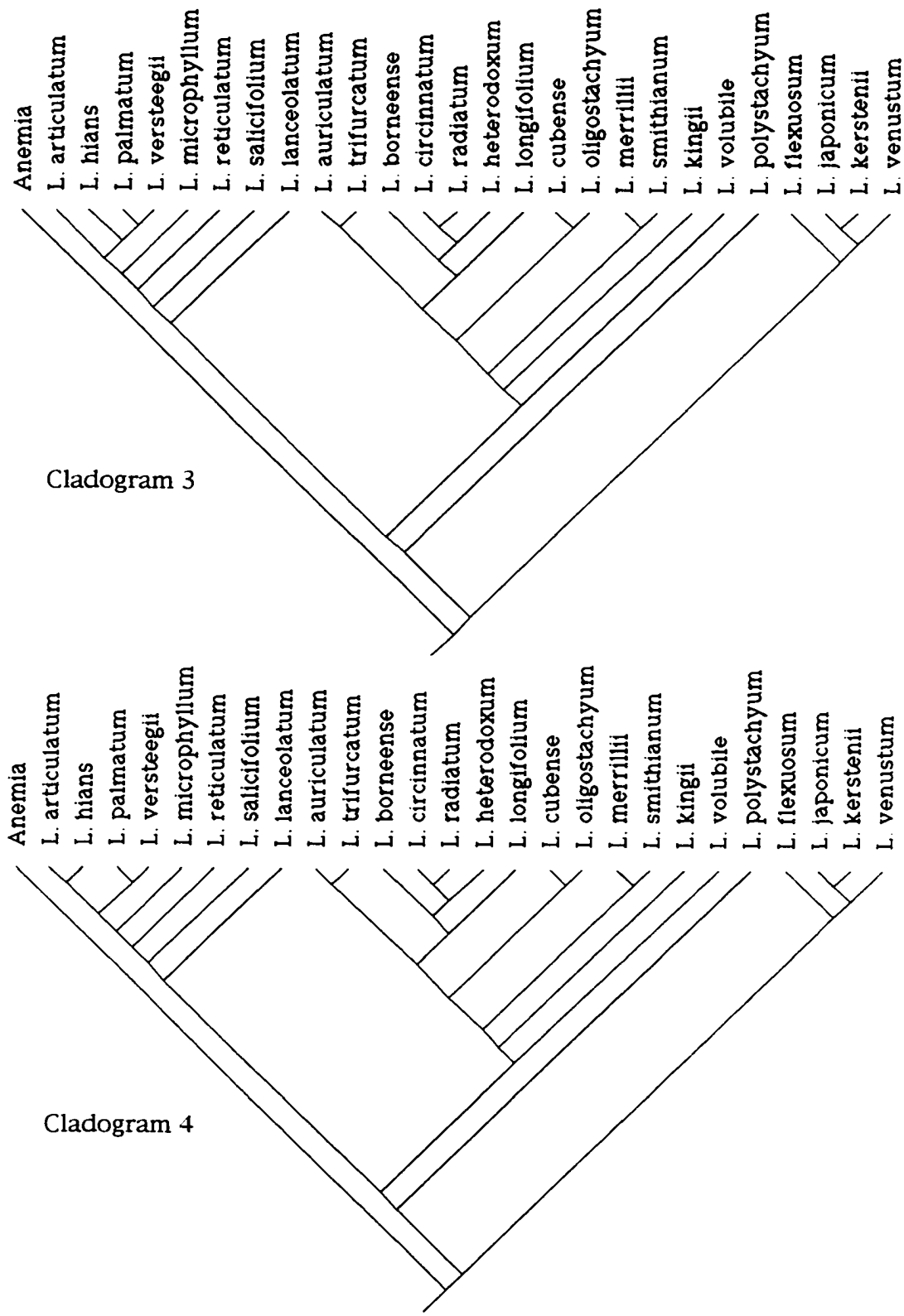


Figure 9.2. Cladograms three and four from a cladistic analysis of *Lygodium*.

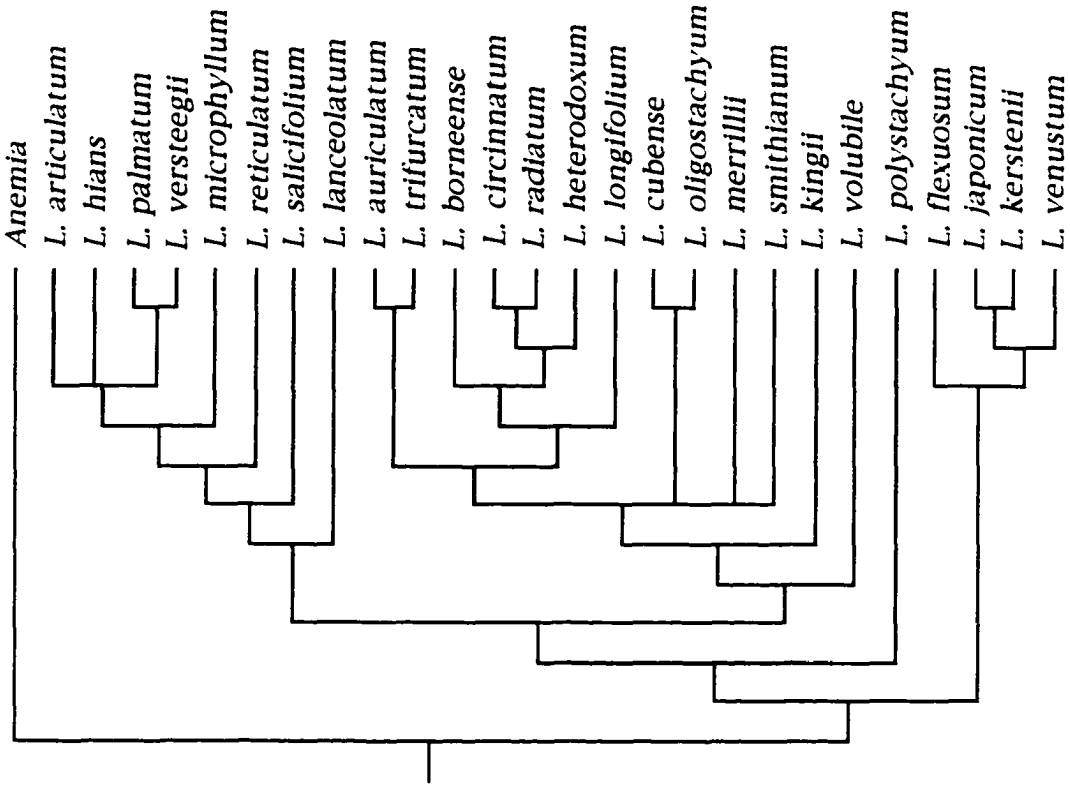


Figure 9.3. The strict consensus tree from the cladistic analysis of *Lygodium*.

three sections: the most basal being the *L. flexuosum* complex with the next basal branch supporting *L. polystachyum* (lying between the *L. flexuosum* subgroup and the rest of the taxa); and a dichotomy producing two monophyletic groups, *L. articulatum-L. lanceolatum* and *L. auriculatum-L. volubile*. These latter clades do not readily conform to the subgeneric schemes presented by Prantl (1881), Reed (1946) or Duek (1978). However, that the ancestral *Lygodium* species are from the "*japonicum* group" is partially supported by the literature (Prantl, 1881). The placement of *L. polystachyum* as the first branch after the "*japonicum* complex" is interesting as it is the only species that exhibits the pinnate branching pattern with pinnatifid segments and in some specimens has sporangia borne on the abaxial margins of the pinnatifid lobules (possible ancestral sorophores).

The clade *L. flexuosum-L. venustum* is supported by only one character - the presence of marginal teeth. Autapomorphies for *L. flexuosum* (the first branch in this clade) are asymmetrical segment base from symmetrical, obscure laesura (from prominent) and the presence of compound C6. The synapomorphies for the *L. japonicum-L. venustum* clade are the long pinna-stalk, subpalmate segment shape, lobed margins, < 30° vein angle from the costa and n=29.

The synapomorphies for the node which supports a dichotomy leading to *L. polystachyum* and to the basal branch for all the other taxa (Fig. 9.3) are segment articulation, fertile pinna-branch once pinnate (from bipinnate) and laesura extending the full length of the radius (from less than the full length). Autapomorphies for *L. polystachyum* include pinnatifid segment shape with lobed margins and < 80 μm mean spore size. This species is one of the most distinct taxa in the genus. The next node produces two branches leading to the monophyletic clades *L. auriculatum-L. volubile* and *L.*

articulatum-L. lanceolatum. This dichotomy is supported by the presence of pulvini at the petiole/pinna-branch junction and glabrous indusia (change in state from pubescent indusia). The former character then undergoes a reversal (loss of pulvini) to support the *L. auriculatum-L. longifolium* group in the *L. auriculatum-L. volubile* clade. The synapomorphies for the *L. auriculatum-L. volubile* clade are verrucate spores, differing macro-ornamentation patterns on proximal and distal faces, presence of a proximal equatorial ridge, and presence of micro-ornamention pattern. These four spore characters undergo reversals higher up in the clade to define the group *L. borneense-L. heterodoxum.*

The branch leading to the *L. articulatum-L. lanceolatum* clade is supported by only one character: a change in segment margin shape from serrate to entire. This character state undergoes a reversal back to serrate margins only once, as an autapomorphy for *L. reticulatum.* The strict consensus tree phylogram in Fig. 9.4 shows the character changes discussed above.

The length of the pinna-stalk, a very stable, unambiguous character morphologically and important taxonomically, supports the *L. japonicum-L. venustum* clade (> 2.5 mm) and the *L. articulatum-L. reticulatum* clade (> 2.5 mm; with a reversal in *L. versteegii*). The branching pattern of the pinna-branch is another taxonomically useful character. The change from the pleisiomorphic pinnate branching to the apomorphic dichotomous branching supports the *L. articulatum-L. versteegii* group within the *L. articulatum-L. lanceolatum* clade. The same change to dichotomous branching represents a synapomorphy for the *L. auriculatum-L. oligostachyum* group in trees 3 and 4 (Fig. 9.2, 9.5), and for the *L. auriculatum-L. longifolium* clade in trees 1 and 2 with subsequent parallel evolution in *L. cubense-L. oligostachyum* (Fig. 9.1).

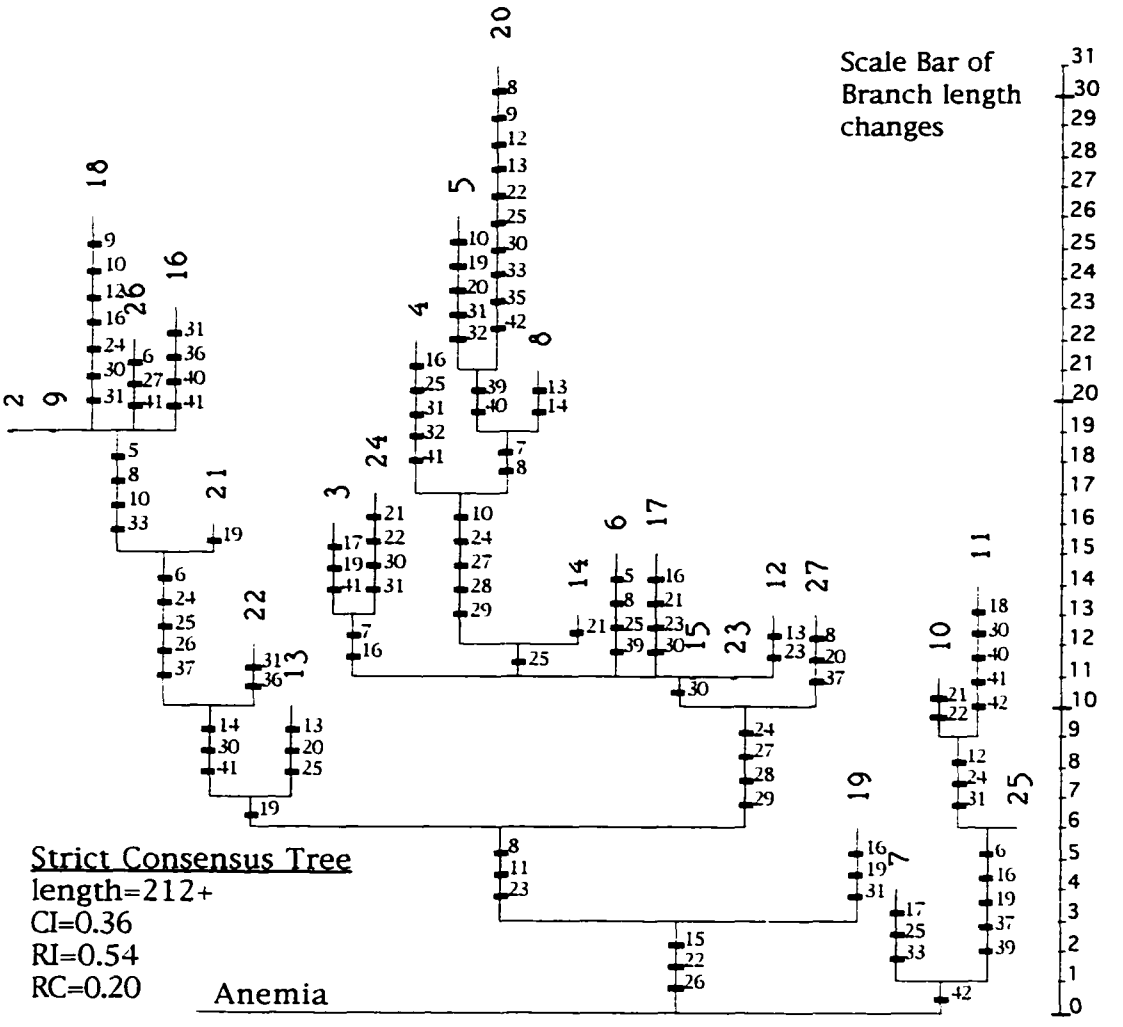


Figure 9.4. The phylogram for the strict consensus tree from the cladistic analysis of *Lygodium*. The taxa are numbered at the end of the branches. A list of the taxa by number appears in Table 9.2. Branch lengths are proportional to the number of unambiguous changes that occur along each branch. Their location and the character numbers changing are on each branch, except where polytomies occur. No changes are shown on these branches. The relative number of changes is indicated in the scale bar on the right of the figure.

These two topologies resolve the polytomy in that group. The former is preferable as dichotomous branching only evolves once in that clade (Fig. 9.2, tree 3, Fig. 9.5).

The following eight characters did not undergo reversals in any of the four trees and, therefore, represent the least ambiguous evolutionary pathway (the character number appears in parentheses): rhizome short- or long-creeping (6), branching pattern of pinna-branch (9), segment base symmetry (17), margins thickened or not (20), mono- or dimorphism (21), spore size (31), vein angle from costa (39), and numbers of clavate hairs on lamina (40). The remainder of the characters, excluding those for the chemical compounds, chromosome numbers, and outgroup analysis, all resulted in reversals (22 of the 42 characters; Table 9.5). Segment shape, pinna-bud hairs, sterile pinna-branch times pinnate, winged segment axes, lamina indument, and segment vein indument are all ambiguous at some of the basal nodes. This ambiguity may be the result of polymorphisms.

Data for the chemical and chromosome number characters are incomplete for at least half of the taxa. Therefore, these characters cannot be traced on individual trees and evaluated in the same manner as the other characters.

Homoplasies are frequent. As can be inferred from the cladogram (Fig. 9.3) reticulate veins have arisen four times in *Lygodium*: as autapomorphies in *L. versteegii*, *L. heterodoxum* and *L. merrillii*, and as a synapomorphy in the *L. lanceolatum-L. reticulatum* clade. The character was removed from the data matrix because of an RI=0.0; if included in the final matrix the trees do not change. Morphological observations on fern genera suggest that reticuate venation has arisen many times (Wagner, 1979). Reticulate spores, on the other hand, also present in many diverse fern groups, evolved only once in

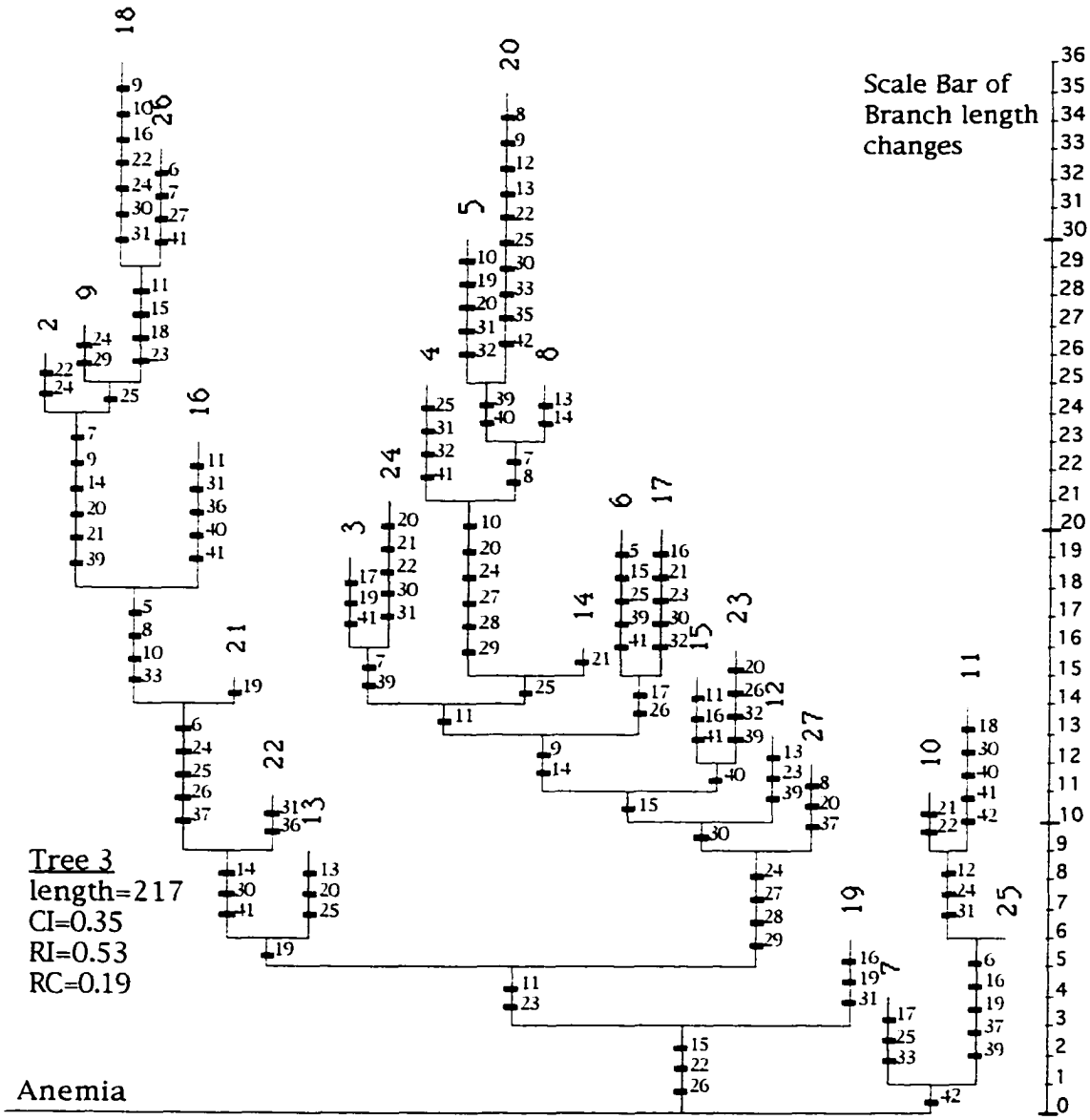


Figure 9.5. The phylogram for tree number three from the cladistic analysis of *Lygodium*. The taxa are numbered at the end of the branches. A list of the taxa by number appears in Table 9.2. Branch lengths are proportional to the number of unambiguous changes that occur along each branch. Their location and the character numbers changing are on each branch. The relative number of these changes is indicated in the scale bar on the right of the figure.

Table 9.5. Characters undergoing reversals in at least two of the four trees (the character number and direction of change is given in parentheses corresponding to Table 9.3; Figs. 9.1-9.5).

1) pinna-stalk length (6) - from long to short pinna-stalk in *L. versteegii* (2→1);

2) pinna-bud (7)- from recessed to prominent pinna-bud in *L. versteegii* (2→1);

3) pinna-bud hairs (8) - a reversal in *L. articulatum-L. microphyllum* branch and *L. circinnatum-L. heterodoxum* branch to the absence of swollen, multicellular-based bud hairs (2→0) - this character has ambiguous internodes;

4) indument on pinna-branch (10) - from glabrous apomorphic state to pleisiomorphic pubescence in *L. palmatum* and *L. circinnatum* (0→1);

5) pulvinus (11) - a reversal to the absence of pulvini in the *L. palmatum-L. versteegii* clade and *L. microphyllum*, and *L. auriculatum-L. merrillii* branch (1→ 0);

6) winged segment axes (13) - in *L. heterodoxum* from the winged to grooved state (2→1); this character is ambiguous at the basal nodes;

7) segment petiole length (14) - in *L. heterodoxum* from petioles absent to petiole length decreasing distally (2→1);

8) articulation (15) - in *L. palmatum-L. versteegii* clade and *L. auriculatum-L. smithianum* group and *L. cubense* from articulate to not articulate (1→0);

9) lamina indument (18) - in *L. palmatum-L. versteegii* clade from absent to present (0→1);

10) margin shape (19) - in *L. reticulatum* from entire to serrate (0→1);

Table 9.5 (continued).

11) fertile pinna-branch times pinnate (22) - in two of the trees (3 and 4) *L. cubense* undergoes reversal from once to twice pinnate (1→2); this character has ambiguous basal nodes;

12) indument on indusium (23) - in *L. palmatum-L.versteegii* clade, *L. kingii*, and *L. cubense*, from glabrous to pubescent (2→1);

13) spore macro-ornamentation pattern (24) - in the clade *L. borneense-L. heterodoxum* from verrucate to tuberculate (2→1);

14) laesura prominent or obscure (25) - in *L. radiatum* from barely visible to prominent (2→0) and in *L. hians, L. palmatum,* and *L. versteegii* (e.g. trees 2 and 3) from obscure to prominent (1→0);

15) laesura length (26) - in *L. articulatum-L. reticulatum* branch, and either *L. oligostachyum-L. cubense* clade and *L. smithianum* (trees 3 and 4) or *L. oligostachyum-L. smithianum* clade (trees 1 and 2) from extending the full length of the radius to less than the radius (0→1);

16) macro-ornamentation pattern distal and proximal faces (27) - in *L. borneense-L. heterodoxum* branch from not the same on both faces to equal patterns on both faces (1→0);

17) proximal equatorial ridge (28) - reversal as for previous character from presence to absence (1→0);

18) micro-ornamentation (29) - reversal as for previous character from presence to absence (1→0);

19) extra-perisporal spherules (30) - in terminal taxa, *L. palmatum, L. trifurcatum, L. radiatum*, and *L. oligostachyum* from absent to present (0→1);

20) laesura widening at equator into a triangular area (32) - in *L. smithianum, L. oligostachyum, L. borneense* and *L. circinnatum* from presence to absence (1→0);

Table 9.5 (continued).

21) veins ending at or before margin (41) - in *L. microphyllum* and *L. versteegii* from ending before margin to at margin (1→0); and

22) marginal teeth (42) - in *L. kerstenii* from present to absent (1→0).

Lygodium, in the *L. reticulatum, L. micropyllum, L. versteegii* clade from a tuberculate spore ancestor.

A change from the basal entire segment shape (based on outgroup analysis with *Anemia*) into the subpalmate form supports the node in the *L. japonicum-L.venustum* clade. Changes in segment shape from entire to palmate show numerous parallelisms. Palmate segments arose three times, in *L. palmatum, L. circinnatum-L. longifolium*, and *L. merrillii.* Bifid and pinnatifid forms arose only once each (*L. auriculatum-L. trifurcatum* and *L. polystachyum*, respectively), while the subpalmate form arose twice, once in the *L. japonicum-L. venustum* clade and again as an autapomorphy in *L. oligostachyum.* Dichotomous branching arose twice from the ancestral pinnate form, in the *L. articulatum-L. versteegii* clade and again in the *L. auriculatum-L. oligostachyum* clade. Even though branching pattern and segment shape have both been used extensively in the classical treatments of *Lygodium*, this analysis shows that it is difficult to employ either of these characters in assessing species for phylogenetic alliances. The following character states also show parallelisms and, therefore, are problematic phyletic determinants (number in parentheses indicates the number of times the character state independently appears in the cladogram): short to long-branching (2); pinna-stalk length from short to long (2); pinna-bud prominent to obscure (3); indument on pinna-branch from present to none (2); number of times pinnate from the pinna-branch from bi- to tripinnate (3 times); presence of narrowly winged axes from grooved or terete axes (4); segment base symmetrical to asymmetrical (3); lamina indument from present to none (2); margins serrate to lobed (2) and serrate to entire (3); thick margins from 1-2 rowed margins (2); fertile pinna-branch times pinnate from 1-2 to 3 times pinnate (2); obscure to prominent laesura (3); spore micro-

ornamentation pattern from absent to present (2); change in spore size from large to small (7); change in vein angle from costa 31°-50° to <30° or >50° (3 and 4 times, respectively); increase in the number of clavate hairs (4) and presence of marginal teeth (2).

Spore macro-ornamentation pattern exhibited few reversals and no homoplasies except in the evolution of granulate spores. This spore pattern evolved twice from reticulate and tuberculate ancestors (*L. palmatum* and *L. japonicum-L. kerstenii,* respectively). This is, therefore, an apparently good phyletic character in *Lygodium* even though it has been thought that various spore macro-ornamentation patterns probably evolved many times in fern families and genera (refer to Chapter 6). It is probable that those taxa with overlapping morphological characters but with different spore patterns represent examples of parallelism and not common ancestry (e.g., *L. heterodoxum* and *L. merrillii; L. smithianum* and *L. salicifolium*). Concomitantly, those taxa with similar morphological and spore characters found in widely separated geographic areas may represent the same taxa or sister taxa (e.g., *L. volubile* and *L. kingii*). The overall similarity in the tuberculate and verrucate pattern is reflected in the reversal from the verrucate spores present in the *L. auriculatum- L. volubile* clade to tuberculate spores in the subgroup of that clade, *L. borneense- L. heterodoxum.* There occurs a loss of reticulate spores in the *L. articulatum-L. reticulatum* clade to tuberculate in *L articulatum.* All the spore types in *Lygodium,* as discussed in Chpater 6, intergrade morphologically as tubercles fuse into verrucae which fuse into ridges which may anastomose into reticuations (with the reverse processes also possible). The other spore characters that do exhibit parallelisms are all subsets of verrucate spores (e.g., proximal equatorial ridge, micro-ornamentation patterns).

Segment articulation is another character that exhibits no homoplasies and few reversals. This character supports a branch of the cladogram at a very basal node (Fig. 9.4, character 15). Prantl (1881) feels that this character is only useful within subgroups to delineate species but has no phylogenetic significance. The evaluation of the cladograms suggests that articulation is a useful phyletic indicator.

The consensus tree (Fig. 9.3) strongly supports the *L. flexuosum-L. venustum* group as basal. This is in partial agreement with Prantl's (1881) original phylogeny of having two ancestral lines with a *L. japonicum* ancestor independent from the *L. articulatum* ancestor. Prantl (1881), however, places *L. flexuosum* on the *L. articulatum* branch.
Almost all the rest of the species of *Lygodium* are the result of an early dichotomy which results in two clades. *Lygodium polystachyum* is basal to this dichotomy (Fig. 9.3). This species has many characteristics that might predict this basal position. The two resulting clades, *L. articulatum-L. lanceolatum* and *L. auriculatum-L. volubile*, indicate that palmate segments and dichotomous branching evolved from taxa with entire segments and pinnate branching. This view agrees with that predicted by classical morpholgy and character polarity assignments (Wagner, 1973). *Lygodium articulatum* and *L. palmatum* represent highly derived taxa. This again is supported by the morphology: both exhibit the most extreme degree of dimorphism and reduction in fertile blade lamina (dimorphism considered derived; Wagner, 1973). The separation of the pinnately branched, entire segment species into two separate monophyletic clades departs from the classical phylogenies. *Lygodium microphyllum, L. reticulatum, L. salicifolium and L. lanceolatum* are considered distinct from *L. volubile, L. smithianum* and *L. kingii. Lygodium salicifolium* is morpholocially indistinct from *L. kingii* except in spore

pattern. In this cladistic analysis *L. salicifolium* is considered a sister species to *L. lanceolatum* and the ancestor of *L. reticulatum:* all are strongly articulate with pulvini and the former two taxa possess tuberculate spores while the latter has reticulate spores. *Lygodium microphyllum* (a direct descendent of *L. reticulatum*) is the most derived and shares an ancestor with *L. articulatum-L. hians* and *L. palmatum-L. versteegii.* Thus, *L. salcifolium* and *L. kingii* are examples of convergence while *L. volubile* and *L. kingii* become sister taxa. The lineage presented in the cladogram reinforces the theory that *L. smithianum* and *L. volubile* represent the same ancestral line that became geographically isolated (Africa-South America disjuncts). *Lygodium longifolium,* a basal member of the *L. borneense-L. longifolium* clade, is removed from *L. circinnatum* to which it is morphologically similar. These taxa have been synonomized in some treatments (see Chapter 10). In this analysis, *L. circinnatum* shares a more recent ancestor with *L. borneense* and *L. heterodoxum* and is the sister taxon to *L. radiatum* (Fig. 9.3). Another disparity occurs between *L. palmatum* and *L. radiatum.* They are chemically similar and only somewhat similar morphologically. That these two taxa are in different clades entirely indicates a parallel evolutionary pathway of simple branching pattern and palmate segments. The overall phyletic predictions based on the cladistic analysis offer innovative alternatives to existing theories and support some of the ideas presented in this work.

The strict consensus tree contains two areas with polytomies (Fig. 9.3). These polytomies are resolved in the individual trees (Figs. 9.1, 9.2). In one case the polytomy involving *L. articulatum-L. hians* is resolved either by having a common ancestor dichotomize, one dichotomy ending in *L. articulatum* and *L. hians,* the other ending in *L. palmatum* and *L. versteegii* (Fig. 9.1, trees 1 and 2). The second scenario involves a branch leading to L.

articulatum and a clade containing *L. hians-L. versteegii*, from which *L. hians* shares a common branch supporting the terminal taxa, *L. palmatum* and *L. versteegii* (Fig. 9.2; trees 3 and 4) There is no evidence to support one topology over the other. However, *L. hians* morphologically seems to represent a New Caledonian variant of *L. articulatum* which would support the former pathway (Fig. 9.1). The second polytomy occurs in the *L. cubense-L. smithianum* clade. This is resolved with either *L. merrillii* as the ancestral species in the *L. auriculatum-L. merrillii* clade with *L. cubense, L. oligostachyum* and *L. smithianum* sharing a common ancestor (Fig. 9.1; trees 1 and 2) or *L. merrillii* as the sister species to *L. smithianum* and both ancestral to *L. cubense-L. oligostachyum* with *L. longifolium* as the ancestral species in the *L. auriculatum-L. longifolium* clade (Fig. 9.2; trees 3 and 4). *Lygodium merrillii* is a reticulate-veined species with large sterile palmate segments and pinnate fertile segments. The topology that places it as the ancestral species to the *L. auriculatum-L. longifolium* clade (Fig. 9.1; trees 1 and 2) does not seem to be as probable as having it the sister species to *L. smithianum*, although either topology is possible.

The original data matrix contained the occurrence of all 10 chemical compounds isolated from *L. japonicum* in the 11 taxa surveyed. Six of these characters had RI = 0.00 and were removed from the analysis. An low RI is indicative of changes concentrated on branches leading to terminal taxa (e.g., not as synapomorphies on internal nodes). When these six characters were put back in the final data matrix the same four trees were obtained. Therefore, whatever information these six characters were providing was also being supported by other characters and thus, the chemical data for these six characters were not informative. The four chemical compounds that were left in the matrix, when removed from the analysis, also resulted in the same four

trees even though these characters had higher RI values (C6 and D4, RI = 0.25; D3 and E1, RI = 0.50; Table 9.3). In some of the original data matrices some of the polytomies were resolved with chemical data. However, in the final analysis these four phytochemical characters were non-informative in the phyletic analysis of *Lygodium.*

There are existing hypotheses as to character state polarity in ferns (Holttum, 1968; Wagner, 1973). Based on outgroup analysis with *Anemia* the following character states are ancestral in *Lygodium*:

rhizome - short-branching
branching pattern of pinna-branch - pinnate
pinna-branch indument - pubescent
pulvinus - absent
pinna-branch - once pinnate
articulation - absent
sterile segment shape - entire
segment base - symmetrical
segment lamina - pubescent
margins of segments - not thickened
fertile pinna-branch- twice pinnate
clavate hairs - sparse
veins exit costa at - 30^{o}-50^{o} angle
spore characters:
laesura prominent, absence of proximal equatorial ridge, absence of micro-ornamentation, laesura does not end in widened area, laesura length is less than the radius.

The basal condition could not be determined in segment axes (terete, grooved or winged), margin shape, or spore macro-ornamentation pattern due to the presence of polymorphisms in *Anemia.*

The following characters, not applicable in *Anemia* (or, if present, polymorphic), are pleisiomorphic in the remainder of *Lygodium* based on analysis of the strict consensus tree with the *L. flexuosum-L. venustum* as an outgroup:

pinna-stalk - < 2 mm
pinna bud - prominent
swollen, multicellular-based pinna-bud hairs - absent

venation pattern - free
segment petioles decrease distally (as opposed to being of equal length)
monomorphic
indusium pubescent
spores tuberculate
spherules on spores
chromosome base number = 28.

Thus, in the cladistic analysis characters such as articulation, pulvini, marginal teeth, margin shape, and spore pattern have been important in supporting the earliest branches, while the classical characters of branching pattern and segment shape have proven to be difficult to interpret due to parallelisms. This analysis represents a new working hypothesis. There are many areas that require further resolution and characters that need complete information sets.

After assessing many morphological trends in *Lygodium* and reviewing the phyletic lines in ferns and directionality in character states presented in the literature (e.g., Holttum, 1968; Wagner, 1973; Mickel, 1974), it is possible to hypothesize the following ideas on the hypothetical ancestry of this genus. *Lygodium polystachyum* may represent one of the primitive forms of *Lygodium*. It produces tuberculate spores and has no articulation. Reticulate spores and articulation are hypothesized as being derived in ferns (Wagner, 1973). The primary pinna-branches have pinnately arranged segments, and the segments are pinnatifid. Veins are free and pinnate. The sporangia are borne in two rows along veins on the underside of the segment lobules and are covered by indusia. This species is not dimorphic and the more typical sorophores are present on some specimens as laminar projections of the lobules. Sorophores occur in all other species of *Lygodium*, but are reduced in length in those highly dissected taxa where the sporangia occur along the veinlets of segment's lacking lamina (e.g., *L. palmatum, L. articulatum, L.*

trifurcatum). This extreme degree of dimorphism is also considered an advanced character. The trends in the evolution of *Lygodium* involve pinnate to dichotomous branching, articulation, dimorphism, and tuberculate or verrucate spore patterns to reticulate ones. Segment development follows from the pinnatifid segments of *L. polystachyum*, to the subpalamte form of *L. japonicum* and diverges along two lines: one leading to palmate segments, and the other to entire segments via the symmetrical or asymmetrical suppression of basal lobes. In the first scenario (pinnatifid to subpalmate) intermediate stages can be seen in *L. kerstenii* (Fig. 10.34). This species contains specimens that resemble *L. japonicum* and *L. polystachyum.* The second case can be observed in *L. flexuosum* through *L. salicifolium* in which the outer lobules become suppressed to produce an entire segment. Many species of *Lygodium* with entire segments have variants which exhibit small outer auricles. *Lygodium merrillii* and *L. heterodoxum* have fertile segments with two inner bifid lobules and often two smaller outer lobes.

The cladistic analysis supports some of these speculations. It places *L. polystachyum* intermediate between the basal *L. flexuosum* -*L. venustum* clade and the rest of the taxa. It has all segment shapes evolving from an entire form as determined by outgroup analysis with *Anemia*. It places *L. flexuosum* more ancestral than would be predicted from the above discussion. It supports the idea that the highly dimorphic taxa are apomorphic and reticulate spores are more derived than tuberculate spores.

The following subgeneric classification is based on the cladograms produced by the analysis of the characters used in the present study of *Lygodium* (Fig. 9.6). This classification is tentative and not formalized due to the many ambiguities. Collections of *Lygodium* from geographic areas that are

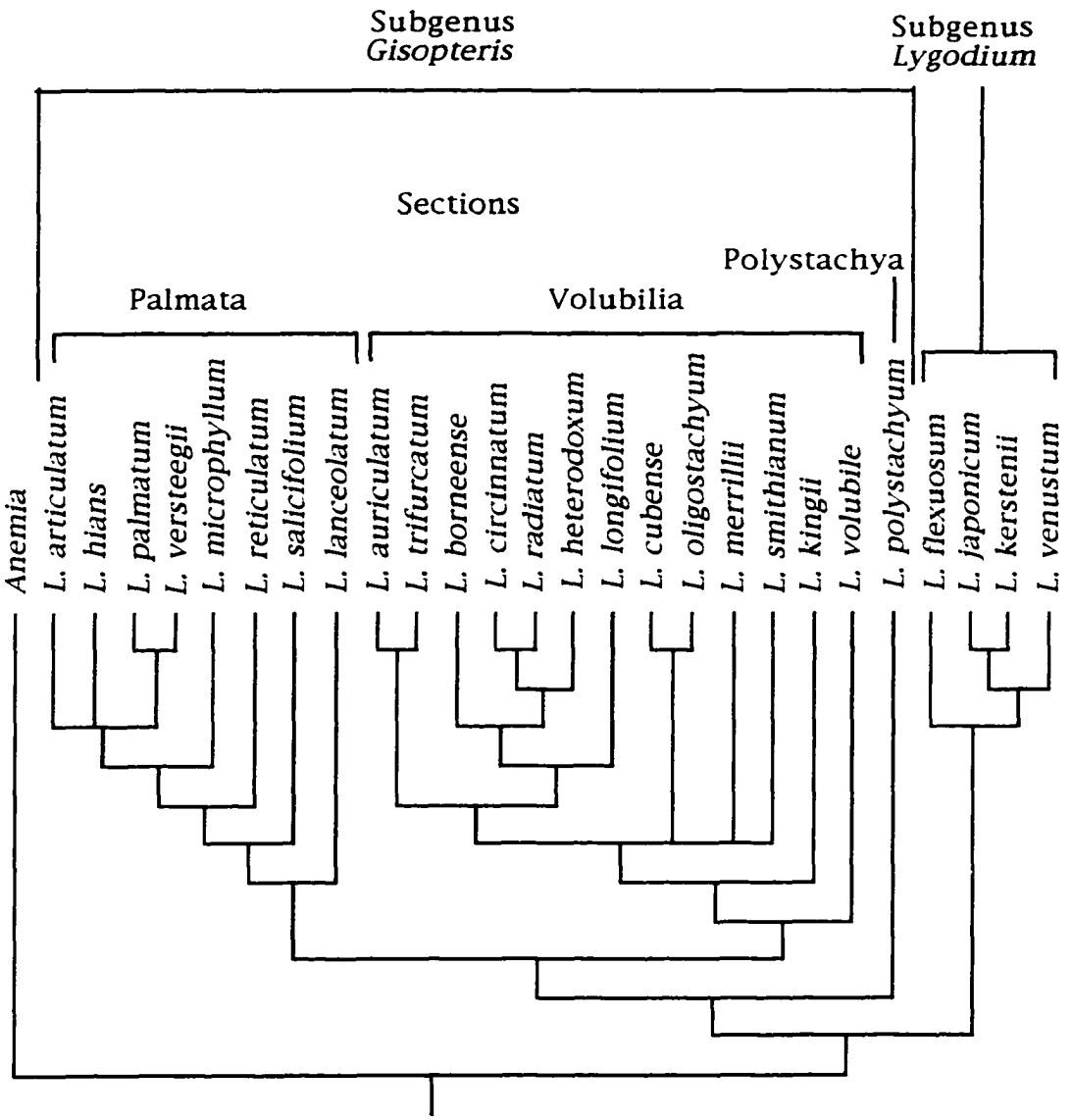


Figure 9.6. The strict consensus tree from the cladistic analysis of *Lygodium* with preliminary subgenera and sections.

at present not well represented will add to the data matrix and help revolve some of the inconsistencies. Chemical and cytological data may prove more informative as data can be obtained from all taxa. As spore characters have been proven useful phylogenetically, a TEM study of the spore layers of all taxa is warranted considering the preliminary results of Tryon and Lugardon (1991; refer to Chapter 6). Additional data will help support or better resolve some of the relationships suggested by this analysis that may be contrary to classical subdivisions. The tentative subgeneric names have been emended from prior names.

Preliminary Classification

Subgenus *Lygodium.* Pinna-branches pinnate to bipinnate, segments with basal auricles (symmetrical or asymmetrical) or subpalmate, margins lobed, lobes often toothed, spores tuberculate to granulate.

L. flexuosum
L. japonicum
L. kerstenii
L. venustum

Subgenus *Gisopteris* (Bernh.) Reed *emend.* Pinna-branches pinnate to dichotomous, segments entire to bifid to palmate, basal auricles present or not, margins, entire to serrate or dentate (rarely toothed) to pinnatifid, articulate or not, spores tuberculate or long-ridged verrucate.

Section Polystachya. Pinna-branches pinnate, segments pinnatifid.

L. polystachyum

Section Volubilia Prantl *emend.* Pinna-branches pinnate to dichotomous, segments entire to bifid to palmate.

L. volubile
L. kingii
L. smithianum
L. merrillii
L. oligostachyum
L. cubense
L. longifolium
L. heterodoxum
L. radiatum
L. circinnatum
L. borneense
L. trifurcatum
L. auriculatum

Section Palmata. Pinna-branches pinnate to dichotomous, segments entire to palmate, margins entire (rarely serrate), spores tuberculate, granulate or reticulate.

L. lanceolatum
L. salicifolium
L. reticulatum
L. micropyllum
L. versteegii
L. palmatum
L. hians
L. articulatum

An ancestral *Lygodium* diverged into two lineages, one giving rise to the Subgenus *Lygodium*, consisting of four species, and the other to the Subgenus *Gisopteris*, consisting of the remainding 22 species. The placement of *L. flexuosum, L. japonicum, L. kerstenii,* and *L. venustum* together in a subgenus is supported by gross morphology, spore morphology and phytochemical data. The ancestral position of this subgenus is upheld by the literature on pleisiomorphic characters in ferns in general (e.g., subpalmate segments, pinnate branching pattern, lack of segment articulation, minimal dimorphism, tuberculate spores). A common ancestor to this clade produced the subgenus *Gisopteris.* At the base is *L. polystachyum.* This species is unique in its pinnatifid segments and sporangia forming abaxially on the margins of the lobules, again character states that might be pleisiomorphic in *Lygodium.* The resultant dichotomy which produced the monophyletic sections, Palmata and Volubilia, introduces some new ideas in the phylogeny of this genus. Dichotomous branching, apomorphic in the genus, evolved twice (once in each section). In each clade, palmate segment shape is apomorphic and evolved twice from species with entire segments. The ancestors of the subgenus *Gisopteris* possessed some combination of subpalmate-pinnatifid segments. Tuberculate spores were the ancestral spore pattern of the subgenus *Gisopteris* with section Volubilia evolving verrucate spores and

section Palmata retaining tuberculate spores and some species reticulate spores. Articulate segments were present in, or arose in, the subgenus *Lygodium*-subgenus *Gisopteris* ancestor: this character is a synapomorphy for all sections of the subgenus *Gisopteris* and is subsequently lost in some of the terminal species (e.g., *L. palmatum, L. versteegii, L. aruiculatum, L. trifurcatum, L. borneense, L. circinnatum*).

The results of the cladistic analysis combined with classical views on fern phylogeny suggest that the precursors of *Lygodium* had a short rhizome, a pinnate branching pattern that lacked both the pseudodichotomous forking of the pinna and the pinna-buds, subpalmate or entire segments without articulation zones or pulvini, marginal sporangia on the abaxial surface of otherwise unmodified pinnules, and tuberculate spores with a prominent laesura that extended the length of the radius, without micro-ornamentation patterns and similar proximal or distal faces. These ancestors have not been found in the fossil record.

CHAPTER X

TAXONOMIC REVISION OF *LYGODIUM*

In this treatment of *Lygodium* I have chosen to recognize many taxa as distinct species, realizing that there are intergradations and polymorphic characters. In many cases the species have been geographically isolated from each other permitting variations over time to produce phenotypically distinct variants. The morphologic plasticity is engendered by common ancestry or parentage and polyploidy. The following are examples of species whose characters intergrade:

New World:
L. cubense
L. volubile
L. oligostachyum

New World/Old World:

Group I	Group II
L. venustum	*L. volubile*
L japonicum	*L. smithianum*
L. kerstenii	*L. kingii*
L. flexuosum	*L. salicifolium*
	L. flexuosum

The cladistic analysis suggests the following subgeneric classification and is not being formalized in this treatment.

Subgenus *Lygodium*
- Section Flexuosa
  - *L. flexuosum*
  - *L. japonicum*
  - *L. kerstenii*
  - *L. venustum*

Subgenus *Gisopteris*
- Section Polystachya
  - *L. polystachyum*
- Section Volubilia
  - *L. volubile*
  - *L. kingii*
  - *L. smithianum*
  - *L. merrillii*
  - *L. oligostachyum*
  - *L. cubense*
  - *L. longifolium*
  - *L. heterodoxum*
  - *L. radiatum*
  - *L. circinnatum*
  - *L. borneense*
  - *L. trifurcatum*
  - *L. auriculatum*
- Section Palmata
  - *L. lanceolatum*
  - *L. salicifolium*
  - *L. reticulatum*
  - *L. microphyllum*

L. versteegii
L. palmatum
L. hians
L. articulatum

Lygodium polystachyum is the ancestral species for sections Volubilia and Palmata. It lies between subgenus *Lygodium* and the rest of the *Lygodium* taxa. These subgroups and sections do not conform with historical subdivisions but intergrade with the ideas of Prantl (1881), Presl (1945), Reed (1946) and those presented in this treatment (see Chapter 9).

The following represents a taxonomically useful ordering for species identification. The distinguishing character is the branching pattern from the primary pinna-branch.

I. Section Palmata. Primary pinna-branches end in segments or divide dichotomously (one or more times) ending in segments, segments palmate or entire to multilobed (lobes discrete):
Lygodium articulatum
Lygodium auriculatum
Lygodium borneense
Lygodium circinnatum
Lygodium hians
Lygodium longifolium
Lygodium palmatum
Lygodium radiatum
Lygodium trifurcatum
Lygodium versteegii

II. Section Volubilia. Primary pinna-branches pinnate.
A. Subsection Flexuosa. Segments subpalmate to pinnatifid:
Lygodium flexuosum
Lygodium heterodoxum
Lygodium japonicum
Lygodium kerstenii
Lygodium merrillii
Lygodium oligostachyum
Lygodium polystachyum
Lygodium venustum

B. Subsection Volubilia. Segments simple, linear to linear-lanceolate to deltoid, rarely with basal auricle:
Lygodium cubense
Lygodium kingii
Lygodium lanceolatum
Lygodium microphyllum

Lygodium reticulatum
Lygodium salicifolium
Lygodium smithianum
Lygodium volubile

Species are described in an order that reflects morphological similarity and not necessarily the phylogenetic alliances predicted in the cladistic analysis.

Lygodium Swartz, Schrader, J. Bot., 1800(2): 106. 1801. *nom. cons.* (Taxon 3: 69-70. 1954). Lectotype. *Lygodium scandens* (L.) Sw. (*Ophioglossum scandens* L.) *emend prop.* Pichi Sermolli, Webbia 12(1): 7. 1956.

Ramondia Mirbel, Bull. Soc. Philom. Paris 2: 179. 1801. Lectotype, selected by Pichi Sermolli, Webbia 12(1): 7. 1956. *Ramondia flexuosa* (L.) Mirbel (≡*Ophioglossum flexuosum* L., Sp. Pl. 2: 1063. 1753).

Ugena Cav., Ic. Desc. 6: 73. 1801. Lectotype, selected by Pichi Sermolli, *ob. cit.*, 8. *Ugena semihastata* Cav.

Odontopteris Bernh., Schrader, J. Bot., 1800 (2): 127, t. 2. f.4. 1801. Type. *Odontopteris scandens* (L.) Bernh. (≡*Ophioglossum scandens* L., Sp. Pl. 2: 1063. 1753.

Gisopteris Bernh., Schrader, J. Bot., 1800(2): 129. 1801. Type. *Gisopteris palmata* Bernh.

Hydroglossum Willd., Abh. Kurf. Mainz. Akad. Nützl. Wiss. Erfurt 2(6): 13, 20. 1802. Lectotype, selected by Pichi Sermolli, *ob. cit.*, 15. *Hydroglossum longifolium* Willd., *ob cit.* 22, t. 2., Herb. Willd., B, photograph NY!.

Ctesium Michx., Fl. Bor. Am., 2: 275. 1803. Type. *Ctesium paniculatum* Michx.

Vallifilix Petit-Thouars, Gen. Nov. Madag. 1. 1808. Type. *Ophioglossum scandens* L., Sp. Pl. 2: 1063. 1753.

Lygodictyon J. Smith, Hooker, Gen. Fil., t. 111 B. 1842. Type. *Lygodictyon forsteri* J. Smith.

Rhizome subterranean, short- to long-creeping, fronds congested or well-spaced, protostelic, branching dichotomously, roots from lower surface, covered with long, shiny, dark reddish-brown to black multicellular hairs,

often appressed, growing tip densely pubescent. Fronds indeterminate, erect, growing 2 to > 10 m tall, borne in two rows on upper surface of rhizome, juvenile plants with fronds branching 1-2 x dichotomously, each side bearing palmate segments or pinnate with pinnatifid segments, segments serrate to biserrate to laciniate; adult fronds dividing dichotomously with one side continuing indefinitely (becoming the rachis), the other ending as a suppressed pinna. Stipe length variable (5-20 cm), often dark brown becoming stramineous, "rhizome" hairs continuous for 2-10 cm up stipe, glabrous or puberulent distally with multicellular hairs. Rachis twining of indeterminate length (circumnutating), brown to stramineous, angular, rarely terete, surface between angles often flattened, glabrous to puberulent, hairs 1-2 celled erect to 3-8 celled acicular, bearing alternate, well-spaced pinnae. Pinna stalk 0.2 to > 1 cm long, angular to winged, rarely terete, dividing to produce two pinna-branches subtended by a dormant bud, glabrous to puberulent. Pinna-bud sunken in a pocket formed by the pinna-branch bases or raised well above the pinna-branch bases, covered with characteristic long, multicellular hairs, septa darker in color, basal cell of hair enlarged to being polycellular and bulbous, apical cell of hair sharply pointed; when injury occurs to distal axes the pinna-bud grows out bearing pinna-branches.

Primary pinna-branches 1-3 x pinnate or ending in simple, bifid, palmate or subpalmate segments or dividing dichotomously and ending in segments, often with a raised area at the petiole/branch junction (most obvious in flexuous branches), angular to winged, glabrous to pubescent with hairs as described for the rachis, the degree of pubescence increases on more distal axes. Segment petiole present in 1-2 x pinnate forms (not in those in which the primary pinna-branch ends in segments), angular or narowly winged, wings

increase distally towards base of segment as does indument, if present, rarely glabrous, puberulent to densely tomentose, often with an obvious articulation zone of cells at the petiole/segment junction so that when the segment abscises the petiole remains on the pinna-branch, if articulation zone present often pubescent. Segments simple deltoid, linear to linear-lanceolate, bifid to palmate (6-7 lobes) to subpalmate (one central lobe with 2-4 outer smaller lobes), 1.5-30 cm long, 0.5-4 cm wide, bases auriculate, cordate, truncate to cuneate and apices acute to acuminate to rounded, hairs, if present, on veins and lamina, unicellular to multicellular, all with glandular club-shaped hairs. Veins free to reticulate, if free, 1-3 times forked, if reticulate with 2-4 anastomosings, ending at or before the margins. Margins entire to serrate to lobulate to pinnatifid with a longitudinal layer of cells, 1-5 cells thick, into which veins merge (or veins may end before layer), veins often extending past serration as a papilla. Fertile and sterile segments monomorphic bearing sorophores to strongly dimorphic with lamina mostly suppressed, primary pinna-branches may be 3-4 times pinnate. Fertile segments entire to subpalmate to pinnatifid to lacking lamina entirely, sporangia borne abaxially, in two rows at the ends of veins covered by an indusium, 2-40 sporangial pairs present, bearing hairs at the base of the sorophore or under sporangia or on indusia. Spores trilete, surface patterns tuberculate, long-ridged verrucate, reticulate or low-tuberculate to granulate, 52-125 μm. There are 26 species of *Lygodium* worldwide.

Key to the species of *Lygodium*

1. Veins reticulate.
   2. Segments strongly articulate.
      3. Swelling (pulvinus) at petiole/segment junction, segments lanceolate, apex acute, spores tuberculate.....*L. lanceolatum.*
      3. Without swelling at petiole/segment junction, segments deltoid, apex rounded to acute, spores reticulate.....*L. reticulatum*

2. Segments not articulate.
    4. Pinna-branches suppressed (0 mm long), each one producing three linear-lanceolate segments, spores reticulate.....*L. versteegii.*
    4. Pinna-branches not suppressed (3-15 cm long), pinnate or dichotomous ending in 3-5-fid segments.
        5. Pinna-branches flexuous, bearing 2-3 bifid to 4-fid segments per side, or dichotomous ending in 3-4-fid segments, spores tuberculate, Mexico to Colombia .....*L. heterodoxum.*
        5. Pinna-branches straight, bearing 3-5 bifid to trifid segments per side, terminal segment 5-6-fid, spores verrucate, China, Indonesia, Philippines and Borneo.....*L. merrillii.*

1. Veins free.
    6. Primary pinna-branches pinnate.
        7. Segments simple, with or without basal auricle.
            8. Segments not articulate.
                9. Segments linear without basal auricle or lobe, Africa..... .....*L. smithianum.*
                9. Segments deltoid to linear-lanceolate, with basal auricle or lobe on one or both sides, southeast Asia.....*L. flexuosum.*
            8. Segments strongly articulate.
                10. Dormant pinna-bud with swollen, multicellular-based hairs.
                    11. Spores tuberculate, India, Asia, Pacifica.....*L. salicifolium.*
                    11. Spores verrucate, endemic to New Guinea.....*L. kingii.*
                10. Dormant pinna-bud without swollen, multicellular-based hairs.
                    12. Segments deltoid, spores reticulate.....*L. microphyllum.*
                    12. Segments linear-lanceolate, spores verrucate or tuberculate.
                        13. Segments with auricle or lobe at base on one or both sides, 2-4 segments per pinna-branch, southeast Asia. .....*L. flexuosum.*
                        13. Segments without basal auricle or lobe, 3-6 segments/pinna-branch, tropical America....*L. volubile.*
        7. Segments subpalmate (central lobe longest) or pinnatifid.
            14. Sterile pinna-branches once pinnate.
                15. Segments uniformly pinnatifid.....*L. polystachyum.*
                15. Segments subpalmate with smaller side lobes and a central long lobe.
                    16. Pinna-stalk 1-3 mm long, pinna-branch with 2-3 segments per side, southeast Asia.....*L. flexuosum.*
                    16. Pinna stalk 5+ mm long, pinna-branches with 3-7 segments per side, tropical America.....*L. venustum.*
            14. Sterile pinna-branches twice pinnate (at least most proximal portion of pinna-branch).
                17. Segments with most veins ending in fine teeth.....*L. japonicum.*
                17. Segments ending in lobes (central lobe often pinnatifid) or undifferentiated margins.....*L. kerstenii.*
    6. Primary pinna-branches not pinnate, dichotomous or ending in a segment.
        18. Pinna-branches ending in segment.
            19. Pinna-stalk 4-10 mm long, segments palmate, 2.5-5 cm long, lobes mostly rounded, margins entire, endemic to United States..... .....*L. palmatum.*
            19. Pinna-stalk 0-2 mm long, segments palmate, 15-25 cm long, lobes acuminate, margins serrate, Costa Rica to Ecuador.....*L. radiatum.*

18. Pinna-branches dichotomous.
    20. Segment 2-6-fid, lobes discrete of approximately equal size or subpalmate.
        21. Segments subpalmate, tripartite, 2-7 cm long, base cuneate..... ..... *L. oligostachyum.*
        21. Segments 2-6-fid, lobes discrete, 10-24 cm long.
            22. Segment margins entire, thickened, spores tuberculate..... ..... *L. circinnatum.*
            22. Segment margins serrate, spores verrucate..... ..... *L. longifolium.*
    20. Segments simple, with or without auricle at base.
        23. Segments simple, without auricle.
            24. Pinna-stalk > 3mm long, fertile dichotomous to 7 times, highly dimorphic, endemic to New Zealand..... ..... *L. articulatum.*
            24. Pinna-stalk < 2mm long.
                25. Fertile segment sporangia only at apex or upper 1/4 of segment, New Caledonia..... *L. hians.*
                25. Fertile segment sporangia over entire margin of segment, segment > 15 cm long, Borneo, Sumatra and Malaysia..... *L. borneense.*
        23. Segments with strong auricle on one or both sides.
            26. Segment articulate with swelling (pulvinus) at segment/pinna-branch junction..... *L. cubense.*
            26. Segment not articulate.
                27. Segments 2-7 cm long, strongly cuneate at base, without swollen, multicellular-based pinna-bud hairs..... *L. oligostachyum.*
                27. Segments 10 + cm long, usually bifid, with swollen, multicellular-based pinna-bud hairs.
                    28. Fertile and sterile segments dimorphic, fertile lamina reduced to 0.2-1.0 mm..... *L. trifurcatum.*
                    28. Fertile and sterile segment monomorphic, auricle most often only on one side of segment base..... ..... *L. auriculatum.*

1. ***Lygodium articulatum*** A. Richard, Essai Fl. New Zealand, Voyage de L'Astrolabe, 96, t. 15. 1832. Type. New Zealand, North Island, Mercury Bay, *d'Urville s.n.* (G, holotype). Figs. 10.27H-M.

Lygodium gracilescens Colenso, Trans. New Zealand Inst. 28: 620. 1896. Type: New Zealand, without locality, without collector, label indicates "Presented by Colenso" (K!).

Rhizome long-creeping, 1-4 mm diam, clothed with appressed, lustrous, reddish-brown to golden, multicellular hairs (2-3 mm long). Fronds 3-20 mm apart, climbing to 10-20 m. Stipes brown basally, stramineous distally, glabrous except for rhizome hairs at base; stipe and succeeding axes abaxially grooved. Rachis ca.1-2 mm diam, hairs sparse, multicellular (4-5 celled, ± 0.5 mm long), reddish. Pinna-stalk 3-7 mm long, glabrous. Dormant pinna-bud recessed in a pocket formed by the bases of two pinnae, thickly covered with septate golden hairs (1-1.5 mm). Primary pinna-branches dichotomously branched 1-4 times, glabrous; angle of branching, 85°- 135° (ave. 110°). Segment-petiole with exaggerated articulation zone at segment base (persistent after segments abscise); sterile segment-petiole 3-10 mm. Segments subcoriaceous, simple, linear to linear-lanceolate, cuneate at base, acute to obtuse at apex, 4-12 cm x 1-1.5 cm, glabrous. Veins free, prominent on both surfaces, 1-2x forked, angle from costa 25°-35°, ending in thickened marginal layer, costa, especially at base of segment, puberulent (as rachis). Margins entire to slightly serrulate, with a marginal layer 3-5 cells wide. Fertile and sterile segments strongly dimorphic; fertile portion of frond dichotomously branched to 7x, angle of branching 120°-130°. Fertile segments with lamina reduced or absent, their petioles 1-5 mm, less distinctly articulate

than the sterile (if lamina present, articulation zone more pronounced); sporangia borne on veins of reduced segments, 4-12 pairs per sorophore; indusium glabrous; sparse multicellular, acicular hairs on veins of sorophore. Spores 82-126 μm (ave. 111.5 μm), densely tuberculate, laesurae not prominent (Figs. 6.1d,e).

Ecology

Lowland to lower montane forests, often at edge of second growth mixed woods, climbing through vegetation (e.g. tree ferns) to 20 + m, forming dense thickets. Elev. 200-450 m.

Distribution

Lygodium articulatum is endemic to North Island, New Zealand, primarily from North Cape to latitude 38° (Bay of Plenty and Kawhai). Fig. 10.1.

Comments

This species is unique in the exaggerated articulation zone of the sterile foliar segments such that the pinna-stalk has an enlarged flattened T-shaped area when the segment abscises. All axes are angular: the pinna-stalk is grooved so that its bud appears to be sunken in a pocket. The highly dimorphic fertile portions of the frond branch dichotomously to 7x. The sporangia form on veins, often with segment lamina lacking and suggesting an elaborately dissected leaflet. The spores are unique in the regular, densely packed tubercles. Spores remain viable only a few days.

This taxon is known as "bushman's mattress" because the long wiry climbing axes develop into tangled masses which make springy bush beds. The Maori used them as roofing thatch (S. Jones, pers. comm.).

Selected specimens examined:

New Zealand. North Island: Auckland, *Fosberg 30231* (GH); *Hunnewell 13369* (MICH); Waitakere Range, *Leland 203* (GH, MICH); *Orchard 3773* (GH); *Pichi Sermolli 6305* (NY); *Trevarthen s.n.* (GH), June 1949; North Auckland, Kaiaka, Herb. E.B. Copeland No. 17803 (NY); Ruatangata, *Satchell s.n.* (MICH); Stokes Point, *Kirk 151* (NY); Wellington, no collector (GH), Herbarium of T. Kirk; without collector, Herb. Richard No. 10 (P); without collector, Voyage Astrolabe (P); without collector, Herb. Richard No. 6 (P).

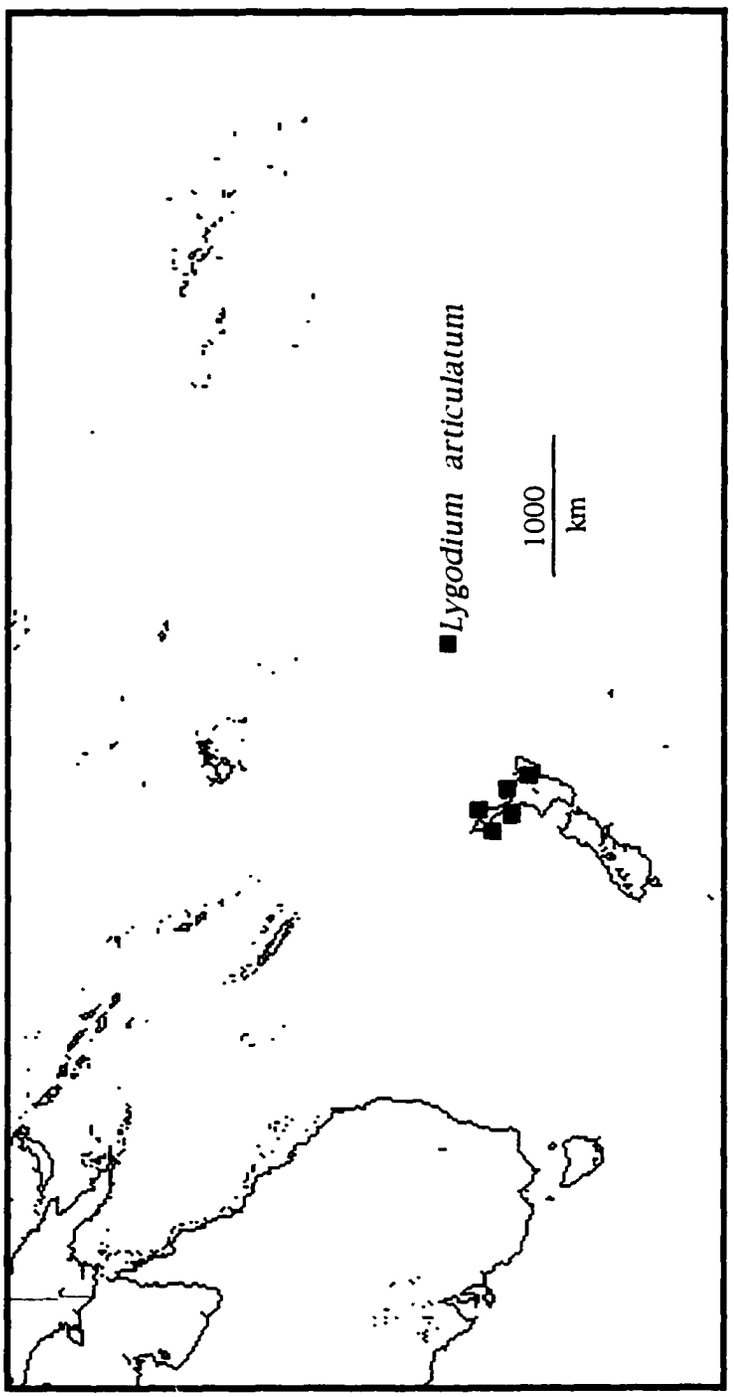


Figure 10.1. The geographic distribution of *Lygodium articulatum*.

2. ***Lygodium hians*** Fournier, Ann. Sci. Nat. 5(18): 35. 1873.

Syntypes. New Caledonia, Mt. Humbold, *Balansa 1564* (P!); Mt. Mou, *Balansa 2737* (P!). Figs. 10.28A-E.

Rhizome long-creeping, covered with reddish-brown multicellular hairs. Fronds well spaced 2.5-3.5 cm apart, climbing to 2 m. Stipe grooved, glabrous except for lowest 1-2 cm. Rachis ca. 1-2 mm, winged, glabrous. Pinna-stalk 1-2 mm, winged. Pinna-bud in pocket formed by pinna bases, covered with multicellular reddish brown hairs. Primary pinna-branches ending in 1 simple segment or dividing dichotomously, each branch ending in a simple segment. Segments coriaceous, simple, linear to linear-lanceolate, 6-9 x 1.5-2.5 cm, cuneate at base, acute at apex, strongly articulate. Veins free, prominent, forking once, ascending at a 25°-30° angle from costa, ending at or before thickened margin. Margins weakly serrulate, marginally thickened, 2-4 cells wide. Fertile and sterile segments semidimorphic. Fertile segments sorophores borne at the apex and along the distal third of the fertile segment, 6-12 sporangial pairs/sorophore, sporangia may not begin on sorophore for 2-4 mm, giving the appearance of pedicellate sporangia; glabrous. Spores 90-120 μm (ave. 100 μm), globose, tuberculate-verrucate (Fig. 6.9a-d).

Ecology

Grows in dense wet forests. Elev. 500-1500 m.

Distribution

Endemic to New Caledonia. Fig. 10.2.

Comments

Lygodium hians is unique in the pinna-branches ending in simple segments and in the position of the sorophores which occur only on the apical

margins of the segments. In some specimens the veins of fertile segment margins often divide giving rise to two or three sorophores. It is also strongly articulate and the segments are long. The juvenile fronds divide dichotomously at 15-24 cm intervals, each dichotomy ending in a bifid or trifid segment. It is easily confused with many other species at this immature stage.

The segment morphology, strong articulation, and dichotomous branching pattern resemble those of the New Zealand endemic, *L. articulatum*. The spores of some of the specimens of *L. hians* are large with exaggerated tubercles and verrucae while the spores of *L. articulatum* are evenly tuberculate but with large tubercles resembling gemmae. It seems possible to hypothesize that *L. hians* is an island species of *L. articulatum* parentage that may have some genetic mutation causing the exaggerated fertile segments and spores. It is also possible that the spores are mitospores resulting from apomixis. Further collections need be studied to determine the viability of the spores and the chromosome number.

Selected specimens examined

New Caledonia. Mount Colnett: *Hurlimann 1979* (NY, P); Mt. Kogley: *Franc 634* (UC); Mt. Mou: *Herb. E.B. Copeland # 11410* (MICH); *Franc s.n.* Jan. 1909 (MICH); *Vieillar* 3376 (P); Mt. Montagne: *Hurlimann 972, 973* (NY, GH-A); Mt. Panié: *Hodel et al. 1434* (UC), juvenile; Massif de Ton-non: *Mackee 19161* (P); *Mackee 15603* (P); *McKee 6391* (GH-A, UC); Lure: *Franc 634* (NY); *Franc s.n.*, Oct. 1908 (P); *M. LeRat 957,* two sheets (P).

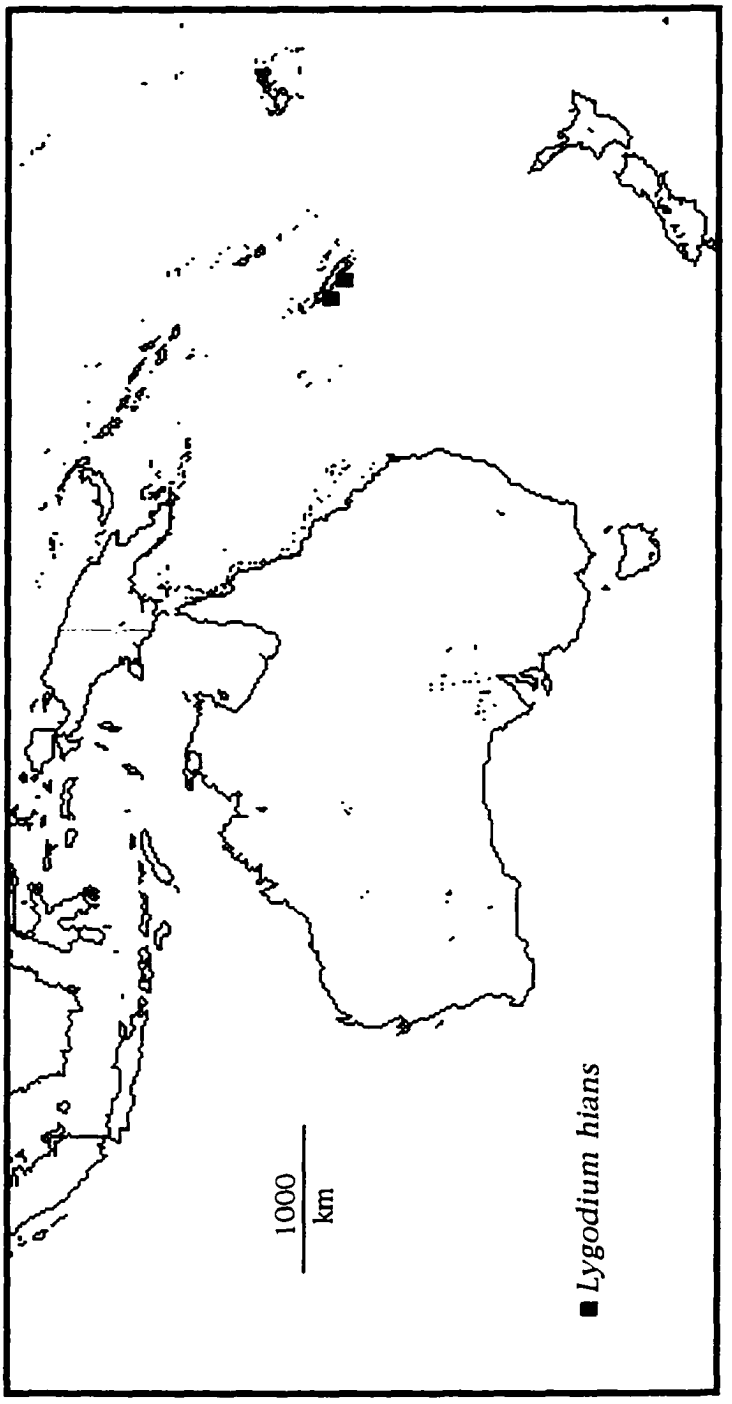


Figure 10.2. The geographic distribution of *Lygodium hians*.

3. ***Lygodium palmatum*** (Bernh.) Swartz, Syn. Fil. 154. 1806. *Gisopteris palmata* Bernh., J. Bot. (Schrader) 1800(2): 129. 1801. *Hydroglossum palmatum* (Bernh.) Willd., Abh. Kurfürstl.-Mainz. Akad. Nützl. Wiss. Erfurt. 2: 25, t. 1, f. 2. 1801. Type. USA. Pennsylvania: Lancaster, *Muhlenberg s.n.*, Herb. Willd. no. 19484 (M, holotype). Figs. 10.29A-F.

Ramonda palmata Mirbel, Bull. Soc. Philom., 2: 179. 1801.

Ctesium paniculatum Michx., Fl. bor. amer. 2: 275. 1803. Type: U.S.A., *Michaux s.n.* (P); Morton photograph 3372 (US).

Rhizome long-creeping, ca. 1-2 mm diam, subterranean 2-4 cm, sparsely covered with reddish-brown to light brown multicellular, septate (septa darker brown) hairs 1-2 mm long. Fronds 1-3 cm apart, climbing to 1-3 m, occasionally longer. Stipes brown at base becoming stramineous distally, glabrous. Rachis ca. 1 mm diam., stramineous, sometimes slightly grooved, glabrous. Pinna-stalk 4-10 mm long, narrowly grooved, with multicellular, acicular, light tan hairs, 0.5-0.75 mm long, in narrow ridge between grooves. Dormant pinna-bud recessed in shallow pocket formed by two pinna-branch bases, glabrous to sparsely covered with hairs as on pinna stalk, often inconspicuous. Primary pinna-branches 5-10 cm apart on rachis narrowly grooved with few hairs in groove, ending in a palmate segment; Segments chartaceous, palmate, with 4-6 lobes, longest lobe 2.5-5 cm long (basal lobes reduced), lobes deeply cut, cordate to auriculate at base, rounded to acute at apex, glabrous or with transparent, silky multicellular (4-6 cells) hairs abaxially, hairs 1.0-2.5 mm long. Veins free, 1-2x forked, ascending at 20°-35° from midvein of lobe, ending at or before margin. Margins entire, slightly thickened. Fertile and sterile segments strongly dimorphic, fertile

primary pinna-branch dichotomously branched to 4 x, angle of branching 100°-110°, all axes winged. Fertile segments with lamina reduced or absent; 3-6 sorophores per segment, 5-10 pairs of sporangia per sorophore, with transparent, acicular hairs (1.5 mm long) abaxially on veins of axes and often on indusia. Spores 62-78 μm (ave. 72 μm), with very low tubercles to granules, some connected to form low ridges, laesurae prominent (Fig. 6.8c,d).

Ecology

Lygodium palmatum is found in mixed deciduous forests (usually at the forest edges), in swampy thickets and *Sphagnum* bogs, climbing over *Nyssa*, dry pine and broadleaved trees and shrubs, along streams, on sandstone cliffs (Kentucky), in pastures and along roadsides. Shaver (1954) reports that in Tennessee *L. palmatum* is found growing with mountain laurel (*Kalmia latifolia*). The species is often associated with acid soils and grows to elevations of 1000 m.

Distribution

Lygodium palmatum is endemic to the eastern United States. It grows in New Hampshire south to South Carolina and west to Tennessee, Kentucky and Ohio with a disjunct population in southwest Michigan. It is rare geographically, but localized dense populations occur. *Lygodium palmatum* is considered a rare species and is protected in a number of states. The report of this species in Florida from two collections, one from Dade County (Lemon City) collected in 1895 (FLAS) and a second specimen without locality (*Calkins 237*, NY) probably represent isolated collections of introduced plants. The report of *L. palmatum* in Alabama (Mobile) may also represent an introduced plant (Dean, 1969). Fig. 10.3.

Comments

This is the only endemic species of *Lygodium* in the temperate New World. It is easily recognized by its palmate sterile segments and highly dimorphic, dichotomously branched, flexuous, fertile segments. The dormant bud is inconspicuous, partially protected by the bases of the pinna-branches and relatively glabrous. The presence of long silken hairs on the abaxial surface of fertile and sterile segments seems to follow a geographic distribution pattern: the glabrous form is found in states north of Maryland and pubescent forms are found south and west of Maryland and in Michigan and Ohio (Fig. 10.3). The spores of *Lygodium palmatum* appear relatively smooth compared to all other species: however they are granular (see spore chapter).

Selected specimens examined.

USA. Connecticut: Windham County, Sterling, *Russell s.n.* (GH); Kentucky: McCreary County, Cumberland Falls, *Wherry & Pennell 13843* (GH), Montgomery County, Jeffersonville, *Wharton 5374* (GH); Massachusetts: Hampshire, *Granby s.n.* (GH), Sept, 1898; Maryland: Riverdale, *Dowell 5325* (GH); Michigan: Kalamazoo County, *Beitel 74213* (NY); New Hampshire: Winchester, *Metcalf s.n.* (GH); New Jersey: Burlington County, *Eiten 1421* (NY); New York: Saratoga County, Gansevoort, *House 26344*; North Carolina: Henderson County, without collector, Biltmore Herbarium 2763 (GH); Stokes County, Danbury, *Pennell 14327* (GH); Ohio: Lawrence County, (NY); Athens, *Chapman s.n.*, Dec. 1932 (GH); Pennsylvania: Monroe County, Pocono Plateau, *Wilcox & Hirshberger s.n.* (GH); Rhode Island: Kent County, Coventry, *Collins & Hope s.n.* (GH) South Carolina: Jocassee, *Hill 19053* (GH) Tennessee: Cumberland County, *Crundell s.n.* (GH); Roane County, Rockwook-Ozone, *Correll 8120* (GH); *Clausen 5392* (NY); Vermont: Stowe, *Evans s.n.*, July 1893 (GH); Virginia: Campbell County, *Freer & Hooks* 4777 (GH); West Virginia: Greenbrier County, *Franklin s.n.* (GH).

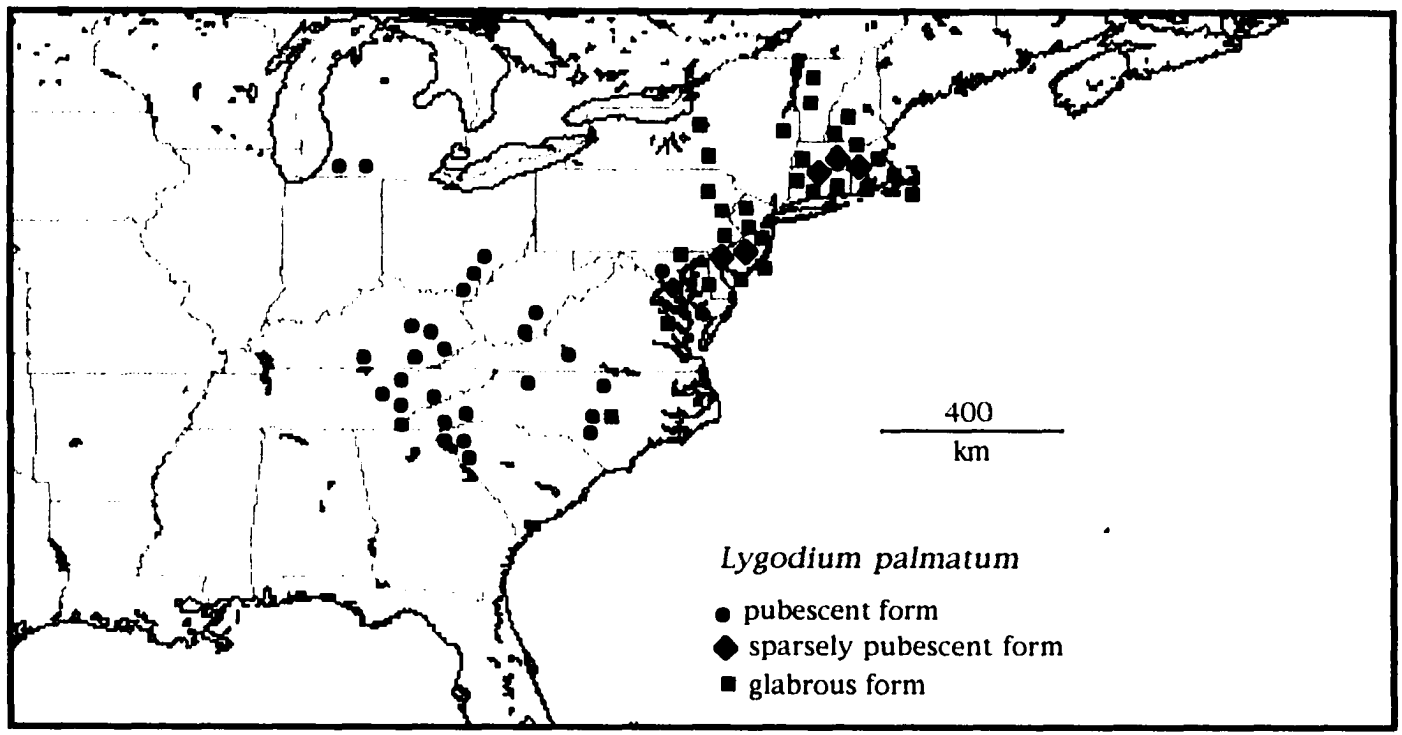


Figure 10.3. The geographic distribution of *Lygodium palmatum*. Variations in degree of pubescence across the species' range are indicated.

4. ***Lygodium radiatum*** Prantl, Unters. Morph. Gefasskrypt., 2: 66. 1881. *Lygodium digitatum* Eaton, Mem. Amer. Acad. Sci. Arts. 8: 217. 1860. non *L. digitatum* Presl, 1825. *nom illeg.* Syntypes. Panama, Gatun, Hayes 25 (YU; isosyntypes, GH, NY!, US); Colombia, Choco, Falls of the Rio Truando, *Schott 77* (?; isosyntypes, F!, MO!). Figs. 10.29G-J.

Rhizome creeping, 4-5 mm diam., with lustrous multicellular black hairs. Frond < 4mm apart, climbing to 5-10 m. Stipe reddish-brown to tan, rhizome-hairs continuing to 5 mm becoming sparse distally. Rachis 1-4 mm diam., wiry, grooved, glabrous. Pinna-stalk 0.2-2 mm. Pinna-bud slightly sunken, covered with reddish-brown to black, multicellular hairs, some with 2-rowed swollen base. Primary pinna-branches 3.5 - 9 cm long, angular becoming winged close to segment base, glabrous (close to pinna-bud may have golden 2-3-celled hairs), each pinna-branch bearing a single palmate segment. Segments papyraceous, palmate, 3-4-fid, occasionally with 5-8 lobes, base truncate to broadly cuneate, apex acute to acuminate to long-attenuate (occasionally bidentate), bearing small clavate hairs, the lobes linear, (10)15-20 (27) x 1.5-3.0 cm, cut 3/4 way to base. Veins free; main vein dividing 2-3 times to become segment midvein (appears to radiate from central point), if 4-fid or 5-fid, outer veins divide again, lobe-veins 1-2 times forked, ascending from main vein at 60°-75°, ending in thin marginal layer, with short acicular one-celled hairs, multicellular 4-5-celled hairs and clavate hairs on main veins. Margins deeply serrate to serrulate, with a thin marginal layer of cells. Fertile and sterile segments monomorphic. Fertile segments bifid to 4-fid (rarely 5-6-lobed), resembling sterile segments; sporangia on sorophores, 5-12 sporangia per sorophore, mostly glabrous. Spores 93-109 μm (ave. 102.7 μm), tuberculate, tubercles irregularly spaced, not dense, laesurae prominent

(Fig. 6.3e,f).

Ecology

Lygodium radiatum often forms thickets in forest shade, wooded swamps, and along streams. It grows in mangrove swamps in Costa Rica. In Colombia it grows 10 m tall at altitudes of 500 m and in Panama twines up to 20 m tall at altitudes up to 1000 m.

Distribution

Costa Rica, Panama, Colombia, Peru, and coastal Ecuador. Fig. 10.4

Comments

This species of *Lygodium* has decidedly palmate segments like those of *L. palmatum.* Its segments are at the ends of the primary pinna-branches. The axes are not noticeably winged. It lacks indument, and segments and axes may be lime-green in color. It is readily separated from *L. heterodoxum* by its free veins. Some Peruvian specimens have 5-8 large lobes that are up to 28 cm long. Segments of juvenile plants often have many lobes (isosyntype *Schott 77*), but those of more mature plants are 3-4-fid.

Selected Specimens Examined

Costa Rica. Puntarenas: *Mickel 2848* (NY); San Jose: *A. Skutch 4929* (F, NY); **Panama.** Colón: *Liesner 1107* (F); Darien: *Terry & Terry 1467* (F); Barro Colorado Island: *W. Maxon 4735* (GH, NY); *Croat 4382* (F); *D. Starry 328* (F); **Colombia.** Santander: *Haught 1357* (UC); Tolima Mariquita: *Murillo* 7 (NY); Chocó: *Gentry & Forero 7159* (NY); **Ecuador.** Coast, without locality: *Wood s.n.* Kew Herb. # 1298 (GH); **Peru.** Loreto: *Killip & Smith 28937* (F); Pasco: *Smith 2011* (MO); Ucaylai:*Vasquez 01* (F); Pasco: *Smith 2011* (F).

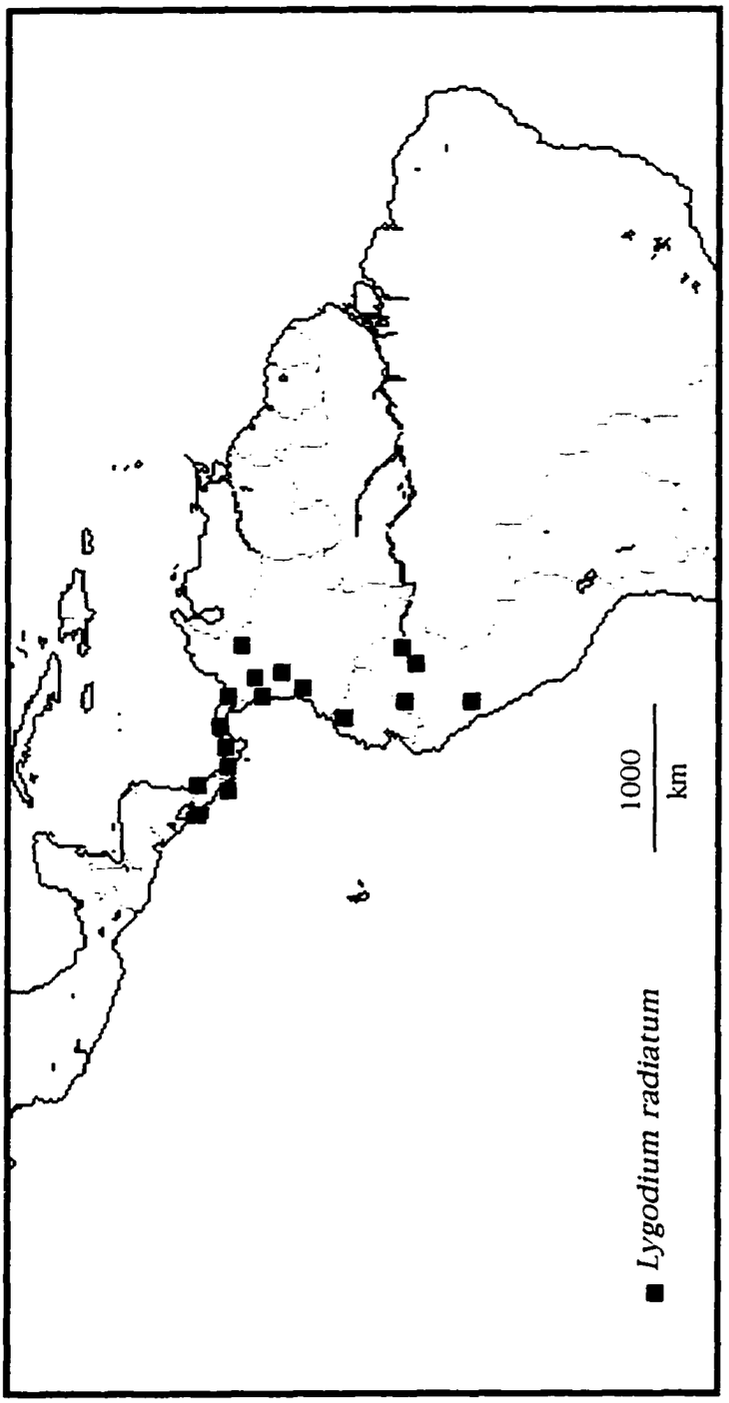


Figure 10.4. The geographic distribution of *Lygodium radiatum*.

5. ***Lygodium auriculatum*** (Willd.) Alston, Reinwardtia 5(1): 16. 1959.

Hydroglossum auriculatum Willd., Sp. Pl. 5: 84. 1810. Type. Philippine Islands, Luzon, *Née s.n.* (MA, holotype). Figs. 10.30A-E.

Ugena semihastata Cav., Icon. Pl. 6: 74. t. 594, fig. 1. 1801. *nom. illeg. excl. syn. Lygodium semihastatum* Desv., Mém. Soc. Linn. Paris 6: 203. 1827. *L. circinnatum* var. *semihastatum* Fosberg, Am. Fern J. 40: 42. 1950. Type: Luzon, Philippine Islands, *Nee s.n.* (MA?).

Rhizome short-creeping, covered with shiny, multicellular, reddish-brown to black, acicular hairs. Fronds 2-5 mm apart, climbing to 5 m. Stipes shallowly grooved, hairs (0.3 mm) sparse in adaxial groove, becoming more frequent toward rachis, rhizome-hairs on base to ca. 10 mm. Rachis ca. 1-3 mm diam., narrowly grooved to winged, glabrous to bearing multicellular, golden, septate hairs (ca. 0.5 mm long), scattered between grooves, and recurring on all subsequent axes. Pinna-stalk less than 2 mm long, grooved. Dormant pinna-bud recessed in pocket formed by the bases of two pinna-branches, densely covered with swollen, multicellular-based hairs with brown-black bases, hairs becoming chestnut to stramineous apically. Primary pinna-branches dichotomously branched 1-2x (1.5-3 cm before split), narrowly winged, each branch ending in a simple or bifid segment; hairs becoming more frequent toward segment petiole. Segment-petiole narrowly winged, lamina wider toward base, sparsely hairy. Segments chartaceous to subcoriaceous, simple, linear-lanceolate to bifid (each lobe linear-lanceolate), 9-24 (to 35) x 1.3-3.5 cm, not articulate, cuneate to auriculate at base, most often auriculate only on one side, cuneate on the other, or truncate on one side, cuneate on other, apex acute. Veins free, 2 times forked, angle from costa 50°-60°, costa prominent on

both surfaces with occasional multicellular hairs (less than 4 cells, ca. 0.8 mm); ending at margin. Margins serrulate; a thin marginal layer. Fertile and sterile segments monomorphic to very slightly dimorphic; fertile segment-petioles winged with scattered hairs. Fertile segments simple, rarely bifid, with slightly reduced lamina, bearing sporangia on up to 75 pairs of sorophores, (basal 1/4th of segment may not contain any sorophores), with 7-15 pairs of sporangia per sorophore, but often 0.5-1 mm of lamina before sporangia begin, giving the appearance sorophores on a pedicel; pubescent with multicellular septate hairs (0.5 mm) on veins and under sporangia; indusium glabrous. Spores 72-92 μm (ave. 85 μm), verrucate, the verrucae of distal face coalescing to form irregular ridges, laesurae prominent, proximal face with enlarged equatorial ridge (Fig. 6.5a-c).

Ecology

Lygodium auriculatum is found in savannas at the foot of hillsides, on cane in the Philippines, amid sword grass on volcanic peaks in Guam, and also on river banks. In Perak it occurs mainly on limestone but has been found on granite on Penang Hill (Holttum, 1954). It is found at elevations of 100-300 m but is reported up to 1000 m in Mindanao.

Distribution

Pacific side of Philippine Islands (Luzon, Mindanao, Mindoro, Negros, Polillo, Samar), Mariana Islands (Guam), Eastern Borneo, and Indochina. Fig. 10.5.

Comments

This species is distinguished by swollen, multicellular-based hairs covering the dormant pinna-bud and by the semi-auriculate ("semi-hastate") base of the simple or bifid segments which can reach a length of 35 cm. Often included

in *L. flexuosum s.l.* (Christenson, 1905; Prantl, 1881), *L. auriculatum* is easily differentiated from it by the dichotomous primary pinna-branches (*L. flexuosum* is most often pinnate). It is also confused with *L. circinnatum*, which branches dichotomously (1x) but most often ends in trifid, 4-fid or 5-fid segments (rarely bifid segments and not semihastate). *Lygodium circinnatum* also bears many hairs on all axes, the segment margins are entire to barely serrulate, and veins end in a prominent thickened marginal layer (8-9 cell layers). The fertile and sterile segments are often dimorphic. The spores of both *L. flexuosum* and *L. circinnatum* are irregularly tuberculate without ridges on the distal face, and do not possess either prominent laesurae or a subequatorial flange.

Nomenclatural Comments

Cavanilles cited *Ophoglossum flexuosum* L. as a synonym of *Ugena semihastata* Cav.: therefore, he should have adopted the epithet *flexuosum* (Alston & Holttum, 1959). The illustration cited is from a collection by Nee in the Philippine Islands and is, however, not *L. flexuosum.*

Selected specimens examined:

Mariana Islands, Guam. Mt. Santa Rosa, *Bryan 1128*, (NY); Sumay, *Conover s.n.* (UC), Feb. 1945; Tonfit River Valley, *Grether 3707* (MICH, UC); Ylig River headwaters, *Rodin 634* (UC); **Philippine Islands: Luzon**, Prov. Bataan, *Williams 210* (UC); Tayabas, Sampalok, Herb. E.B. Copeland 210 (UC, BM), Mar. 1933; Tayabas, *Topping 1291* (NY), *Topping 1287* (NY); Jimmi River, *Womersley and Millar 8589*, (NY). **Mindanao.** T. Urdanetta, Cabadbaran, without collector, distributed by A. Elmer, (NY), Oct 1912; **Mindoro**, Puerto Galeria, *Bartlett 13556* (MICH); **Polillo Island,** Anibawan, *Fox s.n.*, Phil. Nat. Herb. # 8901 (MICH, BM); Mt. Mahagna, Oquendo, *Sulit s.n.*, Phil. Nat. Herb. #

13929 (MICH); **Samar**, *Cuming 337* (BM); Kadapnan, *Castro s.n.*, Phil. Nat. Herb. # 5886 (MICH).

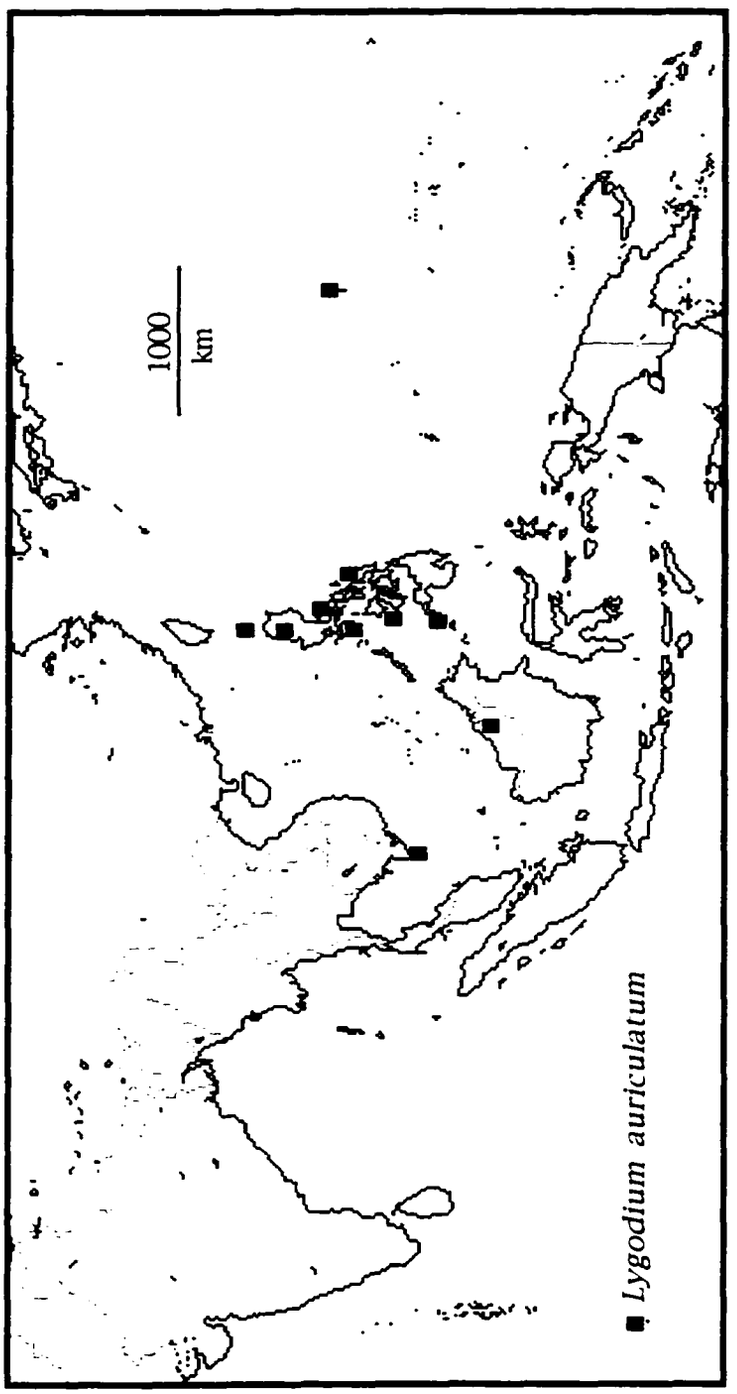


Figure 10.5 The geographic distribution of *Lygodium auriculatum*.

6. ***Lygodium borneense*** Alderw., Bull. Jard. Bot. Buitenzorg 2(20) Appendix: 29. 1915. Syntypes. Borneo, Mt. Uja, *Teysmann s.n.* (BO?, syntype) and *Winkler 2722* (B!, syntype). Fig. 10.30F-J.

Rhizome short-creeping, ca. 5 mm in diameter. Frond approximate, length not indicated. Stipe not observed. Rachis ca. 1-2 mm, angular, glabrous. Pinna-stalk sessile to < 1mm. Dormant pinna-bud slightly sunken, covered with golden multicellular hairs and swollen, multicellular-based hairs. Primary pinna-branches separating dichotomously (1-3 cm before dividing), each dichotomy (5-10 mm long) ending in a simple segment; axes winged; glabrous or with few multicellular hairs between wings. Segments simple or bifid, if bifid, lobes discrete, the whole segment or its lobes oblanceolate, 15-32 cm x 2.5-5 cm, base cuneate, apex acute to acuminate (often bifurcate), occasional hairs on veins. Veins free, twice forked, 45°-50° angle from costa, ending at margin. Margins serrate to serrulate, with thin layer of cells. Fertile and sterile segments monomorphic. Fertile segments bearing 75-100+ sorophores on one side of segment with 5-18 sporangial pairs per sorophore; multicellular (< 3 celled) hairs at base of sporangia, indusia margins erose with occasional 1-2 celled projections. Spores 65-74 μm (ave. 69.2 μm), low-tuberculate (Fig. 6.2 c,d).

Ecology

Growing in damp thickets, often in deep shade in tropical evergreen forests, on limestone outcrops, to 50 m elevation and in Sumatra in fresh water swamps. In Borneo birds use the fronds and pinna-branches to construct nests.

Distribution

Borneo (Sarawak, Sandakan, Niah, Kalimantan, Tawao), Sumatra and Malaysia (Johor). Fig. 10.6.

Comments

Lygodium borneense has extremely large segments with cuneate bases and a suppressed pinna stalk so that the pinna-bud lies between two opposite pinna-branches. It is separated from *L. auriculatum* by the cuneate bases (the latter possesses semi-hastate bases) of the segments. This species and *L. auriculatum* intergrade and more collections should be studied: the spores reported by Holttum (1959) as being unique in their smoothness are actually tuberculate with very low tubercles and cannot, therefore, be used as a unique character.

Selected specimens examined

Borneo. Kalamantan: *Iwatsuki et al. B 510, B 704* (MO); Sarawak: *Allen 2988*, (GH-A); *Brooks 23* (BM, MICH); *Jermy 13937*, 3 sheets (BM); Sandakan: Myburgh Province, *Elmer 20198* (BM,F, GH, MICH, UC); *Boden Kloss 18656* (UC); *Cox 322* (BM); **Sumatra.** *Boden Kloss 14469* (UC). **Malaysia.** Johor: *Holttum s.n.* (BM).

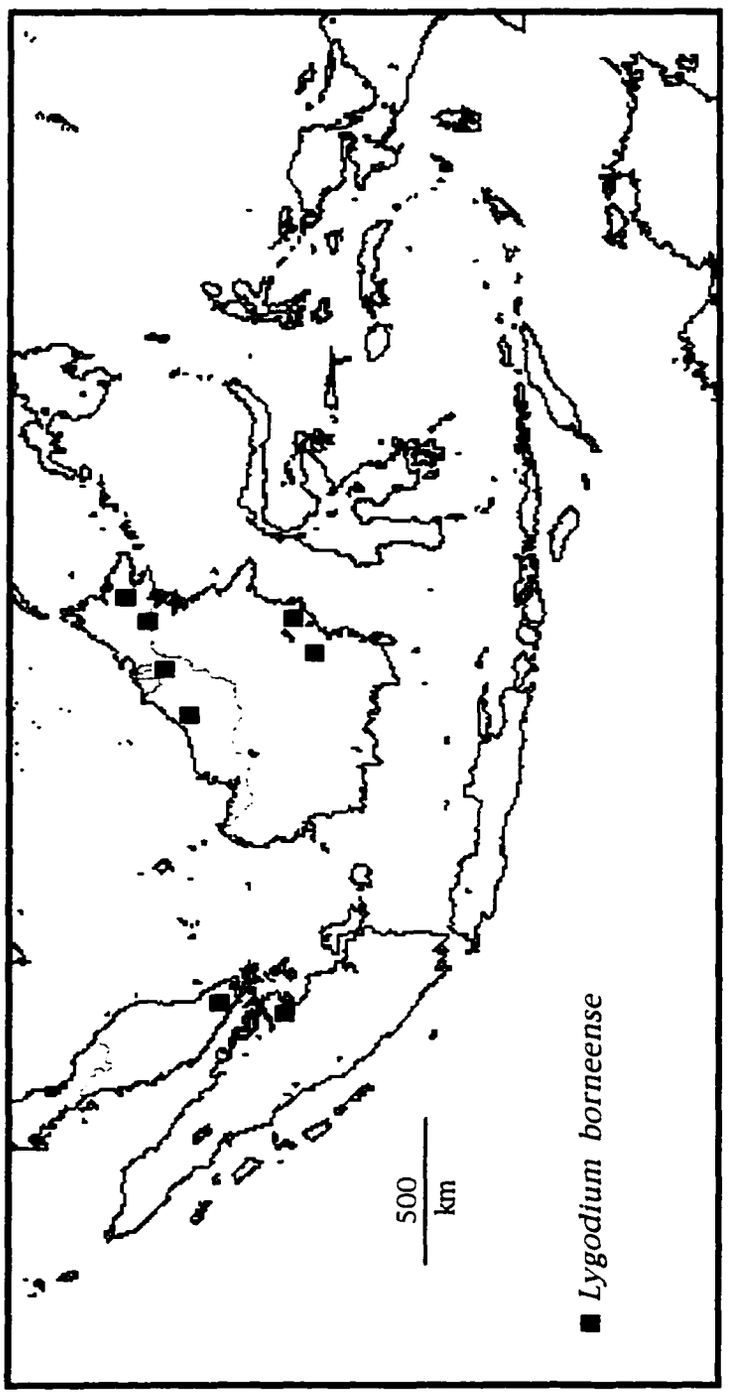


Figure 10.6 The geographic distribution of *Lygodium borneense*.

7. ***Lygodium circinnatum*** (Burm. f.) Sw., Syn Fil., 153. 1806.

Ophioglossum circinnatum Burm. f., Fl. indica 228. 1768. (Basionym).

Hydroglossum circinnatum (Burm. f.) Willd., Abh. Kurfürstl.-Mainz. Akad. Nützl. Wiss. Erfurt 2(4): 24. 1802. Type. Java, Herb. Burman (G, holotype). Fig. 10.31A-F.

Ugena macrostachya Cav.., Icon 6: 75, t. 595, f. 2. Type. *Née s.n.* (MA?).

Lygodium pedatum (Burm. f.) Sw., Syn. Fil. 154. 1806. *Ophioglossum pedatum* Burm. f., Fl. indica, 227, t. 66., f. 1. 1768. *Hydroglossum pedatum* (Burm. f.) Willd., Abh. Kurfürstl.-Mainz. Akad. Nützl. Wiss. Erfurt. 2(4): 24. 1802. Type. Java, Herb. Burman (G).

Lygodium dichotomum (Cav.) Sw. , Syn. Fil., 154. 1806. *Ugena dichotoma* Cav., Icon 6: 74, t. 594, f. 2. 1801. Type. Née *s.n* (MA?).

Ophioglossum furcatum Roxb., Calcutta J. Nat. Hist. 4: 478. 1844. Type.

Lygodium basilanicum Christ, Philipp. J. Sci. Bot., 2C: 179. 1907. Type. Island of Basilan, Philippines, *DeVore & Hoover 28* (K!, holotype, isotype MICH!).

Lygodium conforme C. Chr., Bull. Mus. Paris II, 6: 104. 1934. Type. China, Yunnan, *Colani s.n.*, Herb. École d'Agric. Hanoi n. 2983 (in Herb. C. Christensen, K?).

Rhizome short-creeping, covered with black, shiny multicellular hairs. Fronds approximate, climbing to 3-6 m. Stipe terete, with rhizome hairs continuing through 1.5-2.0 cm at base replaced by scattered multicellular, stramineous, septate hairs. Pinna-stalk 2-3 mm, terete, glabrous. Dormant pinna-bud somewhat recessed (sunken in many herbarium specimens), sparsely covered with stramineous to reddish multicellular hairs. Primary

pinna-branches grooved to narrowly winged, ending (2-6 cm) in trifid to 6-fid (rarely bifid) segments or dividing dichotomously, each dichotomy (1-2 cm) ending in bifid or trifid segment; with scattered multicellular, golden-brown, septate hairs. Segments subcoriaceous, palmate to flabellate, 3-6 lobed, the lobes sometimes discrete, linear to linear-lanceolate, longest lobe 10-18 (32) cm x 2-2.5 (4) cm, base cuneate to cordate (rarely truncate), apex rounded to attenuate, hairy. Veins free, costa of lobe raised, dividing dichotomously 1-2 times, major veins dichotomously forking, side veins forking 1-2 times, ca. 60° angle from costa, < 2mm between secondary veins, sparsely covered with light tan, septate (septa dark brown) 3-4 celled hairs, ending in thickened marginal layer. Margins entire, with a prominent thickened layer of cells (6-10 layers). Fertile and sterile segments dimorphic to strongly dimorphic (lacking most of segment lamina), fertile pinna-branches dividing twice dichotomously, ending in simple or bifid segments (lobes discrete) or one dichotomy dividing again and ending in two entire segments (appearing subpinnate). Fertile segments simple or bifid, lobes discrete, linear, up to 45 cm long, lamina reduced to <1-5 mm surrounding costa, sporangia 2-4 pairs per projection, puberulent, hairy on costa and blade, especially near projections. Spores 54-88 μm, (ave. 70 μm), tuberculate, evenly spaced tubercles, laesura prominent (Fig. 6.2a,b).

Ecology

Lygodium circinnatum has a wide distribution, climbing young undergrowth in rain forests, second growth forests, mangrove swamps, and coconut plantations, often with fronds trailing on the ground. It grows on limestone in Sarawak, on marine strand vegetation in Malaya, and often on sandy dry slopes in China. It grows to 1000 m in elevation.

Distribution

India, Sri Lanka to South China (Hainan, Yunnan, Kwangtung, Kwangsi, Hong Kong,), through Malaysia (New Guinea, Sumatra, Malaya, Borneo, Java), Philippine Islands (Culion, Mindoro, Mindanao, Luzon, Leyte, Samar, Tawi Tawi, Palawan), Admiralty Islands, Caroline Islands, New Hebrides, Solomon Islands, to Australia. Fig. 10.7.

Comments

This species, fairly weedy throughout Asia and Malaysia, may be distinguished from *L. longifolium* by the entire segment margins and veins that end in a very thick marginal layer of cells. Its spores are tuberculate rather than verrucate (*L. longifolium*) and it has no swollen multicellular-based pinna-bud hairs. The latter feature also separates it from *L. auriculatum*, whose segments are semihastate at the base and most often bifid or entire. The juvenile fronds of *L. circinnatum* often have serrate margins and bear white multicellular hairs on veins and lamina.

In New Guinea an extract from the leaves is used to treat tooth aches. In the Philippines the common name is "nito" and it is used for weaving. In China the rachis is used for binding.

Selected Specimens Examined

China. Hainan: *McClure 20133* (GH); Hong Kong: *Wright s.n.* (GH); Kwangsi: *Ko 56113* (GH); Kwangtung: Kochow,*Tsiang 2219* (NY); Yunnan: *Cavalerie 2634* (K); *Wang 79930* (GH); Singapore: *Purseglove P5478* (GH-A); **India.** Sikkim: Darjeerling, *Bonaparte s.n.* (GH); **Sri Lanka.** Kitulgala, *Sledge 1405* (GH); **Borneo.** Sandakan: *Topping 1378* (GH); Sarawak: *Mjoberg s.n.* (NY); **Sumatra.** Banka: *Grushoffner 12* (NY); **Malaya.** Perak: *Allen 4080* GH-A); **Malaysia.** Selangor: *Worthington 13455* (NY); **New Guinea.** Papua: *Croft 1666* (GH-A); *Hoogland 3504* (GH-A); **Admiralty Islands.** *Croft 1173* (GH-A);

Solomon Islands. *Braithwaite 4192* (GH-A); **Indonesia.** Ceram: *Ramlanto 403* (GH-A); **Java.** *Blume s.n.* (GH); **Philippine Islands.** Culion: *Merrill 503* (GH); Luzon: Sorsogon, *Elmer 17396* (GH); *Topping 673, 936* (GH); Mindanao: *Ramos & Edano* (PNH #85268), (GH); Mindoro: *Sulit & Conklin 4609* (GH-A); Palawan: *Bourell 2266A* (GH); Samar: *Vidal 4118* (GH); Tawi Tawi: *Bartsch 162* (GH, P); **New Hebrides.** Vanikoro Island: *Kajewski 689* (GH); **Caroline Islands.** Palau: *Kanchira &Itatusima 4438* (GH).

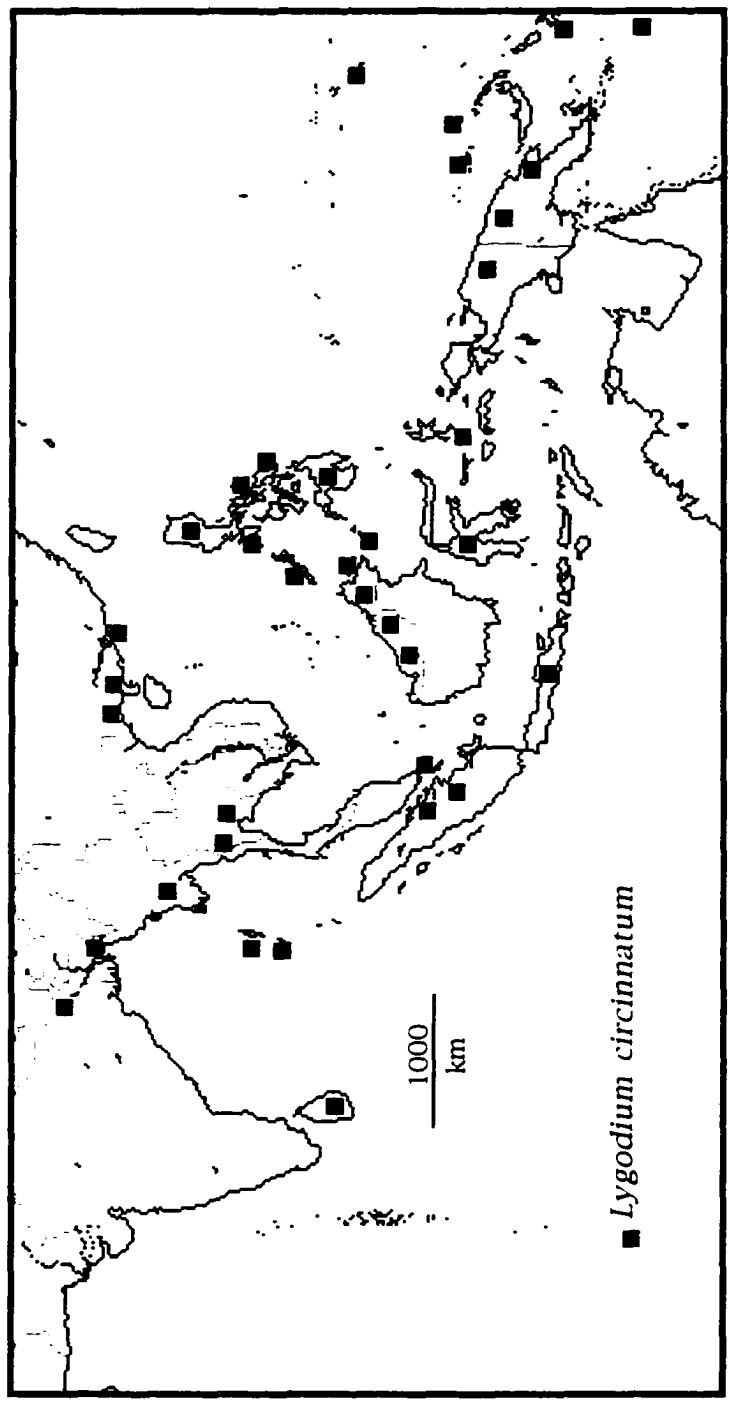


Figure 10.7. The geographic distribution of *Lygodium circinnatum*.

8. ***Lygodium longifolium*** (Willd.) Sw., in Schrader, J. Bot., (1801) 2: 305. 1803. *Hydroglossum longifolium* Willd., Abh. Kurfürstl.-Mainz. Akad. Nützl. Wiss. Erfurt 2(4): 22, t. 2. 1802 (Basionym). Type. India, Malabar, Herb. Willd. (B, holotype, fragment and photo K!). Fig. 10.31G-J.

Lygodium circinnatum var. *cristatum* Alderw., Bull. Dép. Agric. Indes. Néerl., 18: 5. 1908. Type.

Lygodium dichotomum Bedd., Ferns S. India, t. 62. 1863. Type. Travancore, *Johnston s.n.* (K). Non *L. dichotomum* (Cav.) Sw.

Lygodium digitatum Presl, Reliq. haenk., 1: 73. 1825. Type. Philippine Islands, Luzon, Sorzogon, *Haenke s.n.* (PRC, isotype K!).

Lygodium derivatum Alderw., Bull. Jard. bot. Buitenzorg. 3(5): 213. 1922. Type.

Lygodium teysmannii Alderw., Bull. Dép. Agric. Indes. Néerl., 18: 5. 1908. Type. Sumatra, *Teysmann 2304* (B!).

Rhizome short-creeping. Fronds approximate, climbing to 4-10 m. Stipe not observed. Rachis ca. 1-2 mm diam., narrowly grooved, with scattered 1-2-celled hairs. Pinna-stalk sessile to < 2mm. Dormant pinna-bud prominent, covered with long, multicellular reddish-brown hairs and swollen multicellular-based hairs. Primary pinna-branches dividing dichotomously 1-2 times, initial dichotomy < 5cm long, each dichotomy ending in bifid, palmate, 3-4 lobed, or rarely simple segments, grooved to slightly winged, glabrous to bearing short, 2-celled, acicular hairs. Segment-petiole if present, ca. 1-3 mm, winged, glabrous. Segments subcoriaceous, not articulate, segment or lobe linear to linear-lanceolate, 12-24 x 0.5-2.5 cm, cuneate at base, acute to acuminate at apex; glabrous. Veins free, forked 2 times, ascending at 40°-50°

angle from costa, ending at margin, costa prominent, secondary veins obscure, often < 3-5 mm apart; sparsely covered with transparent to white, multicellular, 4-5 celled, hairs. Margins serrate to serrulate, with thin marginal layer. Sterile and fertile segments dimorphic, lamina highly contracted, fertile pinna-branch often twice pinnate, primary branch terminating in a deeply forked 5-fid segment or dividing again, the dichotomy ending in simple, 2- to 4-fid segments with discrete lobes. Fertile segments simple or lobed, the lobes cut almost to the segment base, lamina reduced to 3-7 mm (measured at widest point on segment or lobe), sporangial pairs 4-10 per sorophore, glabrous adaxially, few hairs abaxially on veins and at base of sorophore (hairs as on sterile segments). Spores 100-104 μm (ave. 102.6 μm), verrucate, with prominent laesura and equatorial ridge on proximal face (Fig. 6.6a).

Ecology

Twining on shrubs and palms at the edge of lowland forests in dense undergrowth. In Borneo found in fresh water swamps and in Sarawak on shaded cliffs at 1200 m elevation. Like many species of *Lygodium* it is a successional plant in abandoned plantations (e.g., rubber plantation in Malaya) or disturbed forests. According to Holttum (1959) this species is not found in sites as exposed as *L. circinnatum.*

Distribution

South India (Sri Lanka, Meghlaya, Kerala), China (Hainan) and Malaysia (Malaya, Kingga Island, Singapore Island, Sumatra and Borneo). Fig. 10.8.

Comments

Lygodium longifolium differs from *L. circinnatum* in raised pinna-buds, polycellular swollen based bud-hairs, a serrate to serrulate margin and

verrucate spores. Its segments rarely achieve the size of *L. circinnatum* and are more linear. It has a narrower distribution than *L. circinnatum.* Prantl (1881) and Copeland (1958) reduced it to a synonym of *L. circinnatum,* while Holttum (1959) and Singh and Panigrahi (1984) in their respective studies in Malaysia and India considered them separate taxa. The only other *Lygodium* that it could be confused with is *L. auriculatum,* which has monomorphic segments that are auriculate at the base.

Nomenclatural Comments

The type specimen described by Willdenow consists only of fertile segments. One of characters that readily distinguishes *L. longifolium* from *L. circinnatum* is the type of margin: *L. longifolium* segments have serrate margins and *L. circinnatum* have entire margins. Since the type specimen lacks sterile segments this key character cannot be used in differentiating between these allied species. Alston and Holttum (1958) were able to examine spores from the Willdenow type and confirmed that the specimen was *L. longifolium* by the long-ridged verrucate spores (*L. circinnatum* spores are tuberculate).

Selected Specimens Examined

China. Hainan: *Lam 27393* (GH-A);*Tak 346* (UC); Singapore Island: *Zogg & Gassner 7329* (F, GH, UC); **Malaysia.** Negeri Sembilan: *LaFrankie 2980* (GH-A); Sabah: *Beaman 10308* (MICH); **Malaya.** Johore: *Kramer 9586* (F): *Sinclair 10564* (GH-A); East Coast: *Merchant 4276* (MICH); **Borneo.** Jesselton: *Topping 1916* (GH); Sarawak: *Bell 1973* (MICH); **New Guinea.** Papua: *Croft 166* (GH); **Sumatra.** without localtiy: *Burchard s.n.* (F); **Philippine Islands.** Palawan: *Sulet 14791* (MICH).

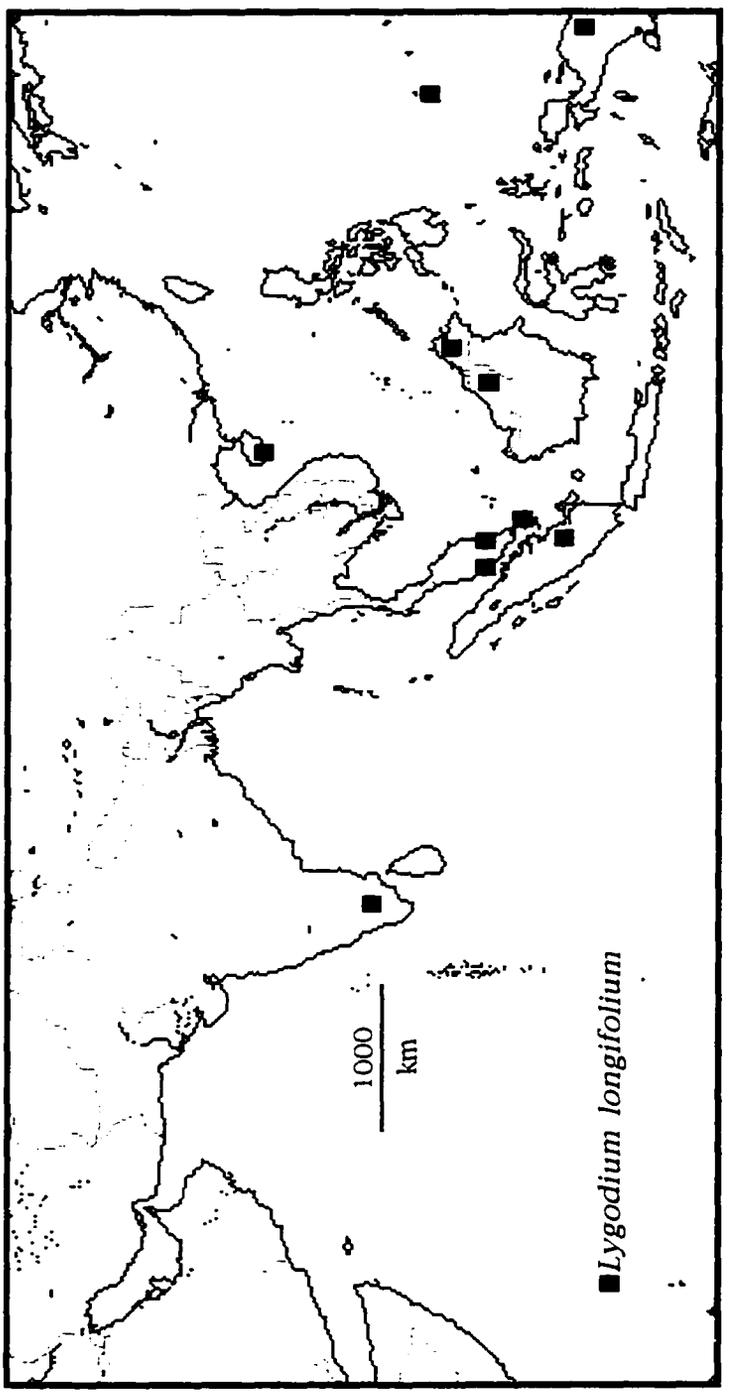


Figure 10.8 The geographic distribution of *Lygodium longifolium*.

9. ***Lygodium trifurcatum*** Baker, Syn. Fil., 437. 1868. *Lygodium circinnatum* var. *trifurcatum* Christ, Monsunia 1:93. 1901. Lectotype chosen by Holttum, 1959, Solomon Islands, *Milne 511* (K!); Solomon Islands, *Milne 591* (K!),*Veitch s.n* (K); Louissade Islands, *Macgillivray s.n.* (K); syntypes. Figs. 10.27A-G.

Lygodium dimorphum Copeland, Philipp. J. Sci. Bot., 6: 67. 1911. *Lygodium novoguineense* Rosenst., Feddes Repert., 9: 427. 1911. Type. New Guinea, Papua, Ambasi, *C. King 134* (BO, holotype; MICH! isotype).

Lygodium flexuosum Gaudich, Freyc. Voy. Bot., 298. 1826 [non(L.) Sw.] .

Rhizome short-creeping, with black multicellular hairs, Fronds climbing to 3-5 (10) m. Stipe not observed. Rachis 1-2 mm diam., grooved, with few multicellular hairs in groove. Pinna-stalk 0-1 mm, grooved. Dormant pinna-bud in pocket formed by bifurcation of winged pinna, covered with golden, long, multicellular hairs (septae dark brown) often interspersed with darker, reddish-brown swollen multicellular-based hairs (15+ cells long). Primary pinna-branches winged, dividing dichotomously, one side ending in a simple segment, the other in a bifid (rarely trifid) segment or two simple segments, bearing erect, 2-3-celled, light brown hairs. Segments subcoriaceous, linear to linear lanceolate, 10-18(22) x 1.5-2.5 cm, often strongly auriculate on one side at base, cuneate on the other, the outermost bifid segments auriculate on both or only on one side, sometimes producing a small orbicular segment, rarely truncate at base, acute to long-attenuate (sometimes weakly bipartite) at apex, the bifid segments lobed to within 1 cm of base, lamina glabrous. Veins free, 2-3 times forked, ascending from costa at 50°-60°, veins ending in marginal layer, costa sparsely pubescent, bearing occasional 3-4-celled thick-

walled, light tan cells. Margins irregularly serrate, thickened. Fertile and sterile segments strongly dimorphic, ultimate fertile segment 3-5 times pinnate. Fertile segments 9-12(18) x 1-1.5 cm (including sporangia), lamina < 0.25 mm surrounding veins, bearing sporangia at end of every vein, 3-4 sporangial pairs per sorophore, or in some specimens less dissected (2 times pinnate) with 10-12 pairs of sporangia per sorophore, pubescent with 3-6-celled, light tan hairs (septa dark brown) on midvein and veins near base of sorophore abaxially. Spores 75-90 μm (ave. 85μm), tuberculate-verrucate, small tubercles often surrounding fused verrucae, laesurae prominent (Figs. 6.7a,b).

Ecology

Lygodium trifurcatum grows in lowland rain forests with dense canopy, often in disturbed places or at edge of forest to 1000 m in elevation, and in lower montane forests at elevations of 2400 m. In the Admiralty Islands it climbs on vegetation in brackish marshes and in Papua, New Guinea grows next to hot streams and in thickets at the edges of mangrove swamps.

Distribution

New Guinea, New Hebrides, Solomon Islands, Admiralty Islands, Louissade Islands. Fig. 10.9.

Comments

Lygodium trifurcatum often resembles *L. auriculatum* in its sterile habit, but differs in its dimorphism. The sterile and fertile segments are of about equal size though the fertile portions lack any appreciable lamina. They appear laciniate and each vein ending bears usually 3-4 sporangial pairs. Many authors consider *L. trifurcatum* and *L. dimorphum* separate species. The only difference discerned in this study is in the degree of fertile

dissection. *Lygodium trifurcatum* is usually 3 times pinnate in the ultimate segments, some specimens having 20+ sporangial pairs (usually 4-6) whereas *L. dimorphum* is more dissected (4-5x pinnate) with uniformly 4-6 sporangial pairs. Further collections and ecological data need to be studied to assess the species status of these forms.

In New Guinea rachises used to weave "buka" baskets and in the construction of armlets and leg bands.

Selected specimens examined.

New Guinea. Papua Milne Bay, Menapi: *Brass 21637, 21640* (GH-A); Papua, Milne Bay, Bolu Bolu: *Brass 24400*(GH-A); Hollandia: *Baim s.n.*(GH); **Solomon Islands.** Auki District, Ura: *McKee 1599* (GH-A); Waimamura, SanCristoba: *Brass 2630* (MICH); Ysabel Group: *Brenchley s.n.* (F); **Admiralty Islands.** Los Negros: *Grether & Wagner 3997* (MICH, UC).

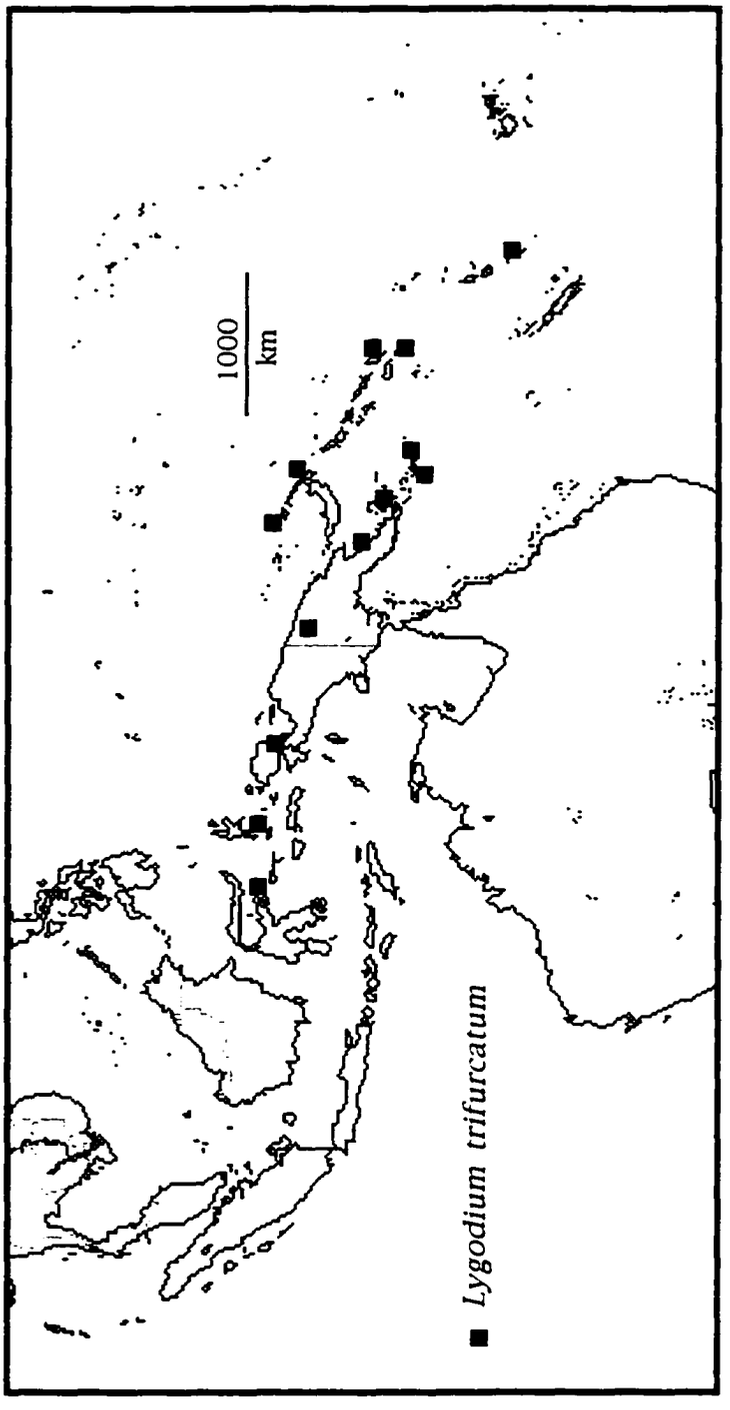


Figure 10.9 The geographic distribution of *Lygodium trifurcatum*.

10. ***Lygodium versteegii*** Christ, Res. Exp. Sci. Neerl. Nouv. Guinea 8: 163. 1910. Type. New Guinea, Noord River, *Versteeg 1400* (BO, holotype; fragment BM!; GH!, P!, isotypes). Figs. 10.28F-L.

Lygodium moskowskii Brause, Jahrb. Syst., 49: 57. 1912. Type. New Guinea, *Moszowski 214.* (B!)

Rhizome short-creeping, bearing swollen, multicellular-based, dark brown, lustrous hairs scattered among typical rhizome hairs; Fronds approximate, climbing 6-8 m. Stipe not observed. Rachis 2-5 mm diam, only slightly grooved, glabrous. Pinna-stalk 0-1 mm, pubescent with erect, white to light tan, 1-2-celled hairs and fewer 2-4-celled, light tan, septate (brown septa) hairs. Dormant pinna-bud inconspicuous in pocket surrounded by whorl of segments, slightly raised, covered with multicellular, golden to light tan, (septae, if visible dark brown), 6-10-celled hairs without swollen multicellular-base. Primary rachis-branches suppressed, each bearing 2-3 simple segments in a whorl around the pinna-bud, pubescent with short erect hairs as described for pinna stalk. Segments coriaceous, oblanceolate, (13)20-25(34) x 2.0-5.5 cm, truncate at base on one side while auriculate on other, the auricle sometimes becoming a separate small rounded or deltoid segment, and the base cuneate, the apex acute to long-attenuate; short hairs (as on axes) on lamina and veins common at base of segment, fewer distally. Veins raised reticulate, ascending at 30° from costa, ending at margin in thickened layer, hairy on the axes. Margins entire to crenulate with multiple cell layers. Fertile and sterile segments dimorphic. Fertile segments 18-25 x 0.3 cm, lamina reduced to < 0.25 mm along costa, covered with 4-7-celled hairs, bearing up to 150 sorophores per segment side, and 3-7(10) sporangial pairs per

sorophore; hairs prominent on indusia and under sporangia, veins anastmosing only once or free due to reduction of lamina. Spores 83-101 μm (ave. 91 μm), reticulate distal face only, proximal face smooth, laesurae prominent (Figs. 6.4a,b).

Ecology

Lygodium versteegii is found in mixed lowland forests up to 1500 m in elevation. In New Guinea seen growin up moss-covered tree at 800 m. It grows on porous soil where there is often a significant dry season.

Distribution

Philippine Islands (Luzon, Mindanao) to New Guinea. Fig. 10.10.

Comments

This species of *Lygodium* is unique in its segment size and dimorphism, particularly in the manner in which the segments arise around the suppressed primary rachis-branches and sessile pinna-stalk. The 4-6 segments appear to arise in a whorl around the rachis, forming a shelf-like ridge that supports the dormant pinna-bud. The rhizome was only observed in one specimen (*de Joncheere 1455*, K) and had unique swollen, multicellular-based hairs resembling those found on the pinna-buds of some species (e.g. *L. auriculatum*) scattered among normal rhizome hairs. These swollen based hairs are lustrous and dark brown.

Selected Specimens Examined

New Guinea, Idenburg River: *Brass 13441* (BM, GH-A, MICH); Papua: *Brass 7138* (MICH); *King 846* (MICH); *Pedermann 7075* (BM); Buso River, *Gawi 25* (GH-K); West Sepik Province, *Croft 1619* (GH-A), *Croft 388* (K); **Philippine Islands.** Luzon: *Escritor* (PNH # 20821, MICH): Mindanao: *Mendoza & Convocar* (PNH # 8700, MICH); **Celebes.** *Joncheere 1455* (K, MICH); *Balgooy 3914* (MICH).

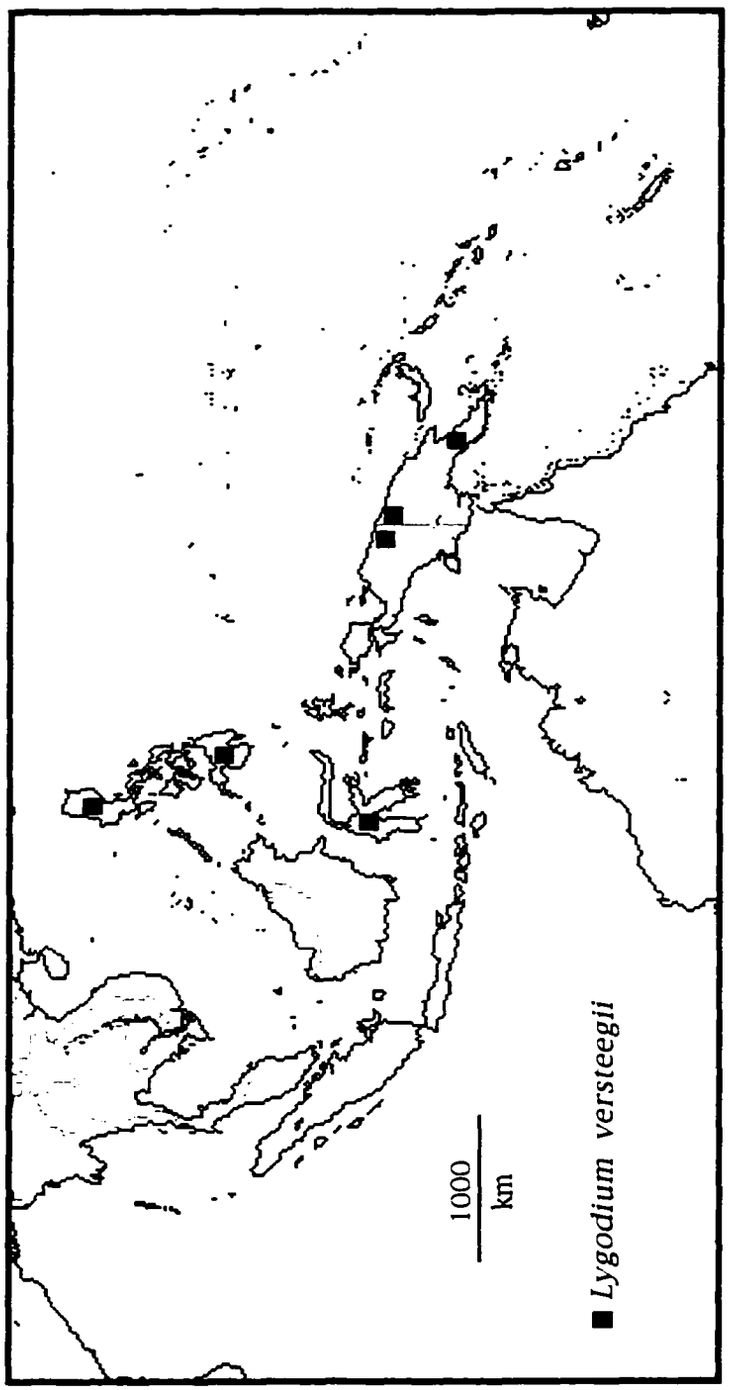


Figure 10.10. The geographic distribution of *Lygodium versteegii*.

11. ***Lygodium heterodoxum*** Kunze, Farrnkräuter. 2: 32, t. 113. 1849.

Hydroglossum heterodoxum (Kunze) Moore, Index. fil. I. cxiii. 1857.

Lygodictyon heterodoxum (Kunze) J. Smith, Ferns Brit. for. 259. 1966.

Type. Mexico, Oaxaca, *Galeotti 6419* bis (B!). Figs. 10.32D-F.

Hydroglossum mexicanum Fée, Mém. foug. 9: 42. 1856.

Syntypes. Mexico, Oaxaca, *Galeotti 6419* (BR), *Jürgensen 763* (B).

Hydroglossum spectabile Liebmann, Mexic. bregn. 299 (reprint 147). 1849.

Type. Mexico, Oaxaca, San Pedro Tepinapa, *Liebmann 936* (C).

Rhizome short-creeping, 0.5-1.0 cm diam. Fronds approximate, 1.5-10 mm apart, climbing to 3-6(10) m. Stipe golden brown, less than 5 mm diam., rhizome hairs 5.0 cm up base of stipe, then with shiny multicellular black hairs 2.5-3.0 mm long (7-8 cells). Rachis 2-4 mm diam., terete. Pinna-stalk 1-4 mm long. Pinna-bud slightly sunken, covered with multicellular (3-6 celled), bicolored (reddish basally to golden apically) hairs. Primary pinna-branches 1-4 times dichotomous, first dichotomy at 4-9 cm, often one side ending in segment while the other divides again, both slightly winged, glabrous or bearing few 2-celled hairs. Segment-petiole 1.5-3 cm long, winged, glabrous. Segments 2-, 3-, 4-fid, palmate, base cordate to auricled, rarely truncate at base, rounded to acute at apex, central segment-lobes longest, linear-lanceolate 15-22 cm long (measured to segment base) and 1.0-3.5 cm wide (above lobe bifurcation). Veins reticulate, ending at margin, 3-5 times anastomosing, 40°-50° angle from main vein, costa prominent, glabrous or bearing few, short, 2-3 celled hairs, prominent clavate unicellular hairs on costa and veins. Margins serrate-serrulate to crenulate with minimally thickened cellular layer. Sterile and fertile segments monomorphic; fertile

pinna-branches dichotomous to 4 times, segment-petioles 8-15 mm, often with multicellular hairs. Fertile segments bifid, less often 3-, 4-fid, cuneate to auriculate basally on both sides or cuneate on one side while auriculate on the other), acute at apex, 5-12 (-18) sporangial pairs per sorophore, lamina puberulent; indusia glabrous. Spores 85-100 μm (ave. 88 μm), tuberculate, with few, unevenly spaced tubercles (Figs. 6.2e,f).

Ecology

Found at elevations to 1500 m in lower montane tropical forests, secondary forests, moist oak forests, along wet roadsides and streams, often in disturbed areas twining around tree trunks and shrubs to 10 m. In Nicaragua reported as climbing to 65 m. In Oaxaca and Tabasco on limestone.

Distribution

Southern Mexico (Oaxaca, Tabasco, Vera Cruz, Chiapas), Guatemala, Belize, Honduras, Nicaragua, Costa Rica and Panama. Fig. 10.11.

Diagnostic Features

This is the only New World species with reticulate venation. A large degree of variation exists in the pinna stalk (from almost none to 4 mm), in the segment bases (from auriculate to cordate to truncate), in the number of dichotomies (1-4+), and in the number of lobes per segement (2-4, rarely 5). Many herbarium specimens show growth from the dormant pinna-bud, a feature that promotes thicket formation.

Lygodium heterodoxum and *L. merrillii* are two net veined taxa with similar morphologic characters probably explained by means of convergence rather than common ancestry. Their distribution presents an interesting problem as *L. heterodoxum* is found in the New World (Mexico to Venezuela) and *L. merrillii* is found in the Philippine Islands, Sumatra and Sarawak (Copeland,

1958). Both exhibit pinnate branching patterns with 2-4-lobed segments in which the lobes are discrete and almost equal in size. The lobes are supplied by veins that arise from the dichotomous branching of the costa, each dichotomy sending off a side vein as necessary depending on the number of lobes. The pinna-branches are often slightly flexuous in *L. heterodoxum*, which is glabrous whereas *L. merrillii* possesses a straight pinna-branch and is puberulent on the pinna stalk, bud, pinna-branches, petiole and often veins. The major difference between the taxa is that *L. heterodoxum* has tuberculate spores whereas *L. merrillii* has verrucate spores. The dormant bud in *Lygodium merrillii* is on a short stalk and is extensively covered with hairs some of which have a slightly swollen base consisting of 2-3 layers of 2-rowed cells (not as exaggerated as in *L. auriculatum*, etc.) whereas its New World counterpart consists of a raised bud with sparse hairs on a 3-4 mm stalk. *Lygodium merrillii* often has reddish-brown axes and bud hairs whereas the same structures are stramineous in *L. heterodoxum*. The sorophores in *L. merrillii* often appear pedicellate as has been seen in some other taxa of *Lygodium* (e.g., *L. borneense* and *L. pedicellatum*). These characters are summarized in Table 10.1.

Selected Specimens Examined

Mexico. Chiapas: *Palacios Rios 02826* (UC); Oaxaca: *Hallberg 1612* (UC); *Mexia 9212* (GH); *Mickel 7220* (NY, UC); Tabasco: *Davidse et al., 29502* (UC); Tehuantepec: *Ross 1062* (UC); Vera Cruz: *Nee 29972* (NY, UC); *Smith s.n.* (UC); **Belize.** *Peck* 599 (GH); Toledo: *Gentle 3997* (GH); **Guatemala.** Alta Verapaz: *Steyermark 44101* (UC); Izabel: *Ortiz 2418* (GH); *Standley 24876* (GH); Petén: *Steyemark 45959* (GH, UC); **Nicaragua.** Zelaya: Mt. Liveco, *Atwood 3034* (GH); *Pipoly 5317* (UC); **Honduras.** Santa Barbara: *Thieme 5663* (GH);

Lancetilla: *Yuncker 4720* (UC); **Costa Rica.** Guanacaste: *Flores 30* (UC); Limon: *Burger et al., 10446* (PMA); Puntarenas: *Sanders et al., 17695* (UC); **Panama.** Prov. Chiriqui: Charco Azul, *Him 344* (PMA).

Table 10.1. A comparison of *Lygodium heterodoxum* and *L. merrillii*.

Characteristic	*L. heterodoxum*	*L. merrillii*
pinna-stalk	± 4 mm	< 2 mm
pinna-bud	not prominent sparsely puberulent	very prominent pubescent with reddish hairs
pinna-branches (number times pinnate)	1x	1x
branch habit	flexuous	straight
number of segments per branch	2 +, terminal 2-3-fid	3-4 +, terminal 3-4-fid
segment shape	2-4(5)-fid lobes discrete	2-4(6)-fid lobes discrete
margins	crenulate to serrulate veins end at margins	irregularly serrate veins often end before margin
indument	axes relatively glabrous	all axes puberulent
spores	tuberculate	long-ridged verrucate

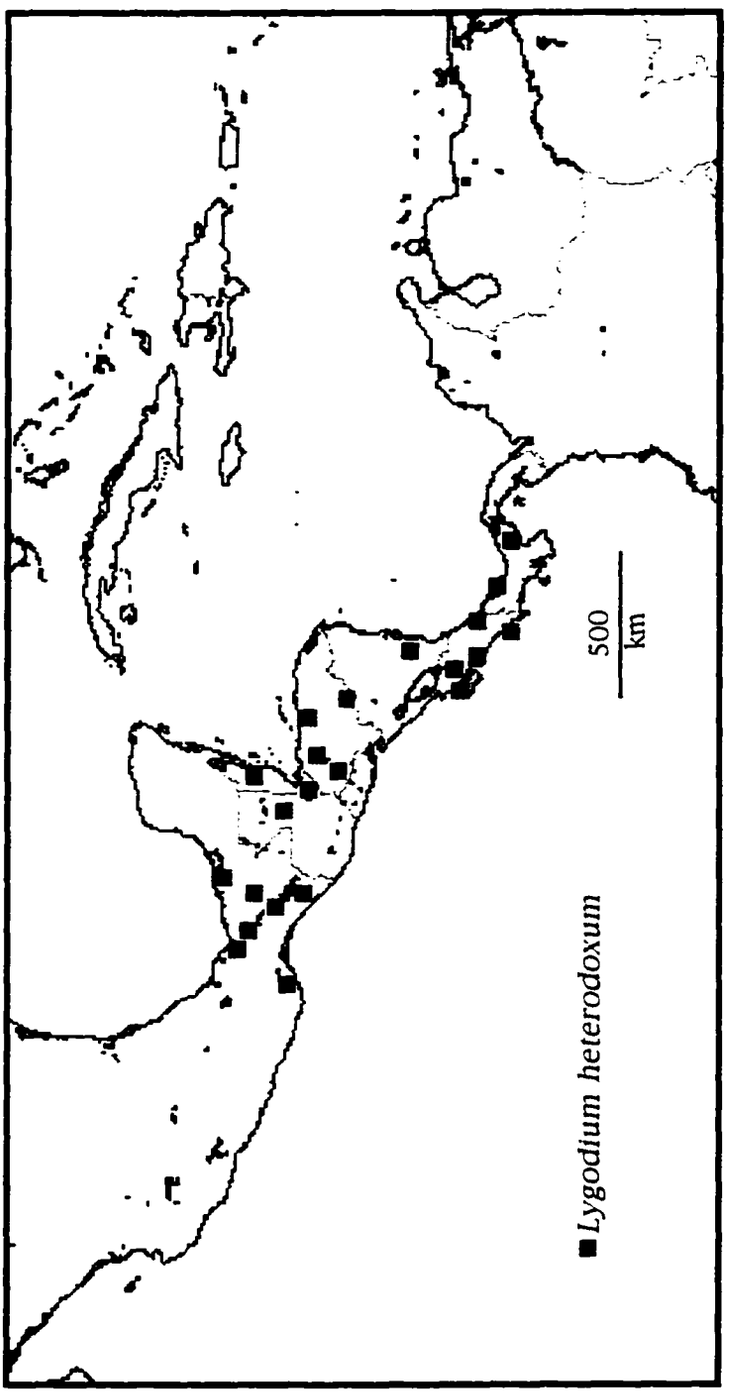


Figure 10.11 The geographic distribution of *Lygodium heterodoxum*.

12. ***Lygodium merrillii*** Copeland, Philipp. J. Sci. 2: 146, pl. 4. 1907.

Type. Philippine Islands, Mindoro, Binabay River, *Merrill 6057*(MICH!, holotype). Figs. 10.32A-C.

Lygodium matthewii Copeland, Philip. J. Sci. Bot. 3: 36. 1908.

Type. Philippine Islands, Luzon, Mt. Maguiling, *Matthew s.n.*. (MICH!; isotypes B!, K!).

Lygodium subareolatum Christ, Fil.Yunnan., 151. 1907. Syntypes. China, Kwei Chow, *Esquirol s.n.* (P; syntype), *Cavalerie s.n.* (P; syntype, photograph of portion of segment, BM!).

Rhizome short-creeping (only juvenile plants observed). Frond climbing to 10 m. Stipe not observed. Rachis 2-5 mm diam., terete, glabrous. Pinna-stalk 0-2 mm, glabrous. Dormant pinna-bud very prominent, covered with long, 8-celled, light tan to reddish-brown hairs (septa dark brown). Primary pinna-branches pinnate, weakly grooved, bearing 2 pairs of widely spaced (> 6 cm) alternate segments, and a terminal 2-5-fid segment, sparsely puberulent with erect, 1-3-celled hairs between grooves, hairs becoming abundant close to pinna-bud. Segment-petioles ca. 7 mm long, decreasing to 4 mm distally, slightly winged, with occasional hairs (as on pinna-branch), the wings increasing in width distally, merging with segment lamina. Segments 2-5-fid, the lobes discrete, linear, outermost shorter, longest 11-15(25) x 1-1.5(5) cm, shortest outer lobes, 5 x 0.8 cm, often unequally cuneate to cordate at base, acute to attenuate at apex, glabrous. Veins reticulate, petiole vein dividing dichotomously and again according to the number of lobes, anastomosing 1-2 (4) times and ending at margin, ascending at 40°-50° from main vein. Margins deeply serrate to serrulate, with thickened layer of cells. Fertile and sterile

segments monomorphic. Fertile segments with longest lobes ca. 15 x 1 cm, outer shorter lobes ca. 5 x 0.5-1 cm; segment-petioles > 10 mm proximally decreasing to 4 mm distally, 11-17 sporangial pairs per sorophore, bearing 4-celled light tan to golden hairs on veins at base of projection abaxially; indusia with crenulate margins, glabrous. Spores 79-94 μm (ave. 87 μm), sparsely verrucate with an uneven surface, verrucae forming ridges with prominent laesurae and proximal equatorial ridge (Figs. 6.6b,c).

Ecology

Grows in forests, to altitudes of 600 m in Philippines and to 800 m in Sarawak.

Distribution

Philippine Islands (Leyte, Luzon, Mindanao, Mindoro, Negros), Borneo (Sarawak), South Sumatra, and China (Tonkin, Kweichow; as *L. subareolatum*). Fig. 10.12.

Comments

Lygodium merrillii has reticulate venation and large, mostly palmate segments arranged pinnately on the primary pinna-branch.
The large form of this species (*L. matthewii*) has never been collected in fertile condition. This species resembles some forms of *L. flexuosum* from Borneo and New Guinea in segment shape but the latter has free veins. There is also some similarity between *L. merrillii* and the New World reticulate-veined taxon, *L. heterodoxum* (see Comments of *L. heterodoxum).*

Nomenclatural Features

Lygodium matthewii, also named by Copeland, is a much larger form of *L. merrillii. L. subareolatum* Christ appears to be a synonym of *L. merrillii.* In his description, Christ indicates an anastomosing venation pattern much like

that of the New World *L. heterodoxum* with a segment dissection between *L. auriculatum* and *L. circinnatum.* A photograph (BM) of a portion of a segment from a Cavalerie specimen at Paris, (where Alston saw the type of Cavalerie; Alston and Holttum, 1959), clearly shows the reticulate venation. This collection was from Kwei Chow. A tracing of a large, 8-fid segment and a fragment of a leaflet with net veins, were also seen from the Herb. Christensen, collector *Bourrel 116 bis*, from Tonkin (BM). Both Cavalerie and sketch were seen by Holttum (1959), who included the collection sites in his distribution of *L. merrillii.* A fragment (F) of a fertile small segment and portion of a lobe of a plant from China determined as *L. subareolatum* with net veins was very similar to *L. merrillii* in segment shape and net vein pattern. Unfortunately, the spores were collapsed and probably immature. One or two spores appeared to have long ridged verrucae. In Christ's description he states that he based his description on the specimens of Esquirol and Cavalerie. Since I have received all the specimens of *Lygodium* from Paris (as well as the types) and this collection was not among them, the Esquirol collection appears to be missing. Because of the tracing of a specimen from Tonkin (BM), the photograph of a segment from Kwei Chow (BM) and the fragment of the collection (without collector) from Field Museum, all with net veins and all from China, I believe *L. subareolatum* to be conspecific with *L. merrillii.* No other net veined taxa occur in China. All other collections determined as *L. subareolatum* that I saw were *L. circinnatum* (with free venation).

Selected Specimens Examined

Philippine Islands. Leyte: *Wenzel 438* (F); Luzon: *Elmer 17993* (F, GH, MICH); *Krukeberg & Braun s.n.* (GH); *Ramos & Edano s.n.* (MICH, NY, UC); *Topping* 1318 (GH, MICH, UC); Mindanao: *Frake s.n.* (GH); Negros: *Price 3059*

(MICH); **Borneo.** Sarawak: *Matthew, s. n.* (MICH); **Sumatra.** Lebong Tandai, *Brooks 24S* (BM).

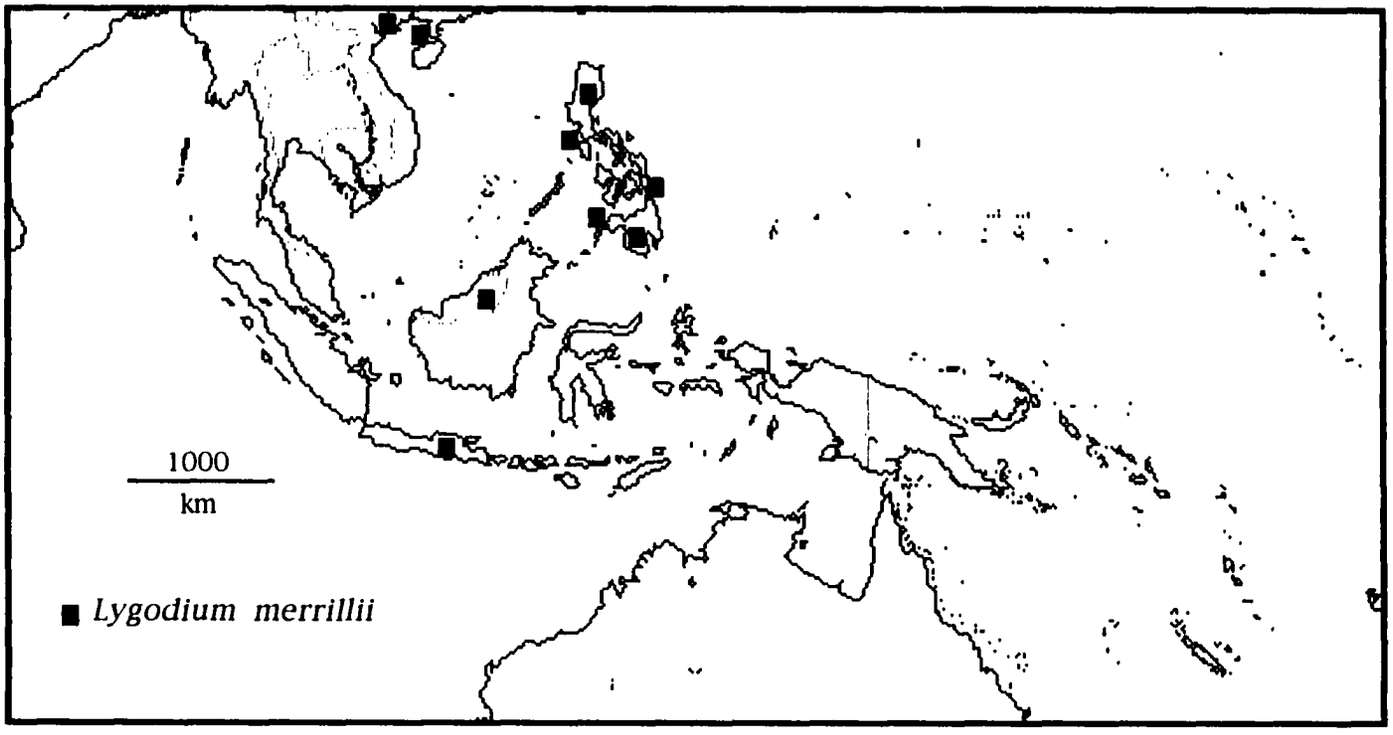


Figure 10.12. The geographic distribution of *Lygodium merrillii*. Specimens from Kweichow Island, China and Tonkin, Viet Nam are collections of *Lygodium subareolatum*, which has been synonomized with *L. merrillii*.

13. ***Lygodium flexuosum*** (L.) Sw., Schrader, J. Bot., 1800, 2: 106. 1801. *Ophioglossum flexuosum* L., Sp. Pl., 1063. 1753. *Hydroglossum flexuosum* (L.) Willd., Abh. Kurfürstl.-Mainz. Akad. Nützl. Wiss. Erfurt 2: 23, t.1, f.3. 1802. *Ramonda flexuosa* (L.) Mirb., Bull. Soc. Philom. Paris 2: 179,t. 12, f.3. 1800. Type. Ceylon, *Hermann 375* (BM), Microfiche Linnaeus Herbarium No. 1243.5 (NY!). Figs. 10.33A-D.

Lygodium scandens (L.) Sw., Schrader, J. Bot. 1800. 2. 106 (1801). *Ophioglossum scandens* L., Sp. Pl., 1063, p.p. 1753. Lectotype, chosen by Alston and Holttum (1959), Ceylon, *Herman 374* (BM; microfiche 1243, NY!)

Hydroglossum pinnitifidum Willd., Abh. Kurfürstl.-Mainz. Akad. Nützl. Wiss. Erfurt 2: 21, *p.p.* 1802. *Lygodium pinnitifidum* (Willd.) Sw., Schrader J. Bot., 1801, 2: 303. 1803. Type. India. Herb. Willd. (B; photograph NY!).

Lygodium altum (C. B. Clarke) Alderw., Malayan Ferns, 114. 1908. *Lygodium flexuosum* var. *alta* C.B. Clarke, J. Linn. Soc., 25: 101, t. 44. 1890. Type. India, Muneypoor, *Clarke 42331B* (K!).

Lygodium flexuosum var. *setulosum* Tard. & C. Chr., Fl. Gen. I.-C. 7: 39. 1939. Type.

Lygodium pilosum Desv., Mém. Soc. Linn. Paris 6: 205. 1827. Type. Java, *Commerson s.n.* (P!).

Lygodium semibipinnatum R. Br., Prod. Fl. Nov. Holl., 162. 1810. Type. *Brown s.n.* (K!).

Lygodium serrulatum Bl., En. Pl. Jav., 254. 1828. Type. *Zippell s.n.*, (according to Alston & Holttum at Buitenzorg).

Rhizome short-creeping, 4-6 mm diam., bearing shiny black rhizome hairs. Fronds approximate, climbing to 8 m. Stipe dark brown at base becoming stramineous distally, rhizome hairs in proximal 3 cm, glabrous distally. Rachis ca. 2-3 mm diam., grooved, glabrous. Pinna-stalk 1-3 mm (rarely to 4 mm; usually < 2mm), grooved. Pinna-bud raised, covered with multicellular golden to reddish-brown hairs (2 mm long), septa reddish. Primary pinna-branches pinnate (rarely 2x pinnate), narrowly winged, with 2-3 alternate segments, ending in a simple or bifid segment; glabrous to pubescent with white, 2-celled (0.25 mm long), appressed hairs. Segment petioles ca. 8 mm proximally, decreasing to 2 mm distally, winged, without articulation zone or with slightly thickened zone at petiole/segment junction, hairs as on pinna-branches. Segments simple, lanceolate-oblong or bearing an auricle on one side or a small lobe on each side, 6-12 cm x 1.5-2.0 cm (measured above auricle or lobes), outer lobes frequently as separate segments, base truncate to cordate, apex acute; indument variable (hairs as on axes). Veins free, 2 times forked, ascending at 30°-45° angle from costa, ending at margin. Margins strongly serrate to serrulate, not thickened. Fertile and sterile segments monomorphic to slightly dimorphic in smaller fertile segments; primary pinna-branch rarely 2 times pinnate; sporangia on sorophores, often very long. Fertile segments simple to auriculate on one side or both, sometimes slightly smaller than sterile ones, deltoid, bearing 2-celled, acicular hairs on axes and lamina abaxially, glabrous adaxially; 4-10(16) sporangial pairs per sorophore. Spores 74-92 μm (ave. 88 μm), evenly tuberculate (Figs. 6.1f,g).

Ecology

Lygodium flexuosum has a wide geographic distribution and may be found in wetter habitats than *L. japonicum*. It climbs over shrubs in forests and over

grasses in savannas. In Thailand, it can be found on dry sandy soil along beaches. It tolerates more shade than sympatric species of *Lygodium* (e.g., *L. scandens*). It grows up to 1600 m in elevation in the Himalayas.

Distribution

India (most all states), Sri Lanka, Nepal, China (Hainan, Kwangi, Kwantung, Yunnan), Myanmar, north Thailand, Malaya, Indonesia, New Guinea, Philippine Islands (Luzon, Negros, Culion, Leyte, Mindanao) and Australia (Queensland). Fig. 10.13.

Comments

This species intergrades with *L. japonicum* (see Comments under that species). It varies greatly in indument: all Philippine and Malayan specimens are so densely pubescent that the axes and often the lamina cannot be seen, (although the fertile portions may have fewer hairs) whereas those of New Guinea and much of China are relatively glabrous. The pinna-stalks may be elongated to 3 mm, which then intergrade with those of *L. japonicum.* This species also is often difficult to separate from *L. salicifolium,* which usually has more segments per primary pinna-branch, segments that are almost always simply linear-lanceolate, an obvious articulation zone at the junction of the petiole and segment covered with hairs, and all segment petioles of equal length. As with *L. japonicum* this species is not entirely distinct from the New World *L. venustum.*

In Indonesia the common name for *L. flexuosum* is "ribu ribu". It is used in the Philippines for handicrafts as is *L. japonicum* and is applied externally in the treatment of skin ailments (e.g. ringworm).

Nomenclatural Comments

According to Alston & Holttum (1959) the type, *Hermann 375,* is part of a sterile frond of a juvenile plant, while the fertile frond preserved with it as

Hermann 374 was called *Ophioglossum scandens* by Linnaeus. Alston & Holttum selected this collection (*Hermann 374*) as the type of *O. scandens* L. which, therefore, becomes a synonym of *L. flexuosum.*

In this treatment, *Lygodium altum* has been synonymized with *L. flexuosum.* The segments of *L. altum* are entire, long (15 x 1.5 cm) with only 2 segments on one side of the pinna-branch and one on the other. The branch ends in a bifid segment. The stalk is 4-5 mm, and petiole and pinna-branches are winged. The segments are not articulate. The type specimen has tuberculate spores as found in L. *flexuosum.* Its distribution is Burma and India. In some respects this "species" is also closely related to *L. salicifolium* and potentially could be considered conspecific with it. *L. salcifolium* has more segments/pinna-branch and the segments are articulate. However, segments are similar to *L. salicifolium* with classic cordate bases and a linear-lanceolate shape (see *N. Parry 380*, K). The other commonality between *L. altum* and *L. salcifolium* is the presence of swollen, multicellular-based dormant bud hairs, which are only found in *L. flexuosum* as the intermediate, 2-rowed stage. Considering the variability in *L. flexuosum*, and the lack of plasticity in *L. salcifolium*, I have chosen to treat *L. altum* as conspecific with *L. flexuosum.*

Lygodium giganteum Tagawa and Iwatsuki could be considered conspecific with *L. flexuosum.* It is a large segment form that has twice pinnate fertile pinna-branches I have not seen the type of this taxon and have only seen one specimen from Thailand (*Anderson 3730* , UC). It has joints at the petiole/pinna-branch junction which are lacking in *L. flexuosum.* The twice pinnate condition of the fertile segments with each secondary pinna-branch bearing 2-3 segments is also not common in *L. flexuosum.* More specimens must be seen to determine its taxonomic placement. However, many collections have been seen of *L. flexuosum* from Thailand and Myanmar

(Burma) with polymorphic segments: one in particular resembles the type description of *L. giganteum* and also the Anderson collection (UC) mentioned above. The segments in these collections are very large and multi-lobed with strongly jointed areas on the primary and secondary branches and these also have tuberculate spores (*Dickason F161*, Burma, MICH; *Conniff 22*, North Siam, GH; *McKee 5998*, Burma, GH). The Anderson collection unfortunately contains immature sporangia. Until more specimens are seen and probably more collections made, this species is questionable and resembles *L. flexuosum* more than any other *Lygodium* species.

Selected specimens examined

India. Assam: *Parry 380* (K); Darjeerling: *Griffith 1206* (GH); Nepal: *Stainton et al., 6780* (GH); Travancore: *Kanothyeshoda 584* (GH). **Nepal.** *Kanai et al., 413* (GH). **Sri Lanka.** *Ferguson 2* (GH). **China.** Hainan: *Lau 3834* (GH); Kwangsi: *Tseng 23120* (GH); Kwangtung: *Merrill 10890* (GH-A). **New Guinea.** Papua: *Maketa 19* (GH); *Kairo 627* (GH-A). **Myanmar (Burma).** Syriam: *Mc Kee 5804* (GH-A); **Thailand.** *Congdon & Hamilton 256* (GH-A); Kanburi, *Smith 429* (GH). **Malaya.** Kepong: *Allen 1360* (GH-A); **Java.** Bogor: *Lorzing 13028* (GH-A); **Philippine Islands.** Luzon: Rizal, *Topping 898* (GH); Bataan, *Topping 835* (GH); Australia. Queensland, Cook district, *Jones 3687* (CBG).

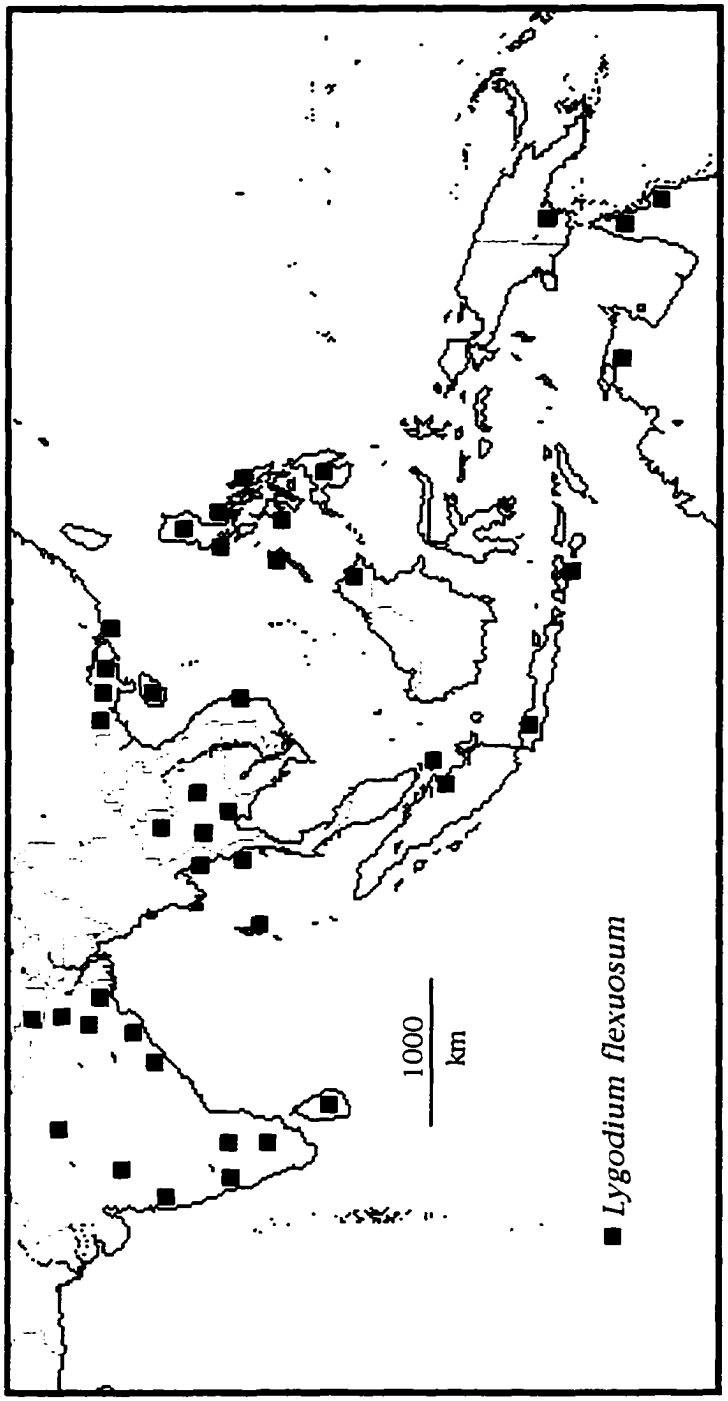


Figure 10.13. The geographic distribution of *Lygodium flexuosum*.

14. ***Lygodium japonicum*** (Thunb.) Sw., Schrader, J. Bot., 1800 (2): 106. 1801. *Ophioglossum japonicum* Thunb., Fl. Jap., 328. 1784. Basionym. *Hydroglossum japonicum* (Thunb.) Willd., Abh. Kurfürstl.-Mainz. Akad. Nützl. Wiss. Erfurt 2(4): 26. 1802. Type. Japan, *Thunberg s.n.* (UPS, holotype; microfiche No. 25222, NY!). Figs. 10.34E-G.

Lygodium chaerophylloides Desv., Mém. Soc. Linn. Paris 6(2): 205. 1827. Type. India, Herb. Desv. (P; photograph BM!(barcode #93671)

Lygodium cochinchinense Desv., Mém. Soc. Linn. Paris 6: 206. 1827. Type. Herb. Desv. (P).

Lygodium dissectum Desv., Berl. mag., 308. 1811. Type. India, *Levallée s.n.* (P!).

Lygodium japonicum var. *elongata* Alderw., Bull. Jard. bot. Buitenzorg. 2(1): 10, t. 3. 1911. Type. (B).

Lygodium mearnsii Copeland, Philipp. J. Sci., 3: 37. 1908. Type. Santo Domingo de Basco, Batan Isl., *Mearns s.n.* (MICH!).

Lygodium microphyllum Link, Hort. berol., 2: 141. 1833. Type. No locality given [LE, photograph BM! (barcode # 93674)].

Lygodium microstachyum Desv., Berl. mag., 308. *Lygodium japonicum* var. *microstachya* (Desv.) Tard. & C. Chr., Fl. Gén. I -C, 6(2): 38. 1939. Type. Philippine Islands, Luzon, Manila, *Commerson 106* (P!).

Lygodium pubescens Kaulf., Enc. Fil., 47, t.1, f.4. 1824. Type. Philippine Islands, Luzon, Manilla, *Chamisso s.n.* (B!).

Lygodium tenue Blume, En. Pl. Jav., 254. 1828. Type. Philippines, *Cumming s.n.* two sheets (B!).

Rhizome short-creeping, ca. 5 mm diam., covered with shiny black to dark reddish-brown hairs. Fronds approximate, 0.5-1.0 cm apart, climbing 2-5 m. Stipe dark brown becoming stramineous; rhizome hairs continuous through 2-4 cm at base of stipe, replaced by long, 7-10 celled light tan hairs with dark brown septa. Rachis ca. 2 mm diam., grooved, with hairs as on stipe, becoming glabous distally. Pinna-stalk 3-5 mm, grooved. Pinna-bud raised, covered with long, light tan multicellular hairs (septa brown). Primary pinna-branches 1-2x pinnate, with 2-4 alternate segments or secondary pinna-branches, narrowly winged, glabrous to densely pubescent with 1-2-celled, light tan acicular hairs and longer multicellular hairs (septa not obvious). Secondary pinna-branches pinnate with 2-3 alternate segments, ending in a segment, often with a long central lobe, narrowly winged, indument variable, if present, hairs as on primary rachis branches. Segment-petioles narrowly winged, < 5mm long proximally, shorter distally, distal segments often sessile, thinly to densely pubescent. Segments subpalmate, 3-5 lobed, base cuneate to cordate, acute at apex, the linear central lobe longest, 2.5-5.0 x 0.5-1.0 cm, outer lobe(s) less than 1/3 as long, often with hairs as on other axes. Veins free, central lobe 2-3 times forked, ascending at 25°-30° angle from costa, the costa hairy on both surfaces, veins ending at margin. Margins lobulate, individual lobes serrulate, not thickened, veins often extending from margin as papilla. Fertile and sterile segments slightly to strongly dimorphic; primary pinna-branches often 3 times pinnate, secondary pinna-branches bearing 3-5 tertiary branches or segments, tertiary branches with 2 pairs of alternate segments and a terminal simple one, the lamina sometimes reduced to varying degrees. Fertile segments subpalmate, 3-5-lobed, the lobes discrete to completely separate, sporangia on sorophores bearing 4-8(15) sporangial pairs, in highly dimorphic forms the lamina is reduced to < 2mm (sterile 5-6

mm wide); hairs as on axes, often present on lamina, veins and indusia. Spores 70-79 μm (ave. 76 μm), evenly low-tuberculate, laesurae prominent (Figs. 6.8e,f).

Ecology

This species grows in habitats subject to a defined dry season at which time the fronds die back. It is found in disturbed forests over a wide geographical range. It is easily naturalized as can be seen in the southern United States: in Florida and Texas mature fronds produce spores and then die back. It occurs in wet sandy soil, swamps, broadleaf evergreen forests and seaside and mountain slope communities to altitudes of 2000 m (India and Burma).

Distribution

India (Assam, Punjab), Sri Lanka, Nepal, China (Anhwei, Chekiang, Hainan, Hunan, Kiangsi, Kwangtung, Kweichow, Nanking, Szechuan, Yunnan provinces), Korea, Japan, Myanmar, Thailand, Vietnam, Indonesia, Philippine Islands (Leyte, Luzon, Negros, Mindanao, Romblon, Sibuyan, Surigao), New Guinea to Australia (Queensland). Singh and Panigrahi (1984) report the species from Thailand and Vietnam. It appears to be absent from Sumatra, Borneo and the Malay Peninsula which experience dry seasons only in the extreme north. The species has been introduced and naturalized in the Southern United States and Puerto Rico. Fig. 10.14.

Comments

There is a large degree of variability in the indument and segment size in *Lygodium japonicum*. Specimens from the Philippines all are densely pubescent while those of China and Japan have little indument (this same geographic variability occurs in *Lygodium flexuosum*). The specimens from Thailand are much smaller than the Japanese forms but have a dense

indument (Tagawa & Iwatsuki, 1989). The naturalized populations in the United States all have fairly short pinna stalks (2-3 mm), whereas those of China have the longest (5-10 mm). Also, specimens from China are often almost monomorphic with very long sorophores with the indument confined to segment veins and lacking on indusia and lamina. A form of this species (= *L. microstachyum* Desv.) has a very long (7-8 x 0.5 cm) central linear lobe, serrulate margins (not lobulate), and segment bases truncate to cuneate. This taxon was described by Desvaux for those forms that had a narrow, linear, elongated central lobe which was common in the collections from the Philippines and China, and on quick perusal appears very distinct from the typical *L. japonicum.* Prantl (1881) synonymized *L. microstachyum* with *L. flexuosum* probably because of the serrate margins without the lobes. However, in all other characters this species represents a geographic form of *L. japonicum.* As in *L. japonicum* these collections have veins that extend beyond the marginal teeth as small papillae.

The type specimen of *L. mearnsi* (synomized here with *L. japonicum*) has the characteristic morphology of *L. japonicum.* This has been noted by Holttum on the type specimen. The spores are slightly shrunken and probably devoid of a perispore, however, they appear verrucate. Other specimens determined as *L. mearnsii* (e.g., *Fenix s.n.*, Philipp. Bur. Sci. # 3651, NY) have a long pinna stalk, segments often having two equal sized central lobes, pubescence on the adaxial costa, biserrate margins, apex of longest lobe often divided, and tuberculate spores. Therefore, in the type specimen we see segment dissection like *L. japonicum* but with probably different spores, while in the Fenix specimen the spores are the same as *L. japonicum* but the segment dissection is different. None of the *L. mearnsii* specimens exhibit net veins. It is possible that this might be a hybrid between *L. japonicum* and *L. flexuosum*

or one of the former specimens and *L. merrillii.* More collections must be examined to determine spore morphology and ploidy levels as well as surveys of the other *Lygodium* taxa in the same geographic range before *L. mearnsii* can be firmly synonomized with *L. japonicum.*

Lygodium japonicum intergrades with *L. flexuosum*: the two species are found within the same geographic range but the latter can tolerate wetter, shadier habitats. *Lygodium flexuosum* most often has shorter pinna stalks, serrate to dentate margins (not lobulate); its segments are entire to auriculate on one or both sides, to tripartite, and are rarely dimorphic. However, these characters are variable. Since the ranges of *L. japonicum* and *L. flexuosum* overlap, it is highly probable that hybridization occurs. There are many specimens that combine the characteristics of both and yet do not have the abortive or larger spores indicative of hybrids. These may represent varieties.

The *Lygodium japonicum* "complex", pantropical in distribution, consists of *L. venustum* in the New World tropics, *L. kerstenii* in Africa and *L. japonicum* in Asia, Malaysia, Indonesia and introduced in the New World. The group exhibits a large degree of polymorphism. The characteristics of the complex are: pinnate branching pattern; 3 to 7-lobed, subpalmate segments, the central lobe 2-3 times longer than the outer lobes, the margin of the outer lobes serrate while that of the central lobe is lobulate, serrate or pinnaifid; dimorphic fertile portions with the extreme forms having a very reduced lamina; and tuberculate spores. In the New World species, *L. venustum,* sterile portions are usually only once pinnate whereas fertile axes may be twice pinnate. This species, therefore, represents the lesser dissected member of the complex as both *L. japonicum* and *L. kerstenii* are usually twice pinnate on sterile pinna-branches to 3-4 times pinnate on fertile branches. The segments may be regularly 5-parted in *L. venustum* with the outer lobes perfectly

symmetrical (as in the sometimes segregated, *L. mexicanum*) to those in which the segments have asymmetrical basal lobes. The segments of *L. japonicum*, in general, are much smaller than those of *L. venustum* or *L. kerstenii*. Concommittently the fertile segments are smaller and more dimorphic. *Lygodium kerstenii* often has segments in which the lower lobe(s) has become reduced and the central lobe contains pointed lobules at the margins so that the segment is nearly pinnatifid. These extreme forms of *L. kerstenii* more resemble the segment dissection of *L. polystachyum* than the other members of the *L. japonicum* complex. The segment variability of *L. kerstenii* was one of the key characters in the original description by Kühn that distinguished it as a separate species from *L. japonicum*. The same segment morphology has been noticed in a few specimens of both *L. venustum* and *L. japonicum* but without the same consistency or regularity of occurrence.

All three species also exhibit a great deal of variability in degree of pubescence and types of hairs present. Many populations of *L. japonicum* in China are pubescent with the abaxial segment surface bearing 1-2-celled erect acicular hairs on both the lamina and veins and longer hairs on the axes. These erect, 1-2 celled hairs are not present to the same degree on pubescent forms of *L. venustum* or *L. kerstenii*, where the indument consists of longer multicellular hairs. In *L. kerstenii* 3-5 celled, appressed hairs are numerous on the adaxial as well as abaxial costa. Small unicellular, clavate hairs are obvious on the lamina and veins of *L venustum* but not the other two taxa. Articulation zones at the petiole/segment junction are present in South American specimens of *L. venustum* and lacking in those populations in Central America. The articulation area is not as discrete as that in *L. microphyllum* where the segment abscises from the petiole leaving a small

crescent of cells attached to the petiole. Taxa of *L. japonicum* and *L. kerstenii* lack articulation.

Veins end at the margin often extending into the marginal layer of cells and into the serrations as papillate extensions in *L. japonicum* and *L. venustum* but rarely in *L. kerstenii* (only some collections from Natal and South Rhodesia). In general, the margins of the segments in this latter species are less serrate and often crenate. Hairs projecting from the segment margins are present only in *L. venustum.*

Both *L. venustum* and *L. kerstenii* possess larger segments, more segments/pinna-branch and are more robust climbers than *L. japonicum.* The above morphological characteristics are all varible to some degree in each of the species discussed. It is possible that this complex represents one taxon undergoing speciation. Secondly, this could simply represent one polymorphic species with various ecotypic character differences and variations. The problem with the latter explanation is that *Lygodium* grows in the same type of habitat in all three geographic areas and one would expect to find the same basic degree of character variability in all three taxa. A third possibility is that there are different ploidy levels present in the taxa, possibly increasing phenotypic variability. *Lygodium japonicum* and *L. venustum* have been found as both diploids and tetraploids (no chromosome counts are available for *L. kerstenii*). Table 10.2 summarizes the character differences and intergradations noted in the above discussion.

Selected specimens examined

China. Anhwei: *Chin 8432* (GH); Chekiang: *Cleo & Wilson 175* (GH-A); Hainan: *Lon-bo 0181* (NY); *Wang 33781* (NY); Hong Kong: *Kuntze 3370* (NY); Kiangsi: *Lau 4729* (GH); Kwangtung: *Tak 00317* (NY); Kweichow: Machang, *Tsiang 9566* (NY); Nanking: *Ching 7352* (NY); Sichuan: *Boufford 24248* (GH); Yunnan:

Ching 7352 (NY); *Wang 73146* (GH); **Taiwan.** Heng chung Experimental Station: *Ream 444*, GH ("*microstachyum*" form); **Japan.** Honshu: *M. Tagasi 1181* (GH); Kyushu: *Mimore et al., 3079* (GH-A); Nagasaki: *Maximowicz 126* (NY): Okinawa: Koza, *Koyama 7326* (NY; "*microstachyum*" form); Suzaki Izu Peninsula: *Kanai s.n.* (GH): **India.** Assam: *Clarke s.n.* (NY); Punjab: *Stewart 2307* (NY); **Nepal.** *BisRam s.n.* (NY); **Myanmar (Burma).** Myitkina: *Smith 73* [mixed collection with *L. flexuosum*(GH)] ; **Vietnam.** Tourane: *Clemens 3003* (NY); **Java.** Bogor: *Kostermans 18140* (GH); **Philippine Islands.** Leyte: *Copeland 21* (GH); Luzon: *Topping 690* (GH); Mindanao: *Fenix s.n.* (GH); Negros: *Elmer 9945* (NY); Romblon Island: *Bartsch 305;* Sibuyan: *Elmer 12447, Fay 1910* (NY); Surigao: *Wenzel 3155* (GH); **Australia.** *Baenitz s.n.*, Flora Silesiaca Breslau (GH); **United States.** Alabama, *Dukes 70* (NY); Florida, Bradford County, *Correll & Correll 54131* (NY); Georgia, Ware County, *Loconte 1121* (NY); Louisiana, Webster, *Thomas 138361* (NY); Mississippi, Pearl River County, *Feibelman 12 (*NY); North Carolina, Fayetteville, *H. Rankin s.n.* (NY); South Carolina:, Dorchester, *Correll* 5356 (NY); Texas, Jasper County, *Correll & Correll 12529* (NY).

Table 10.2. Characteristics of the *Lygodium* species in the "*japonicum*" complex. Three species of *Lygodium* are included in this complex of species. They are *L. venustum,* found in the New World tropics, *L. japonicum* found in Asia and Indonesia, and *L. kerstenii* found in Africa.

Species	Times pinnate		Segment Articu-	Marginal		Club Shaped	Pinna Stalk Length
	sterile	fertile	lation	Teeth	Hairs	Hairs	(mm)
L. venustum							
South America[1]	1	1-2	+	+	+	+	5-10
Central America[2]	1	1-2	+	+	+	+	3-8
West Indies[3]	1	1-2	-	+	-	-	5
L. japonicum							
China	2	3-4	-	+	-	-	3-8
Malaysia and Indonesia	2	3-4	-	+	-	-	3-5
Japan	2	3-4	-	+	-	-	3-4
L. kerstenii	2	3-4	-	-	-	-	3-5

[1] Guianas, Trinidad, Tobago, Venezuela, Colombia, Ecuador, Peru, Bolivia, Paraguay and Brazil.

[2] Mexico, Belize, Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica and Panama.

[3] Cuba, Hispaniola and Puerto Rico.

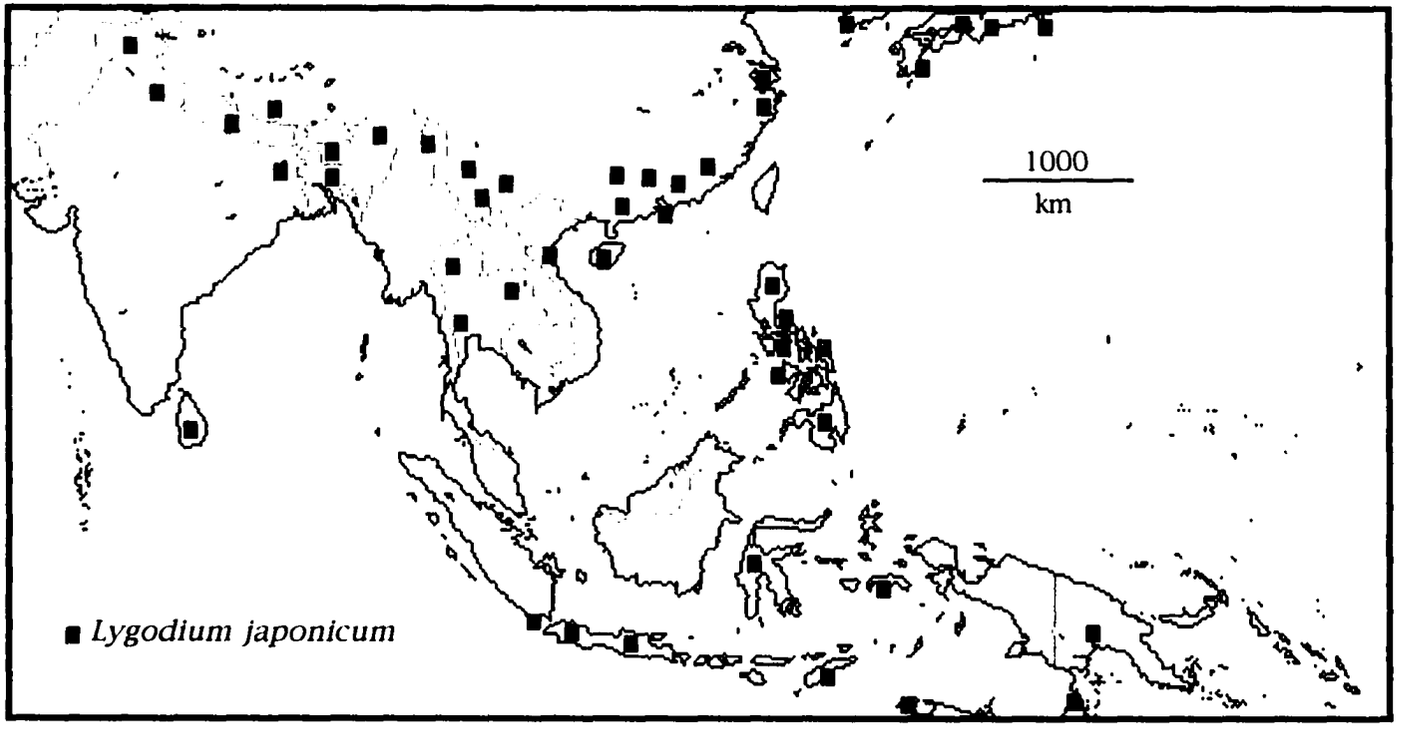


Figure 10.14. The geographic distribution of *Lygodium japonicum*. This species is also naturalized in the southeastern United States.

15. ***Lygodium kerstenii*** Kuhn, Filic. Decken., 28. 1868 [1867]. Syntypes. Madagascar, Nosy-Bey, *Kersten 75* (B!) and Africa, near Mombas, *Kersten 76* (B!). Figs. 34H-L.

Lygodium subalatum Bojer ex Kuhn, Fil. Afr. 170. 1868. Type. Comoro Island, *Bojer* (B!; fragment of type NY!).

Lygodium brycei Baker, Kew Bull. 138. 1901. Type. Rhodesia, Mashonaland, *Bryce s.n.* (K!).

Rhizome short-creeping, ca. 4-5 mm diam, covered with black hairs, subterranean to 4 cm. Fronds approximate, climbing 10-20 m. Stipe shallowly grooved with few reddish multicellular hairs between grooves. Rachis grooved, 1 mm diam., bearing 4-7-celled sharply acicular, transparent hairs with reddish septa. Pinna-stalk 3-5 mm long with hairs as on rachis. Dormant pinna-bud prominent, densely pubescent with 1-2 mm long transparent to light tan septate hairs, septa reddish-brown. Primary pinna-branches twice pinnate with 3-6 pairs of secondary pinna-branches distally or segments proximally, grooved, with 1-2-celled erect hairs and longer multicellular hairs (septa not obvious) on all subsequent axes. Secondary pinna-branches pinnate, each bearing 3-4 pairs of alternate segments, winged, puberulent. Segment-petiole winged, < 1 cm that of proximal segments decreasing distally so that most distal segments sessile, puberulent. Segments subpalmate, usually 3-5 lobed with central lobe 3-4 times length of outer ones, those of distal segments subequal or only on one side, central lobe deltoid to linear lanceolate (often pinnatifid), 2-5 x 1-2 cm, cuneate at base, acute to attenuate at apex, outer lobes discrete, juvenile segments simple, pinnatifid (without basal lobes) as in *L. polystachyum*, without articulation at petiole/segment junction. Veins

free, forked 2 times, ascending at 25°-30° from costa, ending at margin. Margins lobulate, lobes serrulate, not thickened. Fertile and sterile segments monomorphic to dimorphic, primary pinna-branches 3 times pinnate, segment-lamina reduced, tertiary pinna-branches winged, pubescent. Fertile segments usually tripartite, lamina reduced or not, bearing 6-10 sporangial pairs per sorophore, hairy on veins, occasionally on indusia. Spores 65-75 μm (ave. 69 μm), tuberculate, tubercles irregular, laesurae not prominent (Figs. 6.8a,b).

Ecology

In forest and riverine habitats, described by Burroughs (1990) as a "rampant climber on the fringes of tall, evergreen forest at medium altitudes (300-1280 m)". In Africa it is generally uncommon, whereas in Madagascar it is more frequent.

Distribution

Comoro Islands, Madagascar, southern Africa (Zambia, Zimbabwe and Mozambique). Burrows (1990) reports *L. kerstenii* from Kenya, however, I have not seen any specimens from this area. Fig. 10.15.

Comments

Lygodium kerstenii is very similar to *L. japonicum*, except for its segment shape and pinna-branch length. The number of secondary pinna-branches is greater than in *L. japonicum*. In *L. kerstenii* the most proximal secondary branches are always twice pinnate whereas the more distal ones are only once pinnate. The primary pinna-branches usually bear 4-6 secondary pinna-branches or segments in contrast to 3-4 branches or segments in *L. japonicum*. In many specimens the central lobe of the segment is pinnatifid, much like that of *L. polystachyum*. In its juvenile form the initial segments

resemble those of *L. polystachyum*, possibly indicating a common origin. Hooker (1887) noted the taxon as intermediate between *L. japonicum* and *L. polystachyum.* Some specimens resemble *L. japonicum* and may be that species introduced into Africa (Burroughs, 1990). Refer to Comments of *L. japonicum* for further discussion.

Nomenclatural Notes

The species described by Mettenius and Kuhn as *L. boivinii* has reticulate veins on sterile segments only and further collections need to be studied. It is suggested by Christensen (1932) that *L. boivinii* is a hybrid between *L. lanceolatum* and *L. kerstenii.* After examining specimens from various herbaria I have treated *L. boivinii* as a hybrid as it contains abortive spores.

The species described by Bojer and Kuhn as *L. subalatum* is conspecific with *L. kerstenii.* The former was described in Filices Africanae and the latter in Filices Deckenianae both published together in the same paper (1868). Prantl (1881) chose the name *L. subalatum* to describe this species recognizing that both descriptions referred to the same species. Hooker and Baker (1889) synonomized *L. subalatum* under the name *L. kerstenii.* Since Filices Deckenianae was written in 1867 (*L. kerstenii*) and added as an appendix to Filices Africanae (*L. subalatum*) written in 1868, the name L. *kerstenii* has priority over *L. subalatum.*

Selected specimens examined:

Mozambique. Barbosa and Navita: *Schelpe 5309* (GH, MO); *Mendonca 836* (BM); *Schelpe 5465* (GH, B, MO); Sofala: *Mendonca 2371* (BM); Spungabera: *Torre 4276* (BM); **Zimbabwe.** Chipinga District: *Pope et al. 1390* (MO); Inyanga District: Salisbury, *Chase 6133* (GH); Melsetler District: *Müller 2738* (MO); Umtali District: *Schelpe 5434* (BM); **Zambia.** *Fisher 1336* (BM); Fisher *1457* (MO); **South Africa.** Pretoria, Natal District: Durban, *Stray 6725* (MO); Musapi

on Rhodesia border: *Taylor 3306* (GH); **Madagascar.** *Webb 167* (GH); *Bojer 1310* (GH); *Humblot 1587* (UC); **Comoro Islands.** *Hildebrandt 1795* (NY).

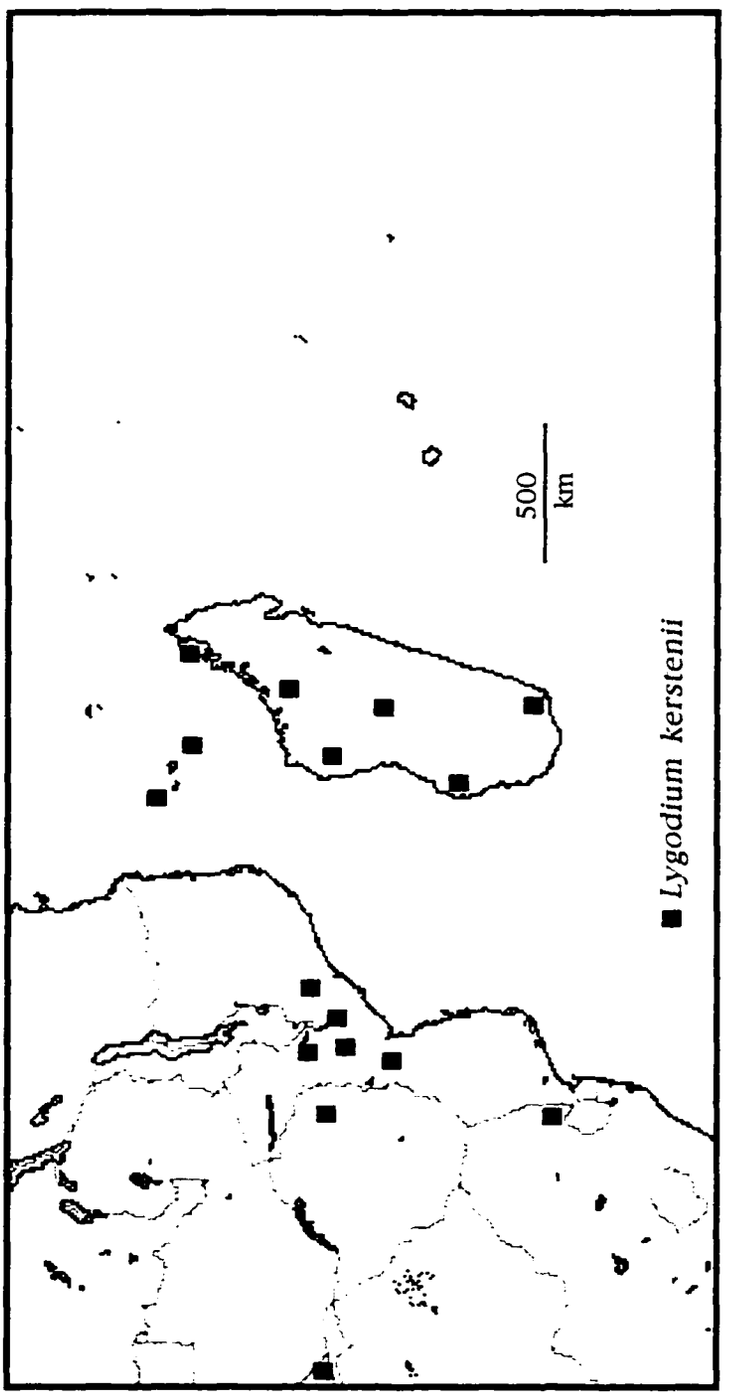


Figure 10.15. The geographic distribution of *Lygodium kerstenii*.

16. ***Lygodium venustum*** Sw., J. Bot. (Schrader), 1801(2): 303. 1803. Type. Jakob Breyne, Cent. I, t. 96. 1678: Tryon and Stolze (1989) question the existence of this specimen. Figs. 10.34A-D.

Hydroglossum hastatum Willd. Sp. Pl. 5: 79. 1810. Type. Brasil, Herb. Willd. (B).

Hydroglossum hirsutum Willd. Sp. Pl. 5: 80. 1810. *Lygodium polymorphum* var. *hirsutum* (Willd.) Farwell, Amer. Midl. Naturalist, 12: 305. 1931. Type. Venezuela, Cumanacoa, Herb. Willd. (B, photograph NY!).

Lygodium polymorphum (Cav.) H.B.K., Nov. gen. sp. I: 31. 1815. *Ugena polymorpha* Cav., Icon. 6: 75, t. 595, f.1. 1801, *nomen illegit.* Basionym. Type. Luzon. Née (P!). H.B.K. thought type wrongly localized and was from tropical America (Alston and Holttum, 1959).

Lygodium commutatum Presl, Abh. Böhm. Ges. Wiss. 4: 370. 1847. Syntypes. Guatemala, *Friedrichstal s.n.* (PRC); Panama, Colón, *Billberg s.n.* (PRC); Peru, *Poeppig s.n.* (PRC).

Lygodium mexicanum Presl, Rel. Haenk., 1: 72. 1825. Type. Mexico, *Haenke s.n.* (PR).

Lygodium mucronulatum Sturm in Mart., Fl. Bras., I(2): 171, t. 14., f. 9. 1859. Type: Guiana, without collector, (B?).

Lygodium palmatilobum Sturm in Mart., Fl. Bras., I(2): 170, t. 14., f. 6. 1859. *Lygodium polymorphum* var. *palmatilobum* (Sturm) Farwell, Amer. Midl. Naturalist, 12:306. 1931. Type. Brazil, Rio de Janeiro, *Dollinger*, Herb. Martii (BR).

Lygodium pohlianum Presl, Suppl. Tent. Pterid., 105. 1845. Type. Brazil, Minas Gerais, *Pohl* (PRC, isotype BR!).

Lygodium schiedianum Presl, Suppl. Tent. Pterido., 110. 1845. Type.

Mexico, *Schiede* (B!, isotype NY!).

Lygodium venustum var. *granatense* Christ, Bot. Jahrb. Syst., 24: 145. 1897.

Type. Grenada, *Eggers 6293* (B!; isotype US).

Rhizome short-creeping, highly branched, ca. 1 cm. diam, covered with black multicellular hairs. Fronds approximate, 3 fronds in 1 cm, climbing to 10-20 m. Stipe dark at base becoming stramineous, covered proximally with rhizome hairs to 1-2 cm, then changing distally to scattered, brown, multicellular, septate hairs. Rachis ca. 2-3 mm diam., grooved, subglabrous; Pinna-stalk 5-10 mm long, pubescent with multicellular (3-4 celled) septate (dark brown) stramineous hairs (0.7-2 mm long), scattered among 1-celled short, erect hairs. Dormant pinna-bud prominent, ca. 3 mm long, covered with stramineous to reddish brown multicellular hairs with brown septa. Primary pinna-branches once pinnate (occasionally bipinnate), bearing 3-10 pairs of alternate segments, grooved to narrowly winged, 1-2 mm diam., glabrous to densely pubescent with hairs as on pinna stalk. Segment-petioles winged, 3-8 mm long proximally, pubescent. Segments subpalmate, usually 3-5-lobed with the central lobe much longer (2/3 length of segment), lateral lobes often pinnatisect or becoming individual segments, the longest lobe 4-8 (-11) x 1-2 cm, glabrous to pubescent adaxially and most often abaxially, with 2-3-celled (1 mm long) white hairs and short, erect, 1-celled acicular hairs. Veins free, forking 2-3 times, the prominent costa entering each lobe, veinlets not as prominent, ascending 25°-30° from costa of longest lobe, often hairy as described for segments, hairs much more frequent on veins than on lamina, ending at margin. Margins coarsely serrate to biserrate to serrulate, not thickened. Fertile and sterile segments monomorphic; primary pinna-branch often 2 times pinnate, secondary pinna-branches with more lamina on wings,

in some localities lamina contracted and semidimorphic; primary pinna-branches with up to 8 pairs of secondary pinna-branches, proximal branches with 3-6 pairs of segments, fewer distally, secondary branches usually ending in entire or bifid segment. Fertile segments subpalmate, more often 3- than 5-fid, pubescent both adaxially and abaxially and on sorophores, sorophores bearing (3)8-20 sporangial pairs; indusia puberulent to densely pubescent, with 1-2-celled, acicular hairs, margins sinuate. Spores 83-95 μm (ave. 90 μm), coarsley tuberculate (Fig. 6.1a-c).

Ecology

Lygodium venustum grows in disturbed places in secondary forests, at edge of roads, in pastures, gallery forest and adjacent cerrado, scrambling over trees and shrubs. This species is ubiquitous in the New World tropics and considered very weedy. It grows in Bolivia at 800 m. Its form varies geographically, especially in segment size and indument.

Distribution

New World tropics: Mexico through Central America (Guatemala, Honduras, Nicaragua, Belize, El Salvador, Costa Rice, Panama) to Venezuela, Colombia, Bolivia, Ecuador, Peru, Paraguay and northern Argentina, Surinam, Guyana, Brazil, Trinidad, Puerto Rico and West Indies (Cuba, Hispaniola, Grenada). Fig. 10.16.

Comments

Lygodium venustum is one of the most diverse species in segment size and indument. The most distinguishing feature is the long pinna-stalk covered with hairs. The most common segment form is 5-fid with a long central lobe, and symmetrical outer lobules, however, segments with a lobe only on one side or trifid also occur. It is not easily confused with *L. volubile* or *L. cubense*

because of the segment shape. The indument may appear to be a decisive characteristic but is not consistent. Pubescence is greater than in other New World species of *Lygodium*, but within the species itself is highly variable (Fig. 10.16). This species is the New World counterpart of Old World taxa, *L. japonicum* and *L. kerstenii*. See Comments, *L. japonicum*, for further discussion.

Selected Specimens Examined

Mexico. Chiapas: *Martinez 8242* (NY); Guerrero: *Langlassi 375* (GH); Hidalgo: *Clark 6850* (NY); Jalisco: *Lott 3229* (NY); Oaxaca: *Mickel and Leonard 5126* (NY); San Luis Potosí: *Pringle 3318* (NY); Salinas: *Nevling & Gomez Pampa 92* (GH); Tabasco: *Fernandez 1323* (NY); Tamaulipas: *Palmer 17* (GH); *King 4020* (NY); Vera Cruz: *Nee & Diggs 24638* (NY); Yucatán: *Lundell 1415* (NY); **Guatemala.** Canoas: *Smith 6410* (GH); **Honduras.** Ceiba: *Yuncker et al., 8566* (NY); Sta. Barbara: *Paz 119* (NY); **Belize.** Carozal: *Gentle 4843* (NY); **Nicaragua.** Managua: *Nichols 1364* (GH); **Costa Rica.** Guanacaste: *Dodge & Thomas 6358* (GH); Limon: *Godfrey 66448* (GH); Puntarenas: *Rossbach 3318* (GH); **Panama.** Canal Zone: *Witherspoon 86098* (NY);*Welch 19864* (NY); **Venezuela.** Amazonas: *Huber 603* (GH); Maracai: *Vogl s.n.* (GH); Miranda: *Pittier 11227* (GH); **Colombia.** Caldas: *Acosta-Arteaga 632* (GH); **Bolivia.** Cochibamba: *White 994* (GH); Santa Cruz: *J. Steinbach 5087* (GH); **Ecuador.** Guayas: *Holm Nielsen s.n.* (GH); Los Rios: *Mexia 6583* (GH); **Peru.** San Martín: *Ferreyia 7868* (GH); Cuzco: *Davis et al. 1282* (GH); **Paraguay.** *Hassler 8207a* (GH); San Bernadino: *Rojas 42470* (GH); **Argentina.** Pilcomayo: *Morel 5255* (GH); **Guyana.** *Smith 2354* (GH); **Brazil.** Amazonas: *Luetzelburg 20416* (GH); Ceara: *Duarte 1296* (GH); Goias: *Irwin et. al., 11900* (GH); Maranhao: *Schatz et al., 918* (MO); Minas Gerais: *L. Williams 6959* (GH); Para: *Spruce s.n.* (GH); **Puerto Rico.** La Muda, *Liogier et al., 29669* (NY); **Jamaica.** *Day s,n.* (NY);

Cuba. San Blas: *Jack 6434* (GH); Pinar del Rio: *Shafer 13736* (GH); **Hispaniola.** Haiti: Dpt. l'Artibonite, *Leonard 8006* (NY); du Nord, *Leonard 9178* (MO); Dominican Republic: Sanchez Ramirez, *Zanoni & Cabral 41250* (NY); Santo Domingo, *Ekman H 14419* (S); *Lioger & Lioger 24090* (NY); Trujillo, *Howard & Howard 9950,* (NY); **Grenada.** St. George: *Proctor 16813* (GH); **Trinidad.** Magdalena: *Holton s.n.* (GH).

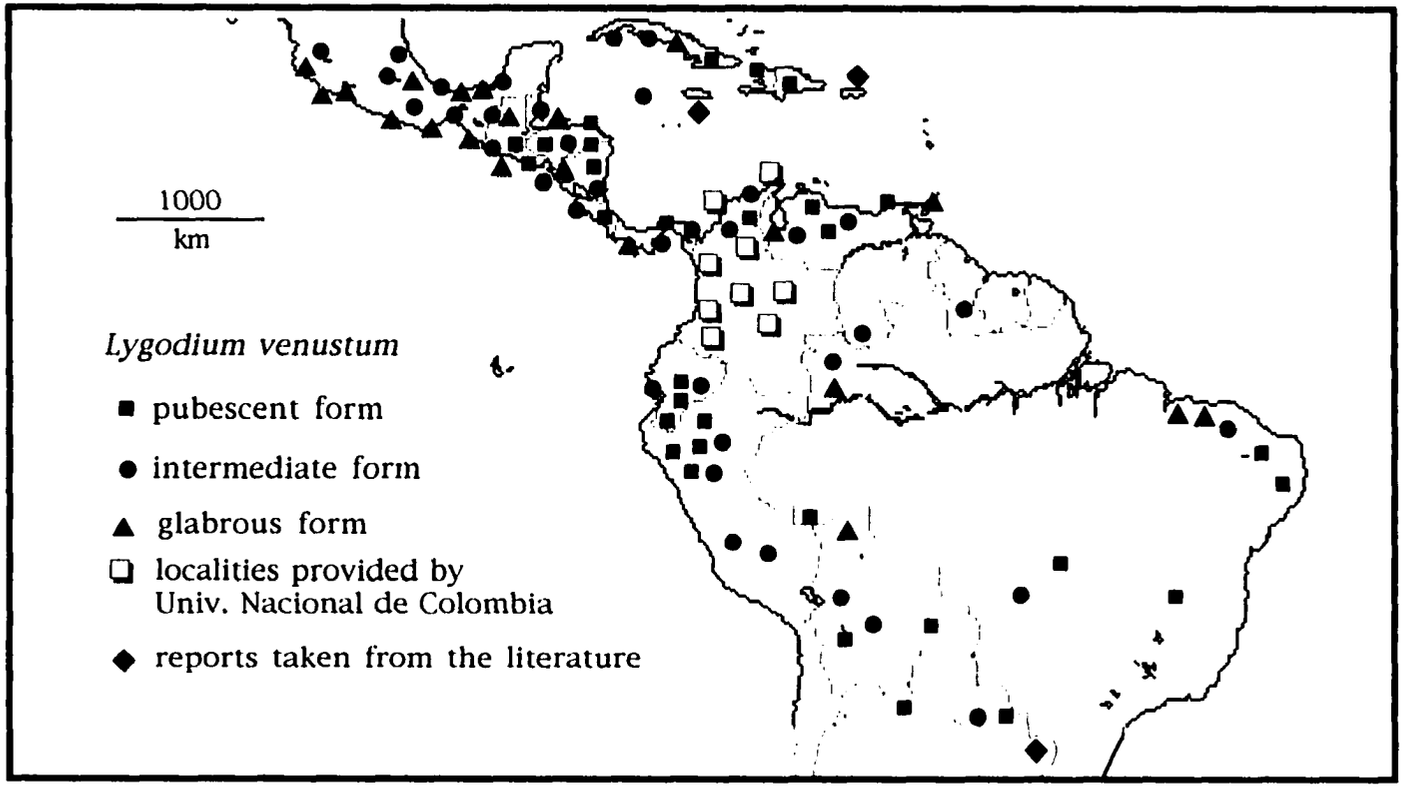


Figure 10.16. The geographic distribution of *Lygodium venustum*. Pubescense of the specimens indicated by the open box symbol (❑) in Colombia was not observed. The locations were provided by Dra. Maria Teresa Murillo Pulido of the Universidad Nacional de Colombia.

17. ***Lygodium polystachyum*** Wallich ex Moore, Gard. Chron. 86: 33. 1859. *Lygodium pinnatifidum* non (Willd.) Sw. *sensu* Prantl, Unters. Morph. Gefässkrypt. 2: 83; t.1, f.11. 1881. Type. Malaya. Penang, *Wallich 177* (K!; isotype B!, two sheets). Fig. 10.33E-J.

Hydroglossum pinnatifidum Willd., Abh. Kurfürstl-Mainz. Akad. Nützl.Wiss. Erfurt 2(4): 21. 1802. Type. *Herb. Willdenow p.p.* (B; photograph NY!).

Lygodium colaniae Tardieu & C. Chr., Natul. Syst. [Paris] 5: 168. 1936. Type. Tonkin, *E. Colani*, Herb. Ecole # 3474 (BM!).

Rhizome short-creeping, approximately 5 mm diam. Frond approximate, up to 11 fronds in 4 cm, climbing to 4-6 m. Stipe dark brown, stramineous distally, glabrous. Rachis ca. 1-2 mm diam., with short, erect, 1-celled, acicular hairs and occasional longer multicellular hairs (hairs sparser than on pinna-branches). Pinna-stalk 0-1 mm long, with abundant short acicular and occasional multicellular hairs. Dormant pinna-bud prominent, densely covered with long chestnut-brown multicellular hairs (6-8 cells; >1.2 mm long) surrounding acicular, septate, dark brown-black hairs with swollen multicellular-bases. Primary pinna-branches pinnate, bearing 5-13 pairs of segments, the terminal segments similar to lateral ones or somewhat exaggerated. Segment-petioles strongly articulate, proximal petioles ca. 1.5-3 mm long, distal ones shorter, all densely covered with 2-celled, erect, acicular hairs (< 0.3 mm long). Segments chartaceous, pinnatifid, oblong-deltate, truncate at base, pinnatifid at apex, lobes cut 2/3 way to costa, 15-30 x 8-15 cm, lobes 6-15 pairs, rounded, 3-10 x 1.5-2.5 mm, bearing multicellular transparent, septate hairs (1.5 mm long) and 1-2-celled, acicular, erect hairs scattered on costa, veins, and occasionally on lamina on both surfaces. Veins

free; costa prominent on both surfaces, veins sometimes obscure, 1-2 times forked (in segment lobe), ascending at 35°-40° from costa. Margins entire, often bearing regularly spaced 3-celled acicular hairs, thickened. Fertile and sterile segments monomorphic to slightly dimorphic. Fertile segments bearing 7-18 pairs of sorophores per segment, the sporangia either on sorophores extending from the lobe margin or abaxially on the segment-lobe itself, with 6-17+ pairs sporangia per sorophore; indusium with multicellular hairs on surface and margin. Spores 69 - 74 µm (ave. 72 µm), tuberculate to verrucate, tubercles unevenly distributed, laesurae not prominent (Figs. 6.3c,d).

Ecology

Lygodium polystachyum grows in open, dry to damp shaded places, usually in disturbed habitats or at forest margins, scandent over secondary vegetation (palm, bamboo), or in mixed evergreen/deciduous forests, on granite bedrock, occasionally on limestone. Holttum (1959) noted that *L. polystachyum* has been found in the jungle climbing slender trees, and is unique in bearing fertile fronds in shade. It grows to elevations of 1200 m.

Distribution

China (Kwangsi), India and southeast Asia (Thailand, Myanmar, Vietnam, and Malaya). Fig. 10.17.

Comments

Lygodium polystachyum is unique in the uniform pinnatifid segments, in sessile pinna-stalks, in having neither the rachis nor subdivisions winged, and in bearing multicellular hairs on the margin of segments and short, erect, single-celled hairs on axes. The segments are articulate with an expanded zone of cells at lamina/stalk junction. The fertile segments are monomorphic

to slightly dimorphic, bearing sporangia on unmodified leaflets and also on sorophores of narrowed lobes. This species exhibits some of the diversity found in *Lygodium* in the placement of the sporangia. It may explain an evolutionary sequence of sporangia primitively marginal that moved to projections when the lamina became contracted. Thus, in its regular pinnatifid segments, its diveristy in sporangial position, and its tuberculate spores, *L. polystachyum* may represent one of the more primitive species.

Selected specimens examined:

India. Hinantha Lushai Hills: *Thakur Rup Chand 4320* (MICH); **Myanmar (Burma).** Dist. Mergui, Maliwun, Victoria Point: *Parker 3155* (NY); Muang Len and Meh Kong: *Rock 2017* (NY); Doi Pang Kop: *Smith 509* (GH): **China.** Kwangsi: *Ching 7320* (NY); **Malaya.** Perak: *Allen 4056* (GH, F); *Winterbottom 1203* (GH); Longkawi Island: *Allen 2782* (GH); Pahang: *Parris & Edwards 037* (MICH); **Thailand.** Prachinburi: *Floto 7804* (GH); Phuket Island: *Hennipman 3707* (NY, MICH); Songkla: *Allen 997* (GH); *Allen 2175* (GH); *Maxwell 87-181* (GH); *Maxwell 90-431* (GH); Singora: *Smith 386* (GH); **Vietnam.** Turong: *Kuntze 7770* (NY); Nathiang: *Poilane 5218* (NY, UC); Mt. Bani: *Clemens 3723* (MICH); Hanoi: *Colani 2984* (MICH); Thai Nguyen Province: *Petelot 3475* (NY); Tonkin: *Petelot 2984*; *Poilane 1743* (NY); *Tsang 30514* (GH).

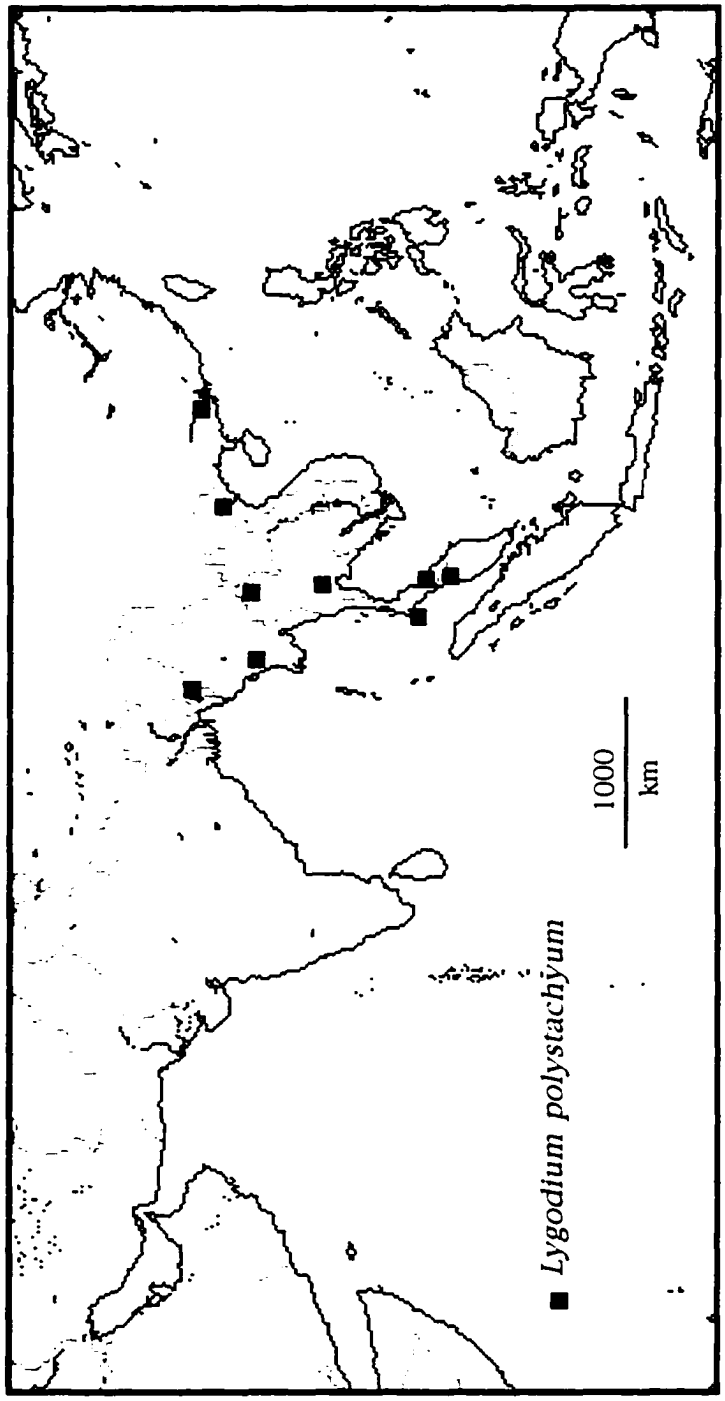


Figure 10.17 The geographic distribution of *Lygodium polystachyum*.

18. ***Lygodium cubense*** H.B.K., Nov. gen. sp. Plantarum 1: 31. 1815. Type. Cuba, near Havana, *Humboldt & Bonpland s.n.* (P, holotype). Figs. 10.35F-J.

Lygodium cubense H.B.K. var. *stenophyllum* Christ, in Engler, Bot. Jahrb. Syst. 24: 145. 1897. Type. Cuba, *Wright 3935* (US, holotype; isotypes GH!, NY!).

Lygodium poeppigianum Presl, Suppl. tent. pterid., 103. 1845. Syntypes. Cuba, *Poeppig s.n.* (B! 3 sheets); San Juan between Taburete and Calajubas, *Otto 229 and 296* (2 sheets)(B!, isotypes K!).

Rhizome long-creeping, 3-4 mm diam., frequently branched, covered with black-brown shiny hairs. Fronds 2-6 mm apart, length not cited. Stipe dark brown, with hairs of rhizome for 10 mm distally, becoming stramineous, with fewer and browner hairs proximally. Rachis wiry, narrowly grooved, 1 mm diam, glabrous to sparsely pilose, with short 3-4-celled hairs. Pinna-stalk 0-1 mm, terete to grooved when present. Dormant pinna-bud prominent, covered with golden to light brown, multicellular (> 4-cells) hairs, septa reddish-brown. Primary pinna-branches flexuous (angle of branching 120°-130°), grooved, forking dichotomously, with a thickening (pulvinus) at site of dichotomy, one dichotomy ending in a linear segment and the other dichotomizing again bearing 2-4 pairs of alternate segments, ending in 2 entire segments or one bifid segment, pubescent with short, curved, acicular, 1-celled hairs, interspersed with longer multicellular hairs. Segment petiole 4-7mm (basal segments), slightly grooved, pubescent with 1-celled curved hairs. Segments coriaceous, weakly articulate, entire or lobed at base usually only on one side, linear to linear-lanceolate, (4) 9-15 (27) x 0.8-2 cm, truncate

to cordate at base, rounded to acuminate at apex, glabrous. Veins free, 2-3 times forked, ascending from costa at 20°-35°, ending at margin, costa prominent, sparsely pubescent. Margins entire to roughly serrulate, slightly thickened. Fertile and sterile segments monomorphic to slightly dimorphic, pinna-branches obviously flexuous, 2 times pinnate, axes winged, bearing three pairs of segments or secondary pinna-branches, terminal segment of ultimate axes entire or lobed at base. Fertile segments smaller, 6-10 x 0.5-1 cm, linear-lanceolate to lanceolate, lobed on both sides, lobes often becoming segments, sorophores very long, bearing 8-13 (-21) pairs of sporangia; margins of indusia sinuate; hairs present abaxially on veins at base of sorophore, increasing in frequency and becoming numerous underneath sporangia, hairs also present adaxially on sorophore vein, white, long (3 mm), 4-celled, septa darker. Spores 85-100 μm (ave. 94 μm), verrucate, the verrucae forming ridges, low-tuberculate between the ridges, laesurae obvious, prominent equatorial ridges on proximal face (Figs. 6.5d,e).

Ecology

Lygodium cubense grows on serpentine soils, limestone and shale, in forests it forms thickets often by roadsides, twining over pine trees and on shrubbery, in open savannas, and in pine barrens up to 300-450m elevation.

Distribution

Endemic to Cuba. Fig. 10.18.

Comments

The distinguishing characteristics of *L. cubense* are the coriaceous texture of the segments, the wiry rachis, and the flexuous pinna-branches dividing dichotomously, with one side terminating in a pair of segments while the other continues. This latter character separates *L. cubense* from *L. volubile*,

of which the primary pinna-branch is pinnate, bearing numerous alternate segments. The flexuous fertile axes and the pulvini at the areas of forking are unique to those definitive specimens of *L. cubense* . Unfortunately, these characteristics often intergrade between *L. cubense* and *L. volubile* making some specimen identification difficult. This species often bears extremely long sorophores on narrowly linear segments. When more specimens are studied and more collections become available from Cuba, it may be shown that *L. cubense* is simply a variety or form of *L. volubile.* One form of *L. cubense* (*L. cubense* var. *stenophyllum* Christ) is simply a variation in segment size and shape: this form contains extremely long, linear segments. The same type of variation can be seen in the central segment lobe of some specimens of *L. japonicum* (*L. japonicum* var. *microstachyum*). In this treatment, *Lygodium pedicellatum* C. Chr. and Maxon has been synonomized with *L. volubile.* It has characteristics of both *L. cubense* and *L. volubile.* The type collection is from Haiti and if synomized with *L. cubense* would, therefore increase the species range to include a population in Hispaniola (refer to Comments, *L. volubile*). No cytological information is available for this *L. cubense*: studies in this area may help explain some of the variablity if tetraploids are found.

Selected Specimens Examined

Cuba. Las Villas: *Shafer 12285* (GH-A); Oriente: *Bro. Clement 216* (GH); Pinar del Rio: *Britton et. al., 7278* (GH, NY); *Shafer 10689* (GH-A, NY); Isles of Pines: San Pedro, *Britton et. al., 14336,* (GH); Los Indios, *Jennings 409* (GH); San Blas: *Jack 6762* (GH); Santa Clara: Trinidad Mountains, *B. Brues s.n.* (GH); *R. Howard 5484* (GH); *Howard 5072* (NY).

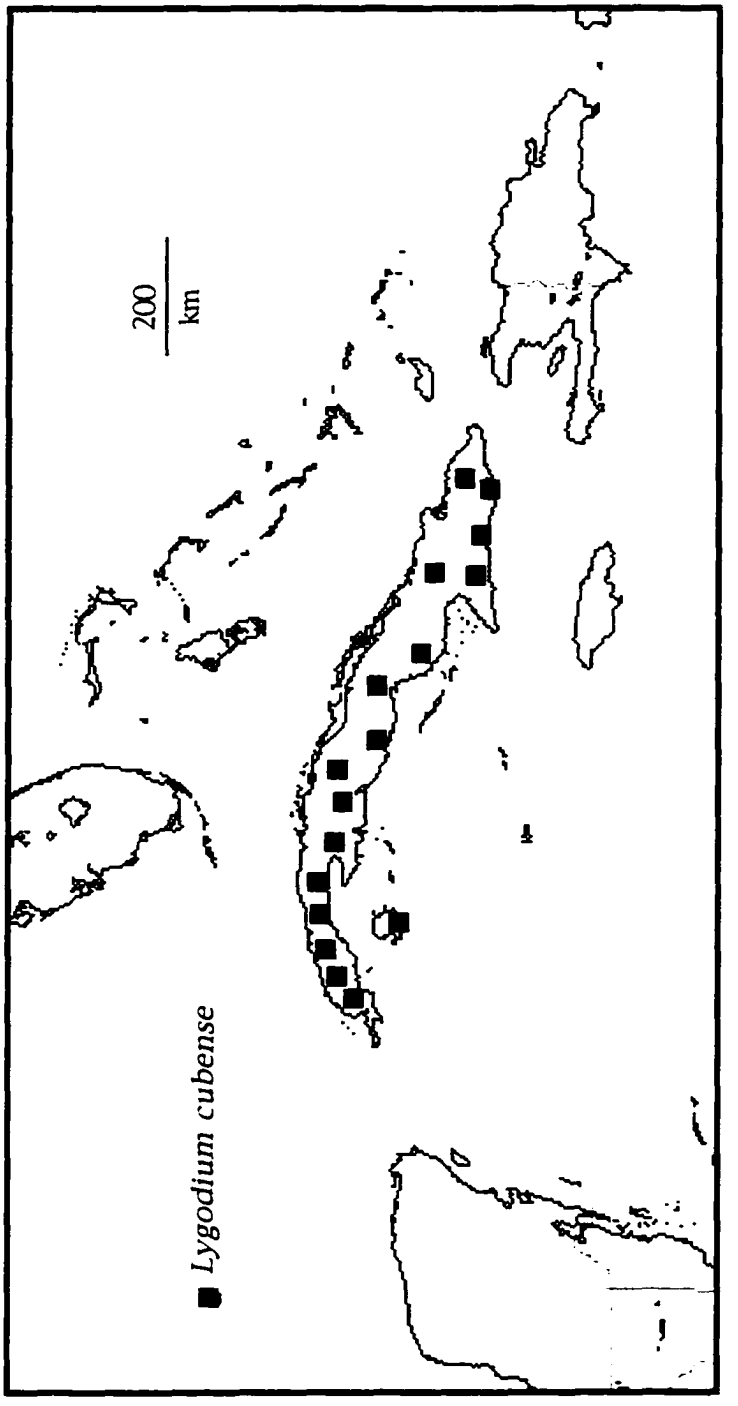


Figure 10.18. The geographic distribution of *Lygodium cubense*.

19. ***Lygodium oligostachyum*** (Willd.) Desv., Mem. Soc. Linn. Paris 6: 205. 1827. *Hydroglossum oligostachyum* Willd., Sp. Pl., 5: 81. 1810. Basionym. Type. Haiti, near Lake Mirogoan, Illustration, Plumier, Traite Foug. fil., 72. t.92. 1705. Figs. 10.35A-E.

Lygodium gracile Baker, J. Bot., 26: 35. 1888. Type. Santo Domingo, *Eggers 2536* (K!; isotypes GH, F!, MO!, P!, US, BM!).

Rhizome short-creeping, subterranean to about 5 cm, covered with black-brown multicellular hairs 4-5 mm long. Fronds approximate, climbing to 3-4 m. Stipe dark brown at base with hairs of rhizome distally, becoming stramineous and glabrous; grooved. Rachis wiry, narrowly grooved, < 2 mm diam., glabrous. Pinna-stalk 1-1.5 mm (< 3mm) long, glabrous. Dormant pinna-bud very prominent, covered with reddish-brown to golden-brown multicellular hairs (4-6 cells), septa dark brown. Primary pinna-branches flexuous (ascending at 110° - 135°), grooved, forking dichotomously with one side terminating in a pair of segments and the other side continuing, bearing 2-3 pairs of alternate segments or secondary pinna-branches, sparsely pubescent. Secondary pinna-branches (when present) narrowly winged, bearing 1-2 pairs of segments, terminal segment entire or lobed on one side, pubescent. Segment-petiole > 4 mm (basal segments), winged, pubescent with 1-2 celled, acicular hairs. Segments small, papyraceous, not articulate to weakly articulate, lanceolate to deltoid to subpalmate, lobed at base usually on both sides, often becoming pinnatisect, 2-7 x 0.5-1 cm, cuneate at base, acuminate at apex, glabrous. Veins free, 2 times forked, ascending at 30°-40° from prominent costa which is sparsely covered with small hairs (< 2 mm). Margins lobed, lobules entire to serrate, not thickened. Fertile and sterile

segments slightly dimorphic, segment-lamina reduced (from 4 mm sterile to 2 mm fertile), axes strongly flexuous. Fertile segments smaller, lamina reduced in some, deltoid to lanceolate, lobed with lobes often becoming segments, abaxially pubescent near fertile margins, sorophores bearing 3-4 sporangial pairs; indusia with 2-celled, erect acicular hairs, and occasional 3-4-celled, septate hairs, margins sinuate. Spores 110-120 μm (ave. 115 μm), long-ridged verrucate, ridges narrow and acute rather than rounded, laesurae prominent (Figs. 6.6d,e).

Ecology

Lygodium oligostachyum grows in riparian woodlands, often on limestone, forming thickets. It is often found growing in forests above the seashore to altitudes of 750 m.

Distribution

Hispaniola, found throughout the Dominican Republic and Haiti and in central Cuba. Fig. 10.19.

Comments

The blade of *L. oligostachyum* is very highly dissected, with small tripartite segments with strongly cuneate bases. The highly flexuous axes, especially obvious on fertile portions of the plant, resemble those of *L. cubense.* However, the dissection of the segments and the 2-3 times pinnate primary pinna-branches separate it from the latter species. *Lygodium cubense* also has coriaceous segments while those of *L. oligostachyum* are more delicate in both texture and overall appearance. The fertile segments bear only short sorophores on often contracted laminae.

Selected Specimens Examined

Cuba: Mina Carlota:*Senn 347* (GH); **Dominican Republic.** Cordillera Central: *T. Zanoni et. al., 30715* (NY): Isabel de Torres: *Liogier 23430* (NY): La Vega: *Valeur 393* (NY); San Cristobel: *De La Cruz et al., 72* (NY); *Zanoni & Mejia 16350* (NY); Samaná; *Abbott 2413* (GH, NY); Santo Domingo: *Liogier 11824* (NY); *Eggers 2536* (NY); *C.Wright s.n.* (NY): *Taylor 247* (NY); *Turckheim 2649* (NY); **Haiti.** Bayeux: *Nash 395* (NY): du Nord: *Ekman 4749* (NY); *Leonard 8741* (GH, NY); Grand'Anse Sul; *Zanoni et al., 24347* (GH).

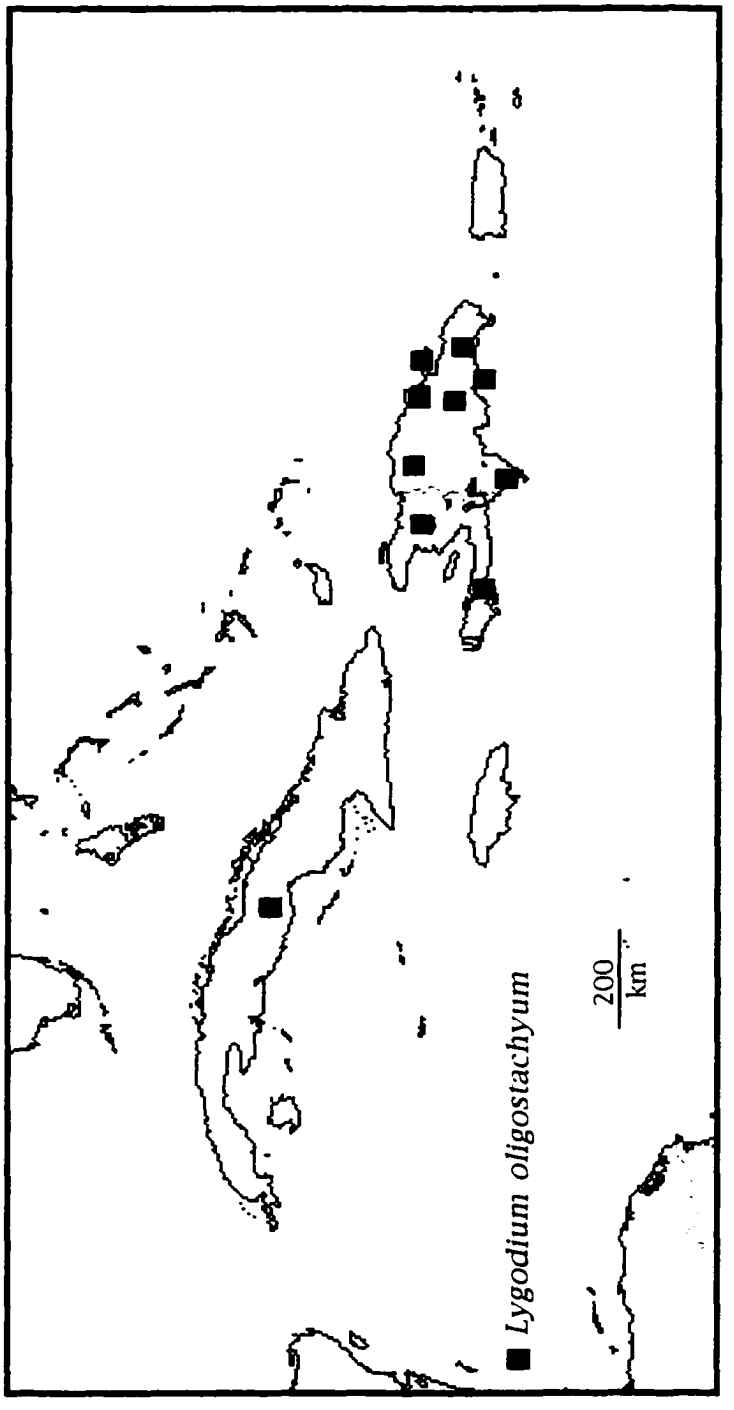


Figure 10.19. The geographic distribution of *Lygodium oligostachyum*.

20. ***Lygodium microphyllum*** (Cav.) R. Brown, Prod. Fl. Nov. Holl. I. 162. 1810. *Ugena microphylla* Cav. Icon 6: 76, t. 595, f. 2. 1801. (Basionym). *Lygodium scandens* var. *microphyllum* (Cav.) Luerss. J. Mus. Godeffroy. 6: 4. 1874. Type: Philippines, Luzon, *Née, s.n.* (MA, holotype). Figs. 10.36A-E.

Ophioglossum filiforme Roxb., Calcutta J. Nat. Hist. 4: 476, t. 26, f. 3. 1844.

Lygodium scandens sensu Swartz, *non Ophioglossum scandens* L., in Schrader, J. Bot. 1800. 2. 106 (1801).

Lygodium scandens var. *intermedium* Cesati. Atti. Accad. Sci. Fis. Nat. Napoli 7: 33. 1876. Type. (RO).

Rhizome long-creeping, ca. 3 mm diam, branching, covered with multicellular hairs, 2-3 mm long, lustrous black changing to reddish-brown on fronds. Fronds 3-10 mm apart, climbing to 10-15 m. Stipes dark brown at base becoming stramineous, covered with reddish brown multicellular hairs (3+ cells), sparse distally, approximately 14 cm to first pinnae. Rachis 1 mm diam, narrowly grooved, glabrous to randomly hairy. Pinna-stalk 3-6 mm, grooved, glabrous to sparsely hairy, hairs short, acicular extending onto pinna-branch for short distance. Dormant pinna-bud prominent, covered with golden to reddish-brown multicellular hairs (4+ cells). Primary pinna-branches grooved, glabrous, pinnate, bearing 4 (5-7) alternate pairs of segments, terminal segments bifid or dichotomous. Segment-petiole 2-4 (-7) mm, articulate (segment deciduous leaving persistent petiole), articulation zone prominent at base of segment. Segments deltoid to lanceolate, rarely lobed, 1.5 -2.5 (-5.5) x 0.9 - 1.5 (-2.0) cm, truncate to cuneate at base, rounded to acute at apex. Veins free, prominent abaxially, 2-3 times forked, ascending at 30°-40°

from costa, ending at margin. Margins minutely crenulate-serrulate to subentire, slightly thickened. Fertile and sterile segments monomorphic to slightly dimorphic. Fertile segments smaller, 1.0-3.0 x 1.0-1.5 cm, articulation zone of petiole less prominent, hairs more common abaxially close to sorophores which extend from veins at margins, 4-6 (-10) sporangial pairs per sorophore; indusia glabrous, margins crenulate. Spores 54 - 78 μm (ave. 60 μm), reticulate, laesurae not prominent (Figs. 6.4e,f).

Ecology

Abundant in many habitats from drier forests to cypress swamps and from open sunny savanna to closed canopy lowland rain forests. In Malaysia this species, often found growing with *L. flexuosum,* can withstand very exposed conditions (Holttum, 1954) . It is found in swamps, at edges of ponds, on river banks, in wet forests, in mangrove swamps and on volcanic soils forming dense entanglements over shrubbery. This species was introduced into Florida in the 1960's (Nauman & Austin, 1978) and is now considered an invasive weed in cypress swamps: a task force has been established to test biological, mechanical and chemical methods of eradication. In New Guinea it grows on kunai grass in full sun much like the growing conditions in the saw grass community in Florida. Elsewhere it may be considered rare but locally abundant. It is found up to 1000 m altitude in Sumatra.

Distribution

India, China, Malaysia, Indonesia, Philippine Islands, South Pacific Islands, Africa and Australia; introduced into southern United States, Jamaica (specimens dated 1891), Guadaloupe (specimen dated 1800), and Guyana (Essequibo Island). Fig. 10.20.

Comments

The distinguishing characteristics of *L. microphyllum* are very uniform. The pinna-stalk is long (> 3 (4-7) mm); the dormant bud is prominent and covered with short pointed hairs; segments are entire, with edges crenulate to crenate; axes are winged; leaflet-petioles are articulate leaving the petiole deciduous on the pinna-branch; the indument is sparse. Holttum (1954) describes the juvenile fronds as often forked twice, each branch with a digitately divided leaflet shaped much like those of *L. circinnatum* but with a broad base. This is an example of the difficulty in identifying juvenile plants of *Lygodium* prior to their acheiving the twining habit. Specimens from India were observed with short to sessile pinna-stalks, longer lanceolate segments and more hairs on pinna-branches (e.g., *Ramamoorthy 1566,* MICH). The indroduced form in the United States has uniformly small segments (1.5 x 1.0 cm). Rarely specimens have been observed with auricled segments as might be found in *L. flexuosum*, and these need close study for accurate identification. If the specimen is fertile, the most accurate means of identification is to observe the spores since this is one of few species with reticulate spores. This species is allied with the reticulate-veined *L. reticulatum* and *L. lanceolatum.* The former has reticulate spores, the latter tuberculate spores.

Pinna-branches from *L. microphyllum* are often used for weaving and making splints (Zamora and Co, 1975). A decoction of roots and leaves has been used to treat dysentery and the young leaves are eaten in Java. Common names include "bibu ribu" (North Borneo), "ribu ribu", "capay alas, capay papua" (Malaysia), and "kachot nu" and "iphao jung" (Thailand).

Nomenclatural Comments

Willdenow (1810), Christensen (1905), Prantl (1859), and Copeland (1959) synonomize *Ugena microphylla* Cav. with *Ophioglossum scandens* L. It has

been determined by Alston and Holttum (1959) that the specimen described by Linnaeus in Species Plantarum (1753) as *O. scandens* (*Hermann 374*) was the fertile part of *Ophioglossum flexuosum* L. collected by Hermann in Ceylon (no. 375). Linnaeus did not realize he was describing the same plant. Hermann (374) was chosen by Alston and Holttum (1959) as the lectotype of *L. scandens* L. Additional citations in Species Plantarum for *O. scandens* are of different species (a specimen of *L. volubile* Sw., a drawing of *L. venustum* Sw., and a drawing of *L. flexuosum* (L.) Sw.). Therefore, *L. scandens* (L.) Sw. becomes a synonym of *L. flexuosum* (L.) Sw. Swartz cites a drawing of Rumphius (Herb. Amb. 6: 32. fig. 2) in his description after citing *O. scandens* L. This drawing was only mentioned by Linnaeus in the 2nd edition of Species Plantarum, not in the first edition where the description occurs. Therefore, this drawing cannot be considered a syntype. The Rumphius drawing is, however, the species we recognize as *L. scandens* (= *L. microphyllum*). It has been interpreted by Alston and Holttum (1959) and Pichi Sermolli (1986) that "...Swartz clearly intended his species to be the same as that of Linnaeus and based his new combination *Lygodium scandens* on the pertinent Linnaean binomial. Thus, *Lygodium scandens* has *Ophioglossum scandens* L. as its basionym..." (Pichi Sermolli, 1986, Taxon 35(4): 685). The next description of *L. scandens sensu* Sw. (*non O. scandens* L.), is Cavanilles' *Ugena microphylla.* There is little doubt that those specimens identified as *L. scandens* (L.) Sw. and as *L. microphyllum* (Cav.) R. Br. are the same. Unfortunately, this important study by Alston and Holttum (1959) had not been recognized so that many collections have been determined as *L. scandens* (L.) Sw.

Selected Specimens Examined:

Australia. Northern Territory: *Specht 661*(GH); Queensland: *Clemens s.n.*, (GH); **Africa. Senegal.** Cape Verde: *J. Thomson s.n.* (BM); **Sierra Leone.**

Freetown: *Johnson s.n.* BM); Yagor(?), Buttu District: *Jones F38* (BM); Makali, Mabonto District:*Jones F33* (BM); **Côte d'Ivoire.** d'Abidjan: *Tardieu-Blot 58* (BM); **Ghana.** Bantama (Komasi, Ashanti): *Box 2907* (BM); **Benin.** *Chevalier 22813* (P): **Nigeria.** 6°30'N4°00'E: *Hambler 106* (BM); **Rep. of the Congo.** Mayumba: *Gossweiler 6080* (BM); **Cameroon.** *Annet 526* (P); **Gabon.** *Halle 1550* (P); **Dem. Rep. of the Congo.** *Kuppier B16* (BM); **Angola.** Saurimo: *Young 615* (BM). **Tanzania.** Zanzibar: Jozani,*Vaughan 2115* (BM); **Uganda.** Namonoe, Mengo District: *Taylor 3224* (BM). **Mauritius Island.** no collector, *P110*, (BM); **China.** Hong Kong: *Woo 253* (MICH); Saratow: *Steen s.n.* (MICH); **India.** Assam: *Mann s.n.* (MO); Mysore: *Saldanha 15612* (MICH); **Sri Lanka.** *Poilane 3943* (MICH): **Thailand.** Udawn Pru: *Hennipman 3674* (MICH); **Sumatra.***Toroes 1847* (MICH); **Malaysia.** Selangor: *Worthington 12364* (MO); **Java.** *Leonhardi s.n.* (MICH); **Celebes (Sulawesi).** *Balgooy 3985* (GH); **Borneo.** Penampang District: *Beaman 8016a* (MICH); **New Guinea.** Bismark Archipelago: *Croft 2072* (GH); Papua: *King s.n.* (MICH); ; **Philippine Islands.** Luzon: *Topping 1329* (MICH); Mindanao: *Clemens*, June 1907 (MICH); Mindoro: *Merrill 6051* (MICH); **Caroline Islands.** Palau: *Fosberg 25694* (MICH); **Solomon Islands.** Santa Ysabel: *Braithwaite 4584* (GH); **Guam.** Mt. Tenjo: *Moore 302*, (MICH); **Marianas.** Saipan: *Fosberg 31757* (MICH); **United States.** Florida: Palm Beach County, *Nauman & Nauman 562* (GH); **Guiana.** Potaro-Siparuni: *Kelloff et al., 892* (NY).

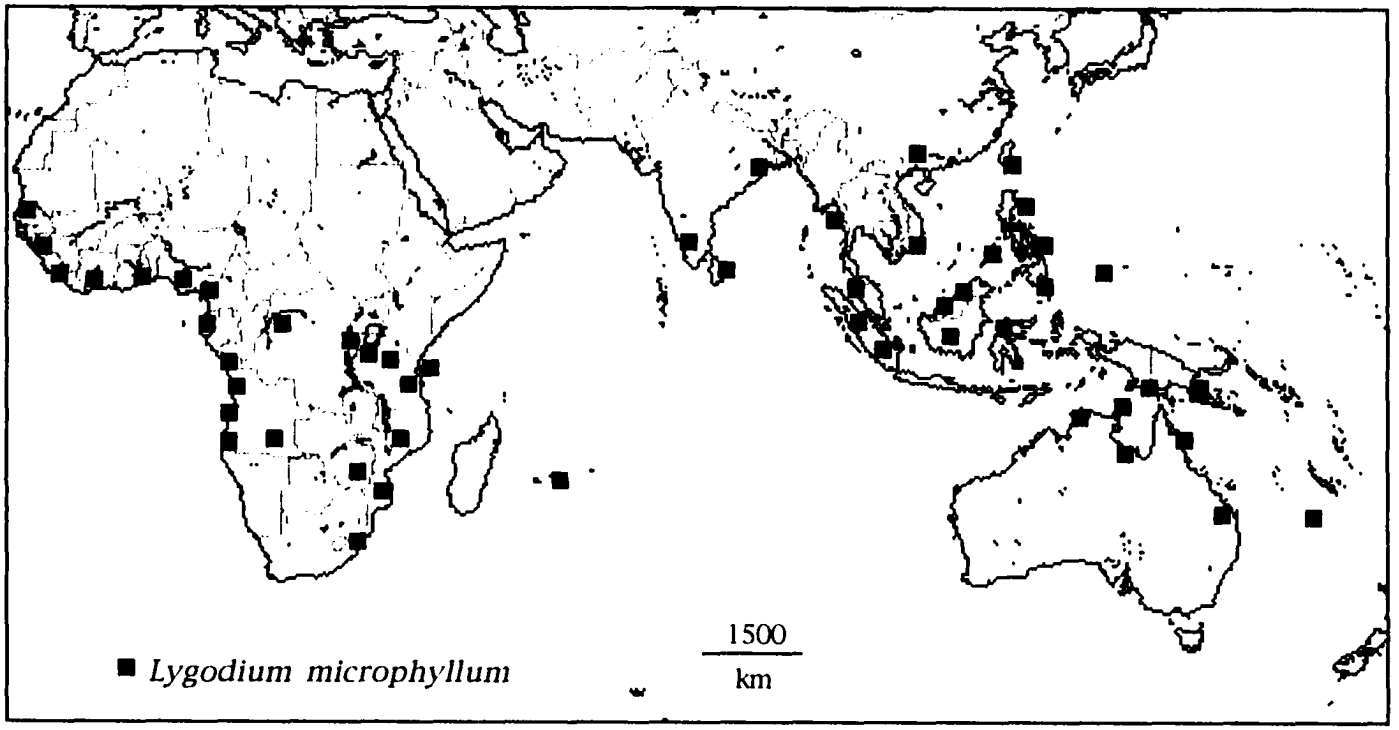


Figure 10.20. The geographic distribution of *Lygodium microphyllum*. This species is also naturalized in Florida in the United States, and in the West Indies and Guyana.

21. ***Lygodium reticulatum*** Schkuhr, Farnkr., 139, t. 139. 1809.

Type. Tahiti, *Forster s.n.* (BM, holotype). Figs. 10.36F-L.

Hydroglossum scandens Presl, Suppl. tent. pterid., 113. 1845.

Syntypes. Society Islands, *Banks s.n.* (BM?), Tahiti, *Forster s.n.* (BM?).

Hydroglossum polycarpum Willd, Spec. 5: 79. 1810. *Lygodium polycarpum* (Willd.) Desv., Prod. Fil. in Mém. Soc. Linn. Paris 5: 204. 1827.

Type. *Sprengel s.n.*, Herb. Willd., (B; photograph NY!).

Rhizome short-creeping, with shiny black hairs. Frond approximate climbing to 3-4 (10) m. Stipe relatively glabrous with occasional brown to reddish multicellular hairs, rhizome hairs proximally 1-2 cm up stipe. Rachis brown to reddish-brown, 1-2 mm diam., slightly grooved, mostly glabrous. Pinna-stalk 3-5(10) mm, grooved, with 2-5-celled dark brown hairs. Dormant pinna-bud raised, bearing some brown-black swollen, multicellular-based hairs amid 2-5-celled dark brown hairs. Primary pinna-branches pinnate, narrowly winged, sparsely covered with 2-3-celled, erect hairs, bearing 5-7 alternate segments, the terminal segment like others. Segment-petiole 2-3 (6) mm long somewhat decreasing distally, winged, with dark multicellular hairs; a swelling(pulvinus) at the petiole/pinna-branch junction. Segments herbaceous, simple, strongly articulate, petiole/segment junction often covered with multicellular (6-7 cells) reddish hairs and erect, brown, 2-celled hairs, deltoid to ovate, occasionally linear-lanceolate, truncate or cuneate to auriculate at base, acuminate to subacute to rounded at apex, 2-7(10) x 0.5-1.5 cm (at segment base), mostly glabrous. Veins reticulate, forking 2-4 times, ascending at ca. 30° from costa, mostly glabrous,

occasionally with multicellular hairs as described for segment/petiole, some veins end before margin. Margins sinuate or crenate to serrate, not thickened. Fertile and sterile segments monomorphic to slightly dimorphic, fertile pinna-branches sometimes twice pinnate. Fertile segments same size as sterile or slightly smaller, glabrous to pubescent, often free veined, 4-10(13) sporangial pairs per sorophore. Spores 96-104 μm (ave. 101 μm), reticulate, and globose (Fig. 6.4d).

Ecology

Lygodium reticulatum grows in humid forests to 1000 m in elevation, climbing on trees at the edge of forests and on stream banks. Reported by Brownlie (1977) as widespread in Fiji.

Distribution

New Caledonia, Society Islands (Tahiti), Fiji, New Hebrides, Australia (North Queensland). Fig. 10.21.

Comments

Lygodium reticulatum often resembles *L. microphyllum* in its articulation, deltoid segments, size of pinna-stalk, narrowly winged axes and reticulate spores. However, it is distinguished from *L. microphyllum* by its reticulate venation and swollen, multicellular-based bud hairs. The fertile segments are often free-veined. The segments in *L. reticulatum* may be longer. The two species intergrade and it would be conceivable to synomize them recognizing the net-veined subset as a variety.

Lygodium reticulatum is distinguishable from *L. lanceolatum* by its reticulate spores. *L. lanceolatum* has tuberculate spores and a longer pinna-stalk. *Lygodium reticulatum* is found from Queensland through Polynesia to Tahiti while *L. lanceolatum* is found in Madagascar. *Lygodium lanceolatum* is

probably a case of island variation and subsequent speciation. It is highly possible that *L. microphyllum* represents the ancestral form from which net-veined *L. reticulatum* and *L. lanceolatum* are derived, though *L. lanceolatum* has tuberculate spores and a shorter pinna-stalk and may be more closely allied with *L. salcifolium.* Of the three species *L. lanceolatum* is the most distinct.

Selected specimens examined.

Australia. Queensland: *Clemens s.n.* (GH); *Tryon & Tryon 7363* (GH); **Fiji.** Kandauu: *Smith 87* (GH); Viti Levu: *Smith 8578* (NY, UC); *Smith* **5486** (GH, UC); **Tahiti.** *Miller s.n.* (GH); **New Hebrides.** *Kajewski 303* (GH, UC); **Society Islands.** Raiatea: *Carlquist 677* (GH, UC); **Marianas.** Rota Island: *Necker R528* (GH); **New Caledonia.** Noumea: *Webster & Hildreth 14423* (GH).

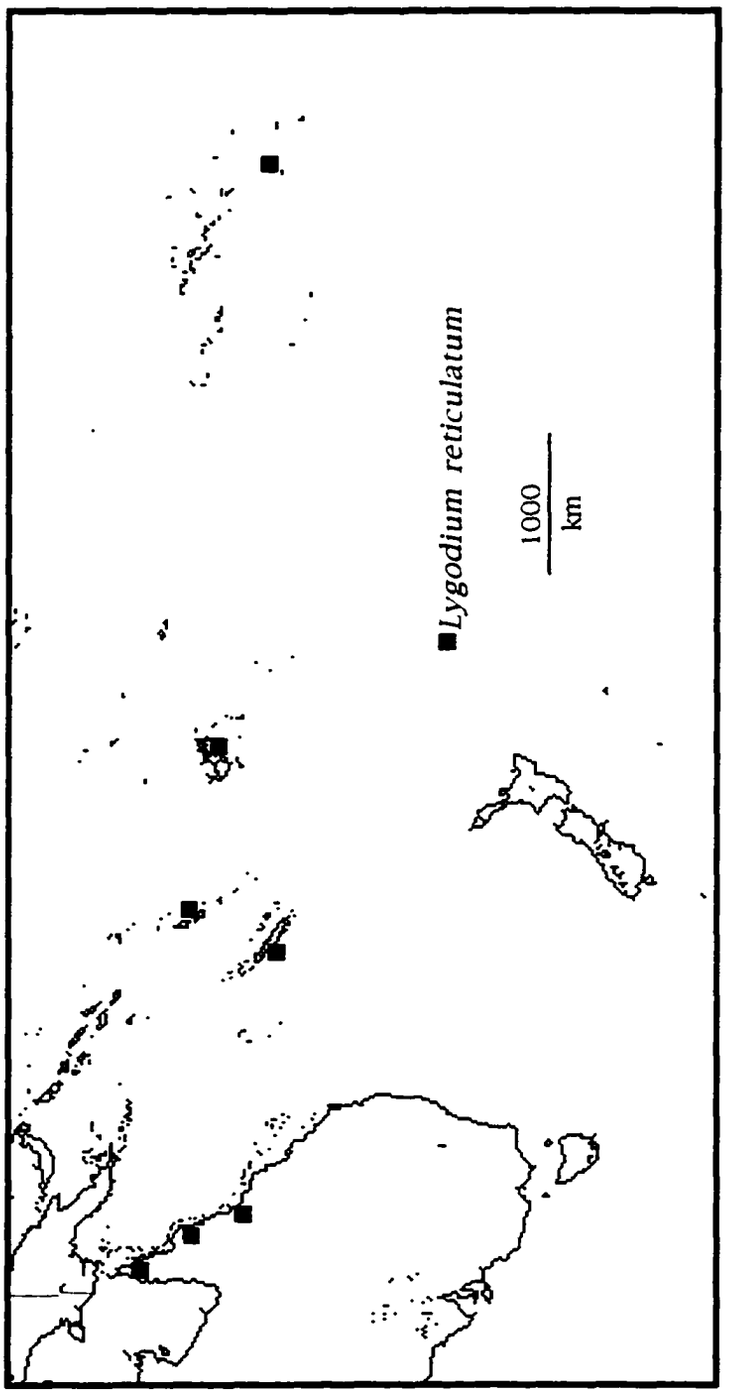


Figure 10.21. The geographic distribution of *Lygodium reticulatum*.

22. ***Lygodium lanceolatum*** Desv., Berl. mag., 5: 308. 1811. *Hydroglossum madagascariense* Poir., Encycl. Suppl. 3: 78. 1813. Type. Madagascar, *Commerson s.n.* (P!). Figs. 10.37A-D.

Rhizome not seen. Fronds climbing to 4 m. Stipe not seen. Rachis 1-3 mm diam., shallowly grooved, ± pubescent with erect 1-2-celled reddish hairs. Pinna-stalk 0 to < 2 mm, mostly glabrous. Dormant pinna-bud prominent in pocket formed by pinna-branch bases, covered with golden-brown to dark brown multicellular septate hairs and swollen, multicellular-based hairs. Primary pinna-branch once to occasionally 2 times pinnate, bearing 3-5 alternate segments, if bipinnate, secondary pinna-branch with 2 pairs of alternate segments, branch(es) ends in either one simple segment or 2 segments; narrowly winged, often with golden to light tan erect, 2-4 celled hairs (septa dark brown). Segment-petioles 5-10 mm decreasing distally, ultimate segments sessile; petioles winged, pubescent with erect reddish to light tan 2-4-celled hairs, prominent raised area (pulvinis) at the petiole/pinna-branch junction. Segments strongly articulate, deltoid to lanceolate, rarely auriculate at outer base, 2.5-8.0 (19.0) x 0.5-1.5 cm, truncate to cuneate at base, acute at apex, with a prominent swelling at segment/petiole junction often covered with hairs as described for petiole, in bipinnate forms the basal segments often smaller and deltoid but the apical segments linear-lancolate. Veins raised-reticulate, dividing at base in three directions from what appears to be the same point, prominent, anastomosing > 8x, ascending at 20°-30° from costa, ending in marginal layer. Margins entire to gently crenulate, with thick layer of cells. Fertile and sterile segments monomorphic to slightly dimorphic. Fertile segments deltoid with lamina somewhat

contracted, strongly reticulate veins, 3-8(12) sporangial pairs per sorophore, or if segments linear with 25 sporangial pairs per sorophore, glabrous. Spores 84-99 μm (ave. 91.4 μm), globose, tuberculate with few low tubercles (Figs. 6.3a,b).

Ecology

A common climber in evergreen forests, often at forest edge. Reported in forest on white sands at 10 m elevation where average rainfall is 2000-5000 mm/year. Grows in vanilla plantations and in the disturbed regrowth of abandoned plantations. Grows to elevations of 1000 m.

Distribution

Madagascar and the Comoro Islands. Fig. 10.22.

Diagnostic Features

Lygodium lanceolatum is unique in its strongly raised reticulate veins, its obvious abscission layer at the petiole/segment junction, its concomitant thickening at the pinna-branch/petiole junction, and in tuberculate spores. The spore pattern distinguishes it from *L. reticulatum* and *L. microphyllum* and the net veins, exaggerated articulation areas and pulvini separate it from *L. salicifolium.*

Selected specimens examined

Madagascar: Province Antananarivo: Mandraka, *Barnett & Dorr 186* (MICH); Perinet, 18°58'S, 42°22'E: *Phillipson 1626* (GH, MICH); Mayotte: *Marie s.n.* (F); Nossi Be:*Viguier & Humbert 110* (F); *Boivin 1955*, 1849(a) and Armange, Borbonia 1849(b) on same sheet (BM); *Hildebrandt 2938* (BM); *Richard 38* (BM); Antsiranana: *Miller 3300* (MICH); Province Tuliar: Ft. Dauphin, *Fosberg 52550* (NY); *D'Arey & Rakotozefy 15384* (MICH); without locality: Herbarium H. F. Hance 122(a) and Kilus Mountains, *J. T. Last*, April 1890(b) on same sheet (BM);

W. Warbur s.n. (BM); *Helsenberg s.n.* (BM); *Forbes s.n.* (BM); *Schlieben 8024* (BM); *Decary 943* (BM). **Comoro Islands.** *Humbolt s.n.* (BM); Mayotte: *Boivin 2885* (P).

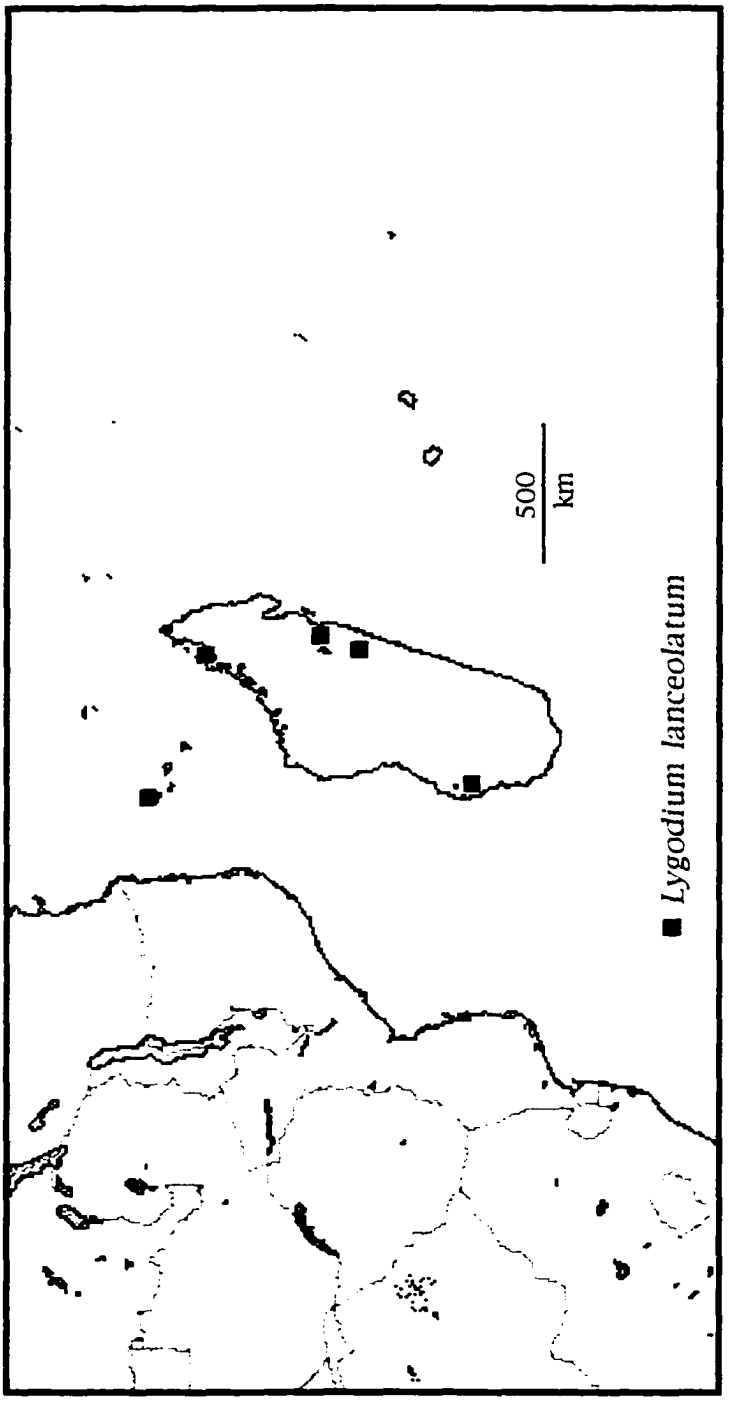


Figure 10.22. The geographic distribution of *Lygodium lanceolatum*.

23. ***Lygodium volubile*** Swartz, J. Bot. (Schrader), 1801(2): 304.

Hydroglossum volubile (Sw.) Willd. Sp. Pl. 5: 78. 1810. Type. Jamaica, *Swartz* (S, holotype). Figs. 10.38E-H.

Hydroglossum expansum Poir. Encycl. méth. Suppl., 4: 77. 1813.

Lygodium expansum (Poir.) Desv., Prodr. pl. Ind. occid. 463 (cited in Prodr. Fil. in Ann. Soc. Linn. de Paris 4: 205. 1827). *Lygodium volubile* Sw. var. *expansum* (Poir.) Prantl, Unter. Morphol. Gefasskr. 2: 78. 1881. Type. Fr. Guiana, Cayenne, *Herb. Desfontaine* (FL).

Hydroglossum heptaphyllum Schrad. in Gött., Gel. Anz. 863. 1824.

Type. Brazil, *Prince Maximilian von Wied-Neuwied s.n.* (M).

Lygodium acuminatum Sturm in Mart., Fl. Bras. 1(2): 174, t. 14, f.2. 1859.

Lygodium volubile var. *acuminatum* (Sturm) Farwell, Amer. Midl. Naturalist., 12: 306. 1931. Type. Brazil, Rio de Janeiro, Mt. Corcovado, near Agua da Serra, *Pohl 3859* (W, PR, or PRC?).

Lygodium hastatum Desv. in Mém. Soc. Linn. Paris 4: 204. 1827.

Type. Brazil, Herb. Desv. (P).

Lygodium hirtum Kaulfuss, Enum. filic. 47. 1824

Lygodium volubile Sw. var. *hirtum* (Kaulf.) Prantl, Unter. Morphol. Gefasskr. 2: 77. 1881. Type. Brazil, Rio de Janeiro, *communicavit Otto*, (B?, PR, PRC?).

Lygodium intermedium Mett. ex Kuhn, Linnaea 36: 168. 1959.

Lygodium volubile var. *intermedium* (Mett. ex Kuhn) Kuhn, Bot. Jahrb. Syst., 24: 145. 1897. Type. Jamaica, *Bertero* (B!).

Lygodium lucens Kaulfuss, Enum. filic. 47. 1824. *Lygodium volubile* Sw. var. *lucens* (Kaulf.) Farwell, Amer. Midl. Naturalist 12: 306. 1931. Type. Brazil, *communicavit Otto,* (B?, PR?, PRC?).

Lygodium micans Sturm in Mart., Fl. Bras. 1(2): 178. 1859. *Lygodium hirtum* Kaulf. var. *lucens* Presl, Suppl. tent. pterid., 104. Type. British Guiana, *Schomburgk 399* (P!, isotype BM).

Lygodium pedatum Goldm.,Nov. Actorum Acad. Caes. Leop.-Carol. Nat. Cur. 13, Suppl 1. 467. 1843. Type. Brasil, Corcovado, *Meyen s.n.* (B!).

Lygodium pedicellatum C. Chr. & Maxon, Svensk. Vet. Akad. Handl. ser. 3, 16(2): 85, t. 19. 1937. Type. Haiti, Jérémie, *Ekman 10217* (S!).

Lygodium puberulum Sturm in Mart. Fl. Bras. 1(2): 174. 1859. Type. Brazil, *Sellow s.n.* (B!, 2 sheets).

Lygodium scandens Schkuhr, Kryp. Gewachse 138, t. 138. 1809. *nom. illeg.* Type. Guyana, Essequibo (?).

Lygodium varium Link, Hort. Bot. Berol. 2: 140. 1833. Type. Brasilia, without collector (B).

Lygodium wrightii Mett. ex Prantl, Unter Morphol. Gefassk. 2: 78. 1881. *Lygodium volubile* Sw. var.*wrightii* (Mett. ex Prantl) Duek, Feddes Repert. 87(5):339. 1976. Type. Cuba, *Wright 925* (B; isotypes P!, US, BM!, HABA, NY!).

Ophioglossum scandens Vellozo Fl. Flum. 11, t. 53. Type. There are no surviving specimens.

Osmunda scandens (L.) Aublet, Hist. pl. Guiane Fr.. 2: 961. 1775. Based on one of the specimens used by Linnaeus to describe *Ophioglossum scandens* L. Type. Tropical America, (BM).

Rhizome short-creeping, branched, covered with black, lustrous hairs, 1.5-2.0 mm long. Fronds approximate, 6 fronds within 2 cm or more evenly and widely spaced (1-2 cm apart), climbing to 8-12 m. Stipe dark brown at base, becoming stramineous, covered with hairs as on rhizome for at least 1 cm proximally, then becoming glabrous. Rachis 2-4 mm diam., often grooved, stramineous, glabrous to sparsely covered with black acicular hairs, 0.5 - 0.75 mm long. Pinna-stalk 0-2 mm, somewhat grooved, glabrous. Dormant pinna-bud prominent, covered with light golden multicellular hairs (4-6 cells), some with a swollen, 2-rowed base (noted only in Cuban specimens). Primary pinna-branches bipinnate (rarely tripinnate), bearing 3-6 alternate pairs of segments, the terminal segments bifid to entire; grooved, often winged, rarely glabrous, usually bearing short acicular hairs, with a small thickening (pulvinus) segment-petiole junction. Segment-petiole 4-10 mm long, articulate, narrowly winged, bearing short acicular hairs (strigose) to longer, silky, appressed hairs (sericeous). Segments subcoriaceous, linear to linear-lanceolate, rarely lobed, 5-15 (23) x 1-3 cm, truncate to cuneate or cordate at base, rounded to acute at apex, rarely with hairs on lamina (Fig. 10.38H). Veins free, prominent, 2-3 times forked, ascending at 30° to 60° from costa, ending at margin; multicellular hairs common on costa, less common on veins, up to 0.5 mm long, appearing sericeous. Margins serrate-serrulate, rarely laciniate or sinuate, thickened. Sterile and fertile segments monomorphic. Fertile segments smaller than sterile ones, usually with more segments per pinna-branch, entire or frequently lobed to pinnatisect, hairs often more common adaxially on veins and lamina and abaxially on costa and veins leading to sorophores; 4-15 (20) sporangial pairs per sorophore; indusia glabrous to sparsely pubescent, hairs more common under sporangium on segment lamina than on indusium, margins of indusia sinuate. Spores

86-98 μm (ave. 95 μm), verrucate, verrucae forming ridges, laesurae and proximal equatorial flange prominent (Figs. 6.7c-e).

Ecology

Lygodium volubile grows in disturbed lowland forests climbing on trees and shrubs but ranges in habitat from forest to savanna and river floodplains to brejos in the Atlantic coastal forest of Brazil. In Guyana, *L. volubile* grows in shaded forests but in São Paulo in strong sun on low shrubs in grasslands. It is associated with limestone as well as red clay soils and is found to 1000 m in altitude.

Distrubution

West Indies (Cuba, Jamaica), Mexico (Chiapas, Vera Cruz), through Central America (Guatemala, Belize, Honduras, Nicaragua, Costa Rica and Panama) to South America (Trinidad, Tobago, Colombia, Venezuela, Bolivia, Ecuador, Guyana, Surinam, French Guiana, Brazil, Peru, Paraguay, northern Argentina). Fig. 10.23.

Comments

The diagnostic features of this species are entire, linear-lanceolate segments, articulation, once pinnate primary pinna-branch, slightly coraiceous segments and monomorphic fertile segments. The species is extremely variable in the following characters: size of segments, base of segment (truncate to cordate), number of segment-pairs per pinna-branch, indument, and presence or absence of winged axes. Fig. 10.23 illustrates the geographic distribution of glabrous, moderately hairy and pubescent populations. The degree of pubescence seems to reflect population differences and lacks obvious geographic continuity. However, most glabrous forms are found in northern South America. There appear to be two forms defined by

the number of segments/pinna-branch: those with 4 or fewer segment pairs and those with 5-6. Many authors have distinguished *L. micans* from *L. volubile* based on this character, along with angle of venation, indument (varieties e.g., *hirsutum*), and size of segment. However, there is far too much variability in these characters to easily divide this taxon. Cuban specimens are less pinnate and more dichotomous, the rachis being somewhat flexuous, a combination of the very flexuous *L. cubense* and the entirely pinnate condition of most South American forms of *L. volubile.*

There is a "*volubile* complex" encompassing one of the most diverse and potentially confusing groups in *Lygodium.* It includes *L. volubile* and *L. cubense* of the New World and *L. salicifolium, L. kingii, L. smithianum* and possibly *L. flexuosum* of the Old World. Spore morphology is verrucate in *L. volubile, L. cubense, L. kingii* and *L. smithianum* and tuberculate in *L. salicifolium* and *L. flexuosum.* All exhibit pinnate branching patterns (usually only once pinnate from the pinna-branch) with entire to slightly lobed lanceolate segments. The exception is *L. flexuosum,* whose extremely variable segment shape places it in an intermediate position between the "*japonicum*" complex and the "*volubile* "complex. As with the *japonicum* complex it is possible that some of these taxa represent a pantropical species that over evolutionary time has become isolated and is undergoing speciation, or high ploidy levels as a result of hybridization might be confusing the taxonomy (e.g. a hexaploid *L. volubile* in Trinidad).

All representative species are articulate to some degree except the African species, *L. smithianum. Lygodium flexuosum* is most often not articulate although a swelling at the petiole/segment junction can be seen in some specimens.

The plasticity in segment morphology in *Lygodium* in general is easily seen in this complex. One can observe on the same plant entire linear-lanceolate segments and segments with outer basal lobules, as well as truncate to cuneate to cordate bases. Indument in this complex, as is common in *Lygodium*, is population based and highly variable. The pinna stalk is often sessile to less than 3 mm in length and the New World taxa do not exhibit the swollen, multicellular-based bud hairs that the Old World taxa possess.

Lygodium cubense is a New World species endemic to Cuba that has a flexuous rachis usually bearing two pairs of coriaceous segments. Forms of this species intergrade with the straight, more classically pinnate arrangement found in *L. volubile*. Table 10.3 compares the morphological characteristics of these related taxa.

Lygodium pedicellatum represents a form of *L. cubense* that is glabrous, with less divided primary pinna-branches, and pedicellate fertile projections. It contains characteristics of both *L. volubile* and *L. cubense*. In Duek's (1978) New World treatment of *Lygodium* he synonomized it with *L. volubile*. In examining many specimens of Cuban *L. volubile* and *L. cubense*, *L. pedicellatum* appears to resemble *L. cubense* in its flexuous dichotomous branching, prominent articulation zone at petiole/segment junction (so that the petiole remains on the pinna-branch after abscission of segment), unequal cordate segment base (cuneate on other side), and veins that coalesce into a very thickened marginal zone. However, it has less dissection in the fertile segments and as such resembles *L. volubile*. The coriaceous texture of segments so characteristic in *L. cubense* is not present. The pedicellate projections are not uncommon in *Lygodium*: this type of sorophore morphology has been observed in some collections of *L. volubile* (Cuba, Oriente, *Shafer 4208,* NY). No other plants except the type collection have

been observed with the same degree of character distinction. Until more material is collected from Haiti it is impossible to determine whether this collection should be synonomized with *L. volubile* or *L. cubense*, treated as a variety of either, or considered a separate species.

Nomenclatural Problems

Lygodium wrightii Mett. ex Prantl has been considered a synonym of *L. volubile* by Duek (1978) in his treatment of the New World taxa. The former species was named by Eaton (Fil. Wright ex Fendl, in Mem. Am. Acad. Camb., 8: 216, 1861-63) with no description. Eaton cites Mettenius' contribution and the type as*Wright 925*, "*prope villam Monte Verde, dictam, Cuba Orentali*, Jan-Jul. 1859". Prantl (1881) describes a specimen collected by Wright in Grisbach's Herbarium (GOET). There are many isotypes of *Wright 925*, all with different labels and specimens. NY has 5 sheets with either *L. volubile*, *L. cubense*, or *L. venustum* singly or in combination. One sheet with the correct label data is a mixed collection of *L. venustum* and *L. cubense*. Since the description of Prantl is describing a *volubile*-like plant, this sheet must not represent an isotype. A specimen from GH of *L. poeppigianum* Presl appears to fit Prantl's description, is a uniform collection, and has appropriate site data. This collection has elliptical segments and may be an island variety of *L.volubile*.

Selected Specimens Examined

Mexico. Vera Cruz*: Lot 1244* (NY); **Belize.** *Gentle P8536* (NY); **Honduras.** *Fryxell 2818* (NY); **Nicaragua.** Zelaya: *Bunting & Licht 1231* (GH); **Panama.** *Hayes 6* (GH, NY); ; **Venezeula.** Bolivar: *Liesner & Gonzalez 11157* (NY); **Guyana.** *Potter 5282* (GH); **Surinam.** *Tryon & Kramer 5623* (GH); **Bolivia.** Santa Cruz: *Vargas 2816* (NY); **Peru.** Loreto: *Diaz & Jaramillo s.n.* (GH); **Brazil.** Para: *R. Spruce s. n.* (GH); Rio de Janeiro: *Smith & Brade s.n.* (GH); Rondonia: *Prance 6784* (GH); Sao Paulo: *Smith 2039* (GH); Vicosa: *Mexia 4819*

(GH); **Paraguay.** *Hassler 6194* (GH); **Argentina.** Misiones: *Rodriguez 42.473* (GH); **Cuba.** Yamaniguey: *Shafer 420* (GH); **Jamaica.** *Crosby et al., 779* (GH); **Trinidad.** *Fendler 31* (GH);*Walker 10322* (NY); **Tobago.** *W. Broadway s.n.* (NY).

Table 10.3. A comparison of characters among the *Lygodium* species in the "volubile complex".

Species	Number times pinnate		Segment Shape	Segments per branch	Articulate	Swelling at pinna branch	Spores[a]	Distribution	Pinna Stalk	Bud Hairs[b]
	Sterile	Fertile								
L. volubile	1x	1x	linear-lanceolate	3-7	+/-	-	verr.	NW[1]	0-2mm	-
L. kingii	1x	1x	linear-lanceolate rarely w/ basal lobes	5-8	+	+	verr.	SE Asia[2]	0-2mm	+
L. salicifolium	1x	1-2x	linear-lanceolate +/- basal lobes	4-7	+	+/-	tuber.	SE Asia[3]	0-2mm	+
L. smithianum	1x	1x	linear-lanceolate	6	-	-	verr.	Af. Ma.[4]	0-2mm	+
L. flexuosum	1-2x	2-3x	lanceolate, often w/ basal lobes, sometimes asymetrically cordate	4-5	-	-	tuber.	SE Asia and Aus.[5]	2+mm	2-3 rows of hairs with 2-celled bases

[a] verr. = verrucate; tuber. = tuberculate. [b] polycellular bud hairs.

[1] NW = New World including the West Indies, Mexico, Central America and South America.

[2] SE Asia = New Guinea, China, Southern Thailand, Malaya, and Burma.

[3] SE Asia = New Guinea, Sumatra, Caroline Islands, India, Vietnam, Siam, S. Andamanka and S. Borneo.

[4] Af. and Ma. = Africa and Madagascar.

[5] SE Asian and Aus. = New Guinea, China, Siam, Java, Vietnam, India, Philippines, Burma, Indonesia, Malaya and Australia.

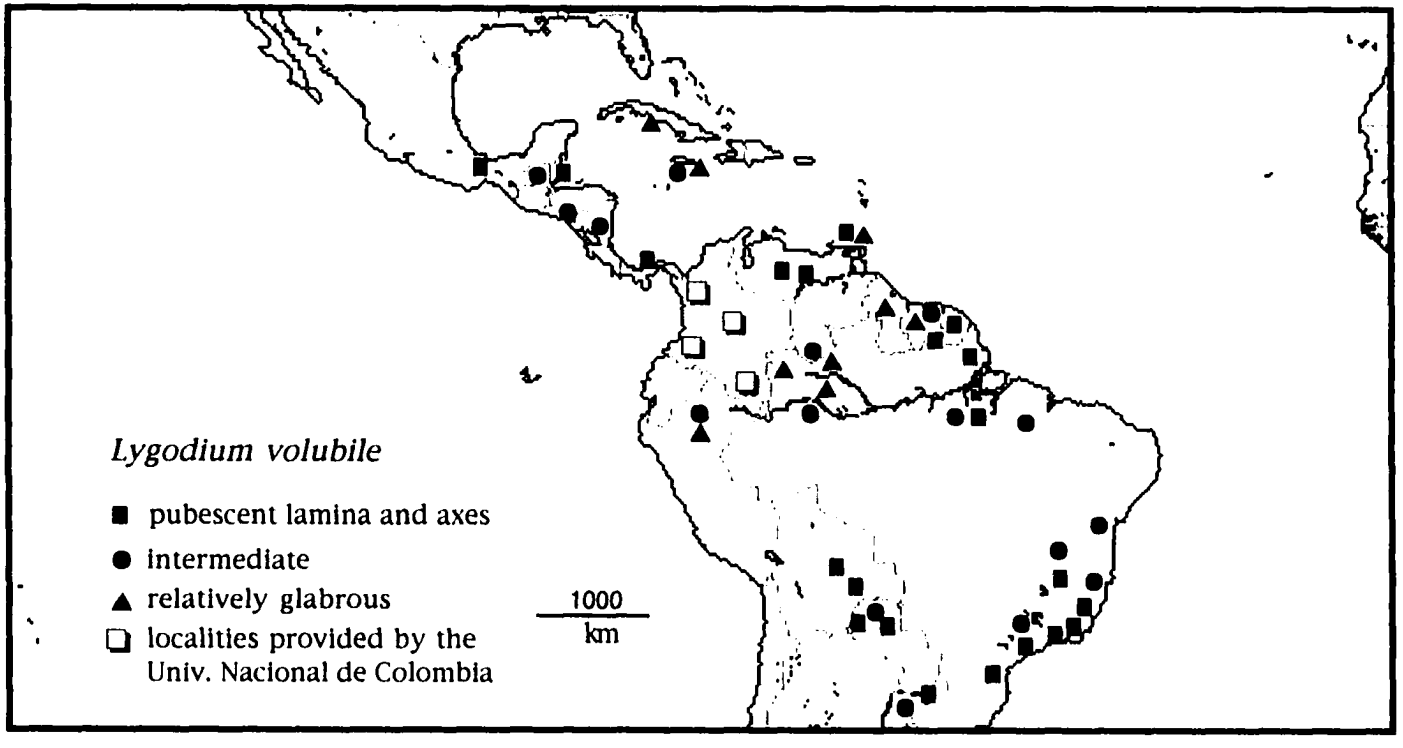


Figure 10.23. The geographic distribution of *Lygodium volubile.* Pubescense of the specimens indicated by the open box symbol (❑) in Columbia was not observed. The locations were provided by Dra. Maria Teresa Murillo Pulido of the Universidad Nacional de Colombia.

24. ***Lygodium smithianum*** Mett. ex Kuhn, Filic. afr., 169. 1869.

Type. Congo, *C. Smith s.n.* (holotype B!, isotype BM!). Figs. 10.38A-D.

Rhizome short-creeping, covered with appressed black shiny hairs. Frond approximate, 15-30 cm apart, climbing 10 m. Stipe reddish-brown becoming tan, relatively glabrous, with some multicellular, septate, tan to reddish-brown hairs, rhizome hairs not present proximally. Rachis 2-3 mm diam., narrowly grooved, glabrous. Pinna-stalk 0-2 mm, grooved, glabrous. Pinna-bud slightly raised, covered with reddish- to golden-brown septate, multicellular hairs (no swollen, muticellular based hairs). Primary pinna-branches pinnate, bearing 4-5 pairs of alternate segments, and often ending in a bifid segment, narrowly grooved (rarely winged), pubescent with multicellular (4-5 cells) golden, septate hairs (septa brown). Segment-petiole 5-7 mm long on proximal segments, becoming shorter distally, winged, lamina of wings becoming greater distally, pubescent with mostly appresed multicellular hairs, or glabrous; no articulation at petiole/segment junction. Segments subcoriaceous, entire, linear to linear-lanceolate, 8-15(20) x 1.0-2.0 cm, truncate to cuneate basally (often unequally), sometimes slightly auriculate on one or both sides, acute to acuminate at apex, lamina glabrous. Veins free, 1-2 times forked, midvein prominent (petiole becomes costa without articulation), ascending at 55°-60° from costa, ending at margins, costa prominent with multicellular hairs. Margins serrulate, not thickened. Sterile and fertile segments monomorphic. Fertile segments 5-6 pairs per primary pinna-branch, petiole more highly winged with multicellular hairs, hairs present adaxially on sorophores, 4-10 sporangial pairs per sorophore. Spores 78-89 μ (ave. 82 μ), long verrucate ridges, very prominent laesurae, and extended equatorial ridge (Fig. 6.6h).

Ecology

Lygodium smithianum grows in secondary rain forest, often in disturbed areas, in seasonally dry forests (July/August dry season), and climbing over shrubs at roadside. In Madagascar it is found at the edges of dense forest on small trees on rolling white sand. Grows at altitudes up to 600m, often on plateaus. In Liberia growing in sun at roadsides.

Distribution

Endemic to Africa and Madagascar. Africa: Cameroon, Ghana, Sierra Leone, Liberia, Gabon, Angola, Dem. Rep. of the Congo. Fig. 10.24.

Comments

Lygodium smithianum has pinnate branches bearing entire, linear segments much like *L. volubile, L. salicifolium* and *L. kingii.* There is no articulation in this species and the petiole appears to simply become the costa. The petiole is narrowly winged and the lamina increases in width towards the base of the segment. The pinna-stalk is absent or very reduced. There is an affinity with the New World species *L. volubile. Lygodium smithianum* differs in the lack of articulation at the petiole/segment junction, in the very reduced pinna stalk (in *L. volubile* it is over 3 mm), in the grooved to narrowly winged axes (there is more lamina in *L. volubile*), and in multicellular hairs often slightly curved toward the axes (in *L. volubile* the hairs are often 1-2-celled and erect). The spores of *L. volubile* and *L. smithianum* are similar.

Lygodium smithianum differs from *L. lanceolatum* in being free-veined and in having verrucate spores. It differs from*L. salicifolium* in the lack of an articulation zone. The spores of *L. smithianum* easily distinguish it from *L.*

microphyllum (reticulate) and *L. reticulatum* (reticulate). (See Comments under *L. volubile* and Table 10.3).

Nomenclatural Problems

Presl (1845:112) named the species without a description. He cited C. Smith's collection from the Congo as the type. This collection was studied by R. Brown who noted it was a new species of *Lygodium.* In various works (including Prantl, 1881), the authorship of *L. smithianum* is attributed to Presl or Presl ex Kuhn. However, Presl's "name" is a *nomen nudum.* Kuhn cited 'Mett. ms.' indicating that the description was taken from Mettenius' work thus, the epithet Mett. ex Kuhn. There are two sheets at Berlin designated as the type collection. One is a mixed sheet (Sheet I) containing only two segments, bearing the C. Smith (Congo) label. The second sheet (Sheet II) consists of a drawing of a complete specimen citing Smith (Congo). The packet contains leaf fragments from Senegal, thus not part of the type collection. Since Mettenius described this species from the fragments of Sheet I and the drawing (Sheet II) both these sheets must represent the syntypes. The complete specimen from which the drawing came is in Herb. Lucien Hauman, which is at Brussels (BRVU). A excellent example of *L. smithianum* is an isotype at BM.

Selected Specimens Examined

Sierra Leone. Taia River: *Fay 1205* (NY, MO, F); **Liberia.** Monrovia: *Wren 351* (F); *F. Cook 151* (MO); Central Province, Ganta: *Harley F6* (GH); Western Province: *Baldwin 10281* (GH); Bong City: *Fay 1229* (F); **Côte d'Ivoire.** Foret du Banco: *Halle s.n.* (P); Abidjan: *Leeuwenberg 1791* (MO); **Ghana.** Kibi: *Adams 971* (NY); Asuanse: *Box 2042* (BM); **Nigeria.** Ikom(?), Cross River: *Ariwaodo 868* (MO); *A. Kitbon s.n* . (BM); **Cameroon.** Bipindi: *Zenker 1876* (MO); *Leeuwenberg 7882* (MO); Yaúnde: *Zenker and Staudt 455* (BM); *Schlechter*

12399 (BM); **Gabon.** Mussima and Morimba(?): *Halle and Cours 5970* (P); Ogue-Ivindo (Makokou): *Dorr and Barnett 4297* (MO); Asok: *Breteler and Wilde 128* (MO); Estuaire Region, 0°33'N 9°40'E: *Thomas and Wilks 6366* (MO); Prov. Haut-Ougairé (Franceville): *Frei s.n.* (MICH); *Imhoof s.n.* (MICH); Mitzic, 0°47'N 11°34'E: *Jeffrey s.n.* (GH); **Rep. of the Congo.** *Lebrum 267* (BM); **Dem. Rep. of the Congo.** Kinshasa Valleé, Luvanium: *Lewalle 3126* (NY); Kinshasa: *Lewalle 3126* (P); Leopoldville: *Gupffert 265* (MO); **Angola.** Golungo: *Welwitsch 80* (BM).

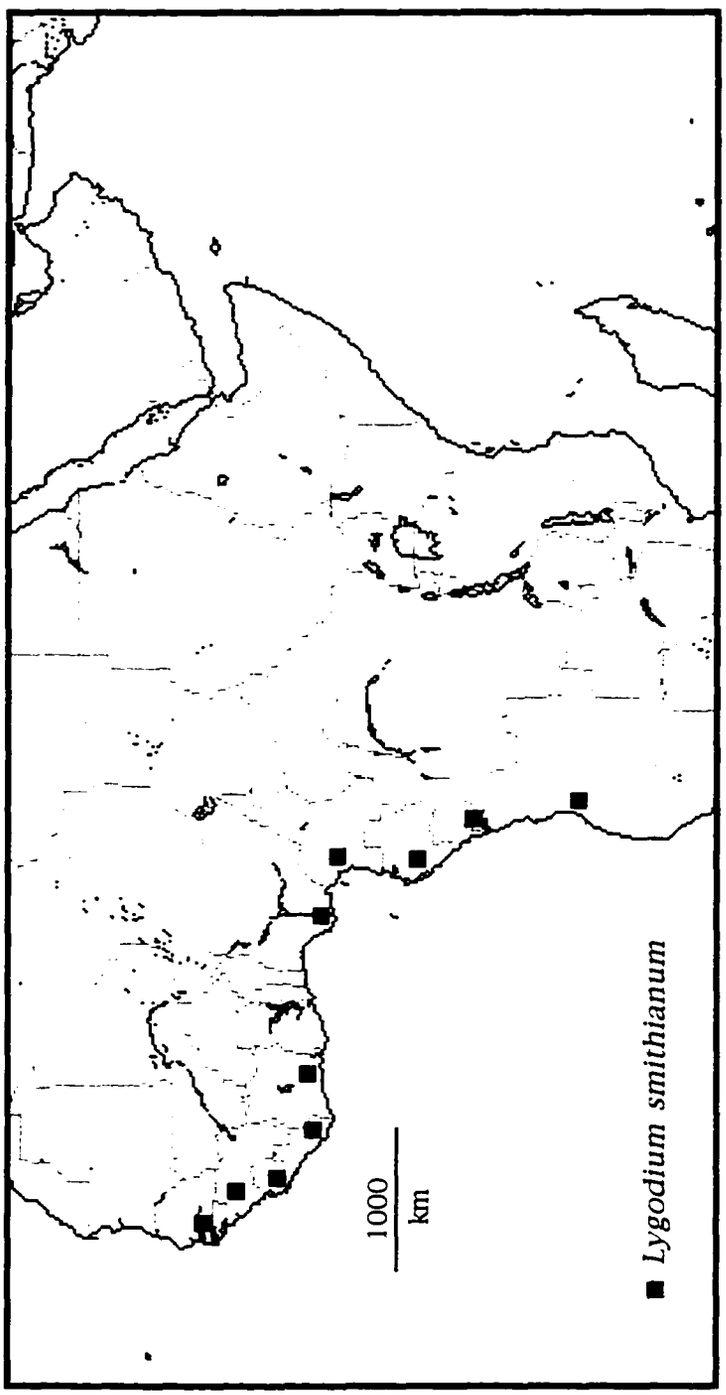


Figure 10.24. The geographic distribution of *Lygodium smithianum*.

25. ***Lygodium salicifolium*** Presl, Suppl. tent. pterid., 102. 1845.

Type. Singapore, *Cuming 365* (K!, isotype BM!). Figs. 10.37E-H.

Rhizome short-creeping, 3-7 mm diam. Frond approximate, 4-6 fronds within 2 cm, climbing to 5-7 m. Stipe with hairs of rhizome continuing 4 cm up stipe. Rachis 1-3 mm in diam., narrowly grooved, pubescent with few 2-4-celled, light tan, acicular hairs with brown septa and short, erect, 1-2-celled hairs. Pinna-stalk 0-1 mm, often bearing 5-celled, reddish-brown septate hairs. Dormant pinna-bud prominent with stramineous, multicellular hairs surrounding reddish-brown to dark brown lustrous, swollen, multicellular-based hairs. Primary pinna-branch pinnate (rarely 2 times pinnate), grooved to narrowly winged, bearing 4-6 alternate segments, ending in two simple or one bifid segment, branch often curved toward axis, sparsely covered with multicellular, acicular, septate, brownish hairs, intermixed with 2-celled, erect hairs; often with swelling (pulvinis) at segment/petiole junction. Segment-petiole 5-7 mm, proximal and distal segments of uniform size, grooved to narrowly winged, pubescent, hairs as on primary pinna-branch. Segments subcoriaceous, simple, rarely with basal lobes, linear to linear-lanceolate, 4-9(-17) x 1.5-2.0 cm, truncate to cuneate to occasionally slightly cordate at base, acuminate to long attenuate at apex, rarely obtuse; articulate, thickened petiole/segment junction covered with multicellular hairs, lamina glabrous. Veins free, 1-2 times forked, ascending at 40°-50° from costa, ending at margin; costa with occasional 5-celled stramineous hairs, septa brown. Margins serrulate to barely serrulate, not thickened. Fertile and sterile segments monomorphic. Fertile segments bearing 5-10(-16) sporangial pairs

per sorophore, glabrous. Spores 67-79 μ (ave. 73.8 μ), tuberculate, tubercles scattered (Fig. 6.1h).

Ecology

Lygodium salicifolium grows in second growth rain forests at altitudes to 800 m, and at forest edges, forming thickets. In Caroline Islands it is found on volcanic clay soil at the edge of clearings with *L. circinnatum*, *Nephrolepis*, *Hibiscus*, and *Scleria*.. Holttum (1959) reported it growing in teak forests in western Java. This species is usually confined to areas with a short dry season.

Distribution

China (Yunnan), Taiwan, India (Assam, Kerali, Bihar, Pradesh, Tripura), Myanmar, Vietnam, Thailand, Sumatra, Malay Peninsula, Borneo, Java, New Guinea, Andaman Islands, Nicobar Islands, Caroline Islands (Palau), Prince of Wales Island. Fig. 10.25.

Comments

Lygodium salcifolium has an obvious articulation between petiole and segment, often covered with stramineous, septate, 4-celled hairs. The segment apex is acuminate. The species intergrades with *L. flexuosum.* In Ferns of Malaya Holttum (1954) unites the two species. However, in Flora Malesiana (1959) Holttum recognizes both species. In this latter study he noted that *L. salcifolium* grows in regions with a short dry season while *L. flexuosum* tolerates a longer dry season and has a wider distribution. He suggests that hybridization betweent the two species might occur. In all specimens examined in this study no abortive spores were seen. Altson & Holttum (1959) cited the following diagnostic features of *L. salicifolium*: articulation at junction between petiole and segment; all petiole segments of equal size (distal segments smaller or segments sessile in *L. flexuosum*); basal segments usually simple or, if lobed, the lobes small and spreading at right angles (in *L.*

flexuosum elongate oblique basal lobes or often with free quaternary leaflets). In this study the articulation zone seemed the most consistent character (*L. flexuosum* has only a small thickened area, if any) as there is too much variation in segment shape and petiole size. *Lygodium salicifolium* has its New World counterpart in *L. volubile* (see Comments under *L. volubile;* Table 10.3).

Nomenclatural Notes

Lygodium kingii has been treated as a distinct species in this study. All characters are similar to those of *L. salicifolium* except for spore morphology. In *L. salcifolium* spores are tuberculate while those of *L. kingii* are long-ridged verrucate (as in *L.volubile* and *L. smithianum*). Since this character does not vary intraspecifically, the two are not being considered conspecific. Study of more specimens of *L. kingii,* to determine its variability, and knowledge of its natural growth habit may determine if it should be reduced to a form of *L. volubile, L. smithianum* or *L. salicifolium.*

Selected Specimens Examined

China. Yunnan, Menla: *Shing et al., 6683* (MICH); Yunnan: *Zhanhuo 91-116* (GH-A); Shing *6683* (GH-A); **Myanmar (Burma).** Myitkyna, Lawa: *McKee* 6278 (GH); Namkham: *Dickason 230, 233* (MICH); **Thailand.** Chantabon: *Smith 387* (MICH); Kaosabap: *Smith 527* (GH); Koh Chang: *Smith 440* (GH); Udawn Loey: *Tagawa et al., 1109* (GH); **Malay Peninsula.** Perak: *Spane 36232* (MICH); **Sumatra.** Koealoe: *Bartlett 7269* (GH, MICH,UC); **Borneo.** Sarawak: *Clemens & Clemens 20706* (GH); **New Guinea.** Papua, Milne Bay: *Brass* 23927 (GH); Papua, Aisa River: *Brass 1420* (UC); Papua, Ambasi: *King* 178 (MICH); Papua: *Croft & Marsh 893* (GH-A); *J. Croft 1676* (GH-A); **Caroline Islands.** Koror Island: *F. Fosberg 2349* (UC); Palau: *Canfield 512* (NY); **Prince of Wales Island.** without collector or site (GH).

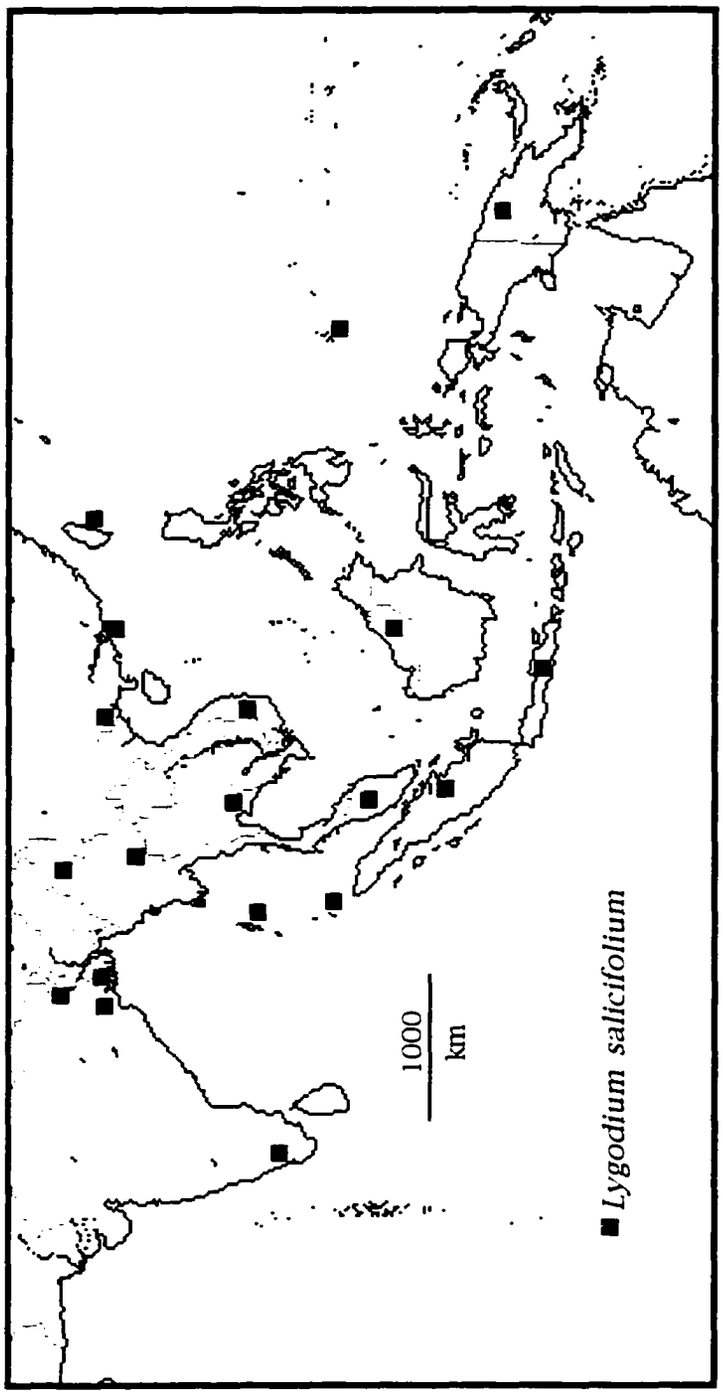


Figure 10.25. The geographic distribution of *Lygodium salicifolium*.

26. ***Lygodium kingii*** Copeland, Philipp. J. Sci. Bot., 6: 68. 1911.

Syntypes. New Guinea, Papua, *King 178, 282, 362* (MICH!).

Rhizome short-creeping, 2-3 mm in diameter, sparsely covered with dark brown, septate multicellular hairs. Frond length not recorded. Stipe not observed. Rachis 2-3 mm in diameter, slightly grooved. Pinna stalk 0 to < 1 mm. Dormant pinna-bud in pocket formed by pinna-branch bases, covered with multicellular, septate hairs intermixed with swollen, multicellular-based hairs. Primary pinna-branch grooved, once pinnate, bearing 5-6 alternate segments, swelling (pulvinus) at petiole/branch junction. Segment-petioles 7-10 mm decreasing distally, grooved, glabrous. Segments strongly articulate, linear-lanceolate, rarely with basal auricles , 5-12 x 1-2 cm, cordate to truncate basally, rarely cuneate, apex attenuate to acute, bearing 3-4-celled hairs at the base of the segment, rarely on costa. Veins free, dividing 2-3 times, ascending 40°-50° from costa, ending at margin. Margins serrulate to serrate, slightly thickened. Fertile and sterile segments monomorphic. Fertile segments bearing 5-10 sporangial pairs/sorophore, indusia with margins erose, multicellular hairs under sporangia on sorophore, midvein, and occasionally on indusium. Spores 67-90 μm (ave. 83 μm), long-ridged verrucate, laesura prominent (Fig 6.5g).

Ecology

Lygodium kingii grows in forests to 800 m altitude, along rivers, and on dry slopes of mountains.

Distribution

Endemic to New Guinea. Fig. 10.26.

Comments

It is difficult to separate *Lygodium kingii* from *L. salcifolium* except by the spore morphology (the latter has tuberculate spores). In many respects the taxa resemble the New World *L. volubile* and may be an example of convergent evolution or a previous pantropical taxon that has undergone geographic isolation and speciation.

Selected specimens examined

New Guinea. Papua: Black Water Creek, *Croft* 1676 (GH-A); Leie, *Croft and J. Marsh 893* (GH-A); Mamba River, *King s.n.* (P); Morobe District, Boana, *Clemens 41446* (MICH, UC); Papua, *Brass 1420* (GH); Sattleburg, *Bamler 70* (P).

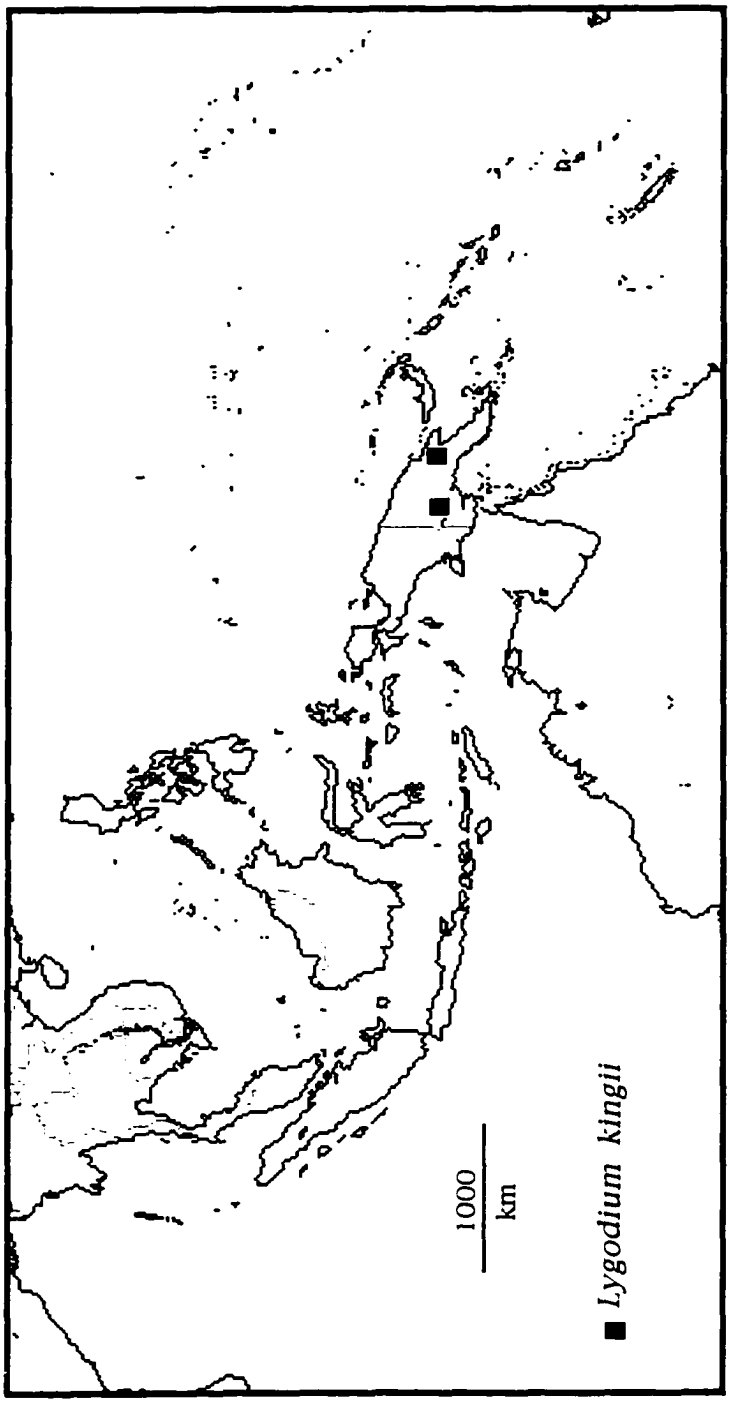


Figure 10.26. The geographic distribution of *Lygodium kingii*.

Hybrids

I. ***Lygodium*** **×** ***fayae*** Jermy and T. Walker, Bull. Br. Mus. nat. Hist. (Bot.) 13(2): 252. 1985. Type. Trinidad. Cedros Ward, Irois, *Walker T6960* (BM!; isotype, NY!). *L. volubile* Sw. (= *L. micans* Sturm) x *L. venustum* Sw.

This hybrid combines the characteristic long linear-lanceolate segments of *L. volubile* with the symmetrical basal lobes of *L. venustum.* The sporangia are on long sorophores and spores are abortive. This hybrid is difficult to distinguish from *L. volubile.* There are specimens of *L. volubile* that have viable spores with the most proximal segments bearing symmetrical lobes, especially on fertile blades. Walker (1985) considers *L. micans* and *L. volubile* distinct species and this hybrid one between *L. micans* and *L. venusutm:* it is rare in Trinidad. Specimens of the parents, from the same locality as the Type of *L. x fayae* are deposited at BM and NY (*Jermy 2437, Walker T10322* of *L. micans;* and *Walker T10558* from a close locality (Tarigua Ward) of *L. venustum).* Walker compares the morphology of parents and hybrid in his Cytotaxonomic studies of the ferns of Trinidad (1985).

Selected specimens examined

Trinidad. *Crosby 46* (NY); Guayaguayare, *Fay 635* (F).

II. ***Lygodium heterodoxum*** **×** ***venustum***

This hybrid originally recognized from Oaxaca, Mexico by Mickel and Beitel (1988) combined the segment morphology of *L. venustum* with the net veins of *L. heterodoxum.* The pinna stalk is 2-6 mm, dormant pinna-bud is prominent with reddish-brown multicellular hairs. The primary pinna-branches are

pinnate, slightly flexuous, bearing 3-4 segments, and have a swelling (pulvinis) at the branch/petiole junction. Axes are puberulent and grooved to narrowly winged. The segments are deltoid to linear-lanceolate to subpalmate. Fertile primary pinna-branches are often twice pinnate proximally becoming once pinnate distally. The fertile segments bear sorophores with up to 25 sporangial pairs per sorophore and abortive spores. The sterile and fertile segments are free to net veined. The fertile blades resemble *L. merrillii* and *L. flexuosum* in which the segments are often 4-fid with the two innermost lobes long and the outermost auricled.

Selected Specimens Examined

Mexico. Oaxaca: Juchitan, *Mickel & Hellwig* 4179, 2 sheets (NY); Yoro: Aldea Las Minas, *Gomez 128* (NY).

III. ***Lygodium kerstenii*** × ***lanceolatum*** (*Lygodium boivini* Mett ex Kuhn, Fil. Afr., 168. 1868).

This hybrid was described by Kuhn as *Lygodium boivini* (Type: Madagascar, Nossy Be, *Boivin s.n.*, B!). It has sterile segments with irregularly lobed margins, often pinnatifid resembling those of the Madagascar specimens of *L. kerstenii* combined with the net veins of *L. lanceolatum.* The fertile segments are almost entire, rarely net veined, with sporangia borne on very long sorophores as in *L. lanceolatum.* The spores are abortive, often enlarged, globose with smaller satellite spherules (Fig. 6.10a-c).

Selected Specimens Examined

Madagascar. Nossy Be: *Boivin s.n.* (BM, F); *Decary 2090* (BM); fragment of Type?, Ex. Herb. Mett., without collector or number (NY).

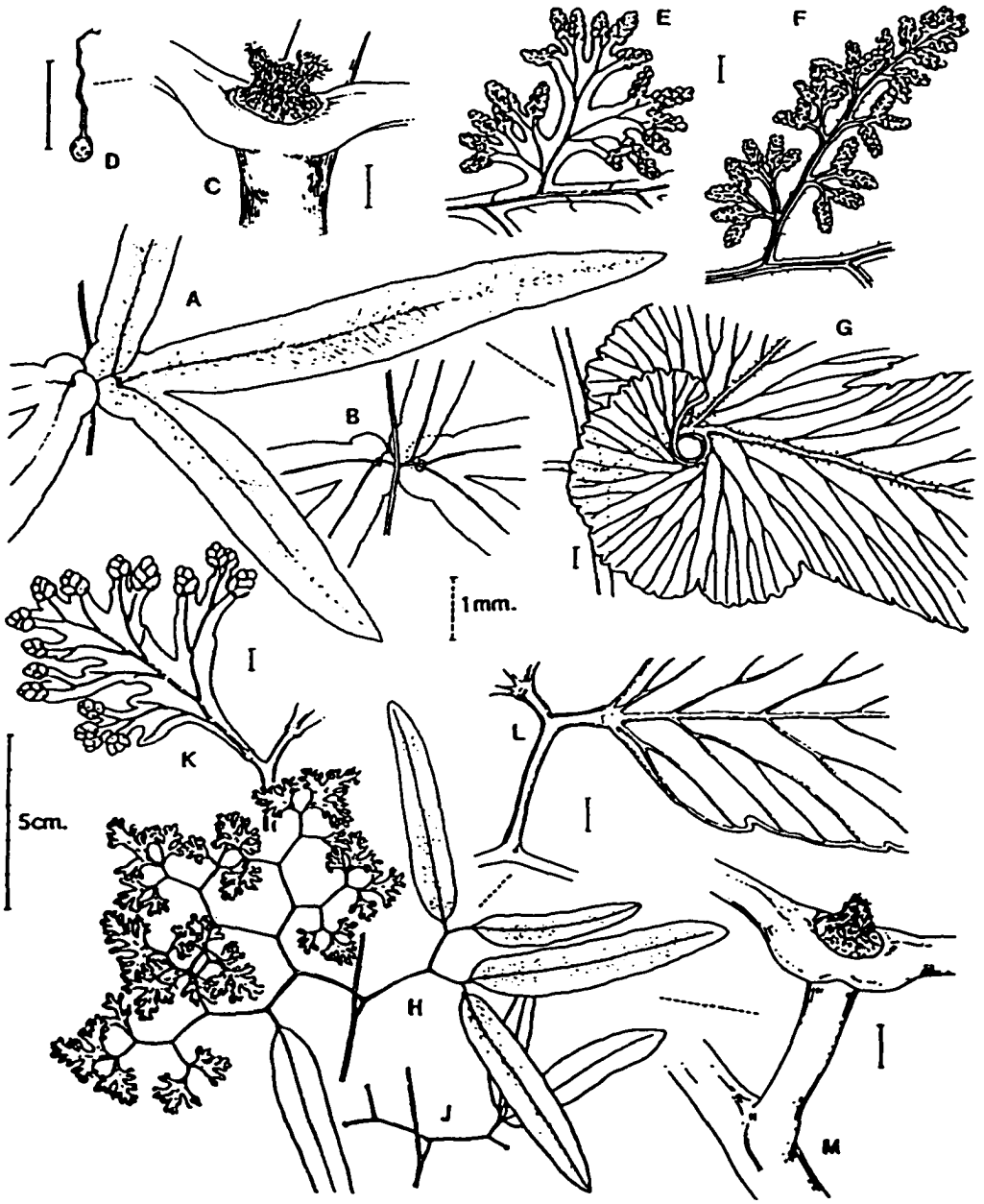


Fig. 10.27. A-G. *Lygodium trifurcatum*. A, B. Sterile pinna-branch and strongly auriculate segments adaxial (A) and abaxial (B) view (*Brass 24400*, GH-A); C, D. Pinna-stalk and pinna-bud detail with swollen, multicellular-based bud hair (*King 134*, MICH); E. Fertile segment detail (*Brass 24400*, GH-A); F. Fertile segment with reduced lamina (*King 134*, MICH); G. Segment base and venation detail (*Brass 24400*, GH-A). H-M. *Lygodium articulatum* [*Trevarthen s.n.*, GH-A; (L, *Pichi Sermolli 6305*, NY)]. H. Pinna-branches with sterile and fertile segments; J. Abscised segments leaving petioles on pinna-branch; K. Fertile segment detail; L. Petiole and segment base with strong articulation zone, venation and margin detail; M. Pinna-stalk and pinna-bud detail.

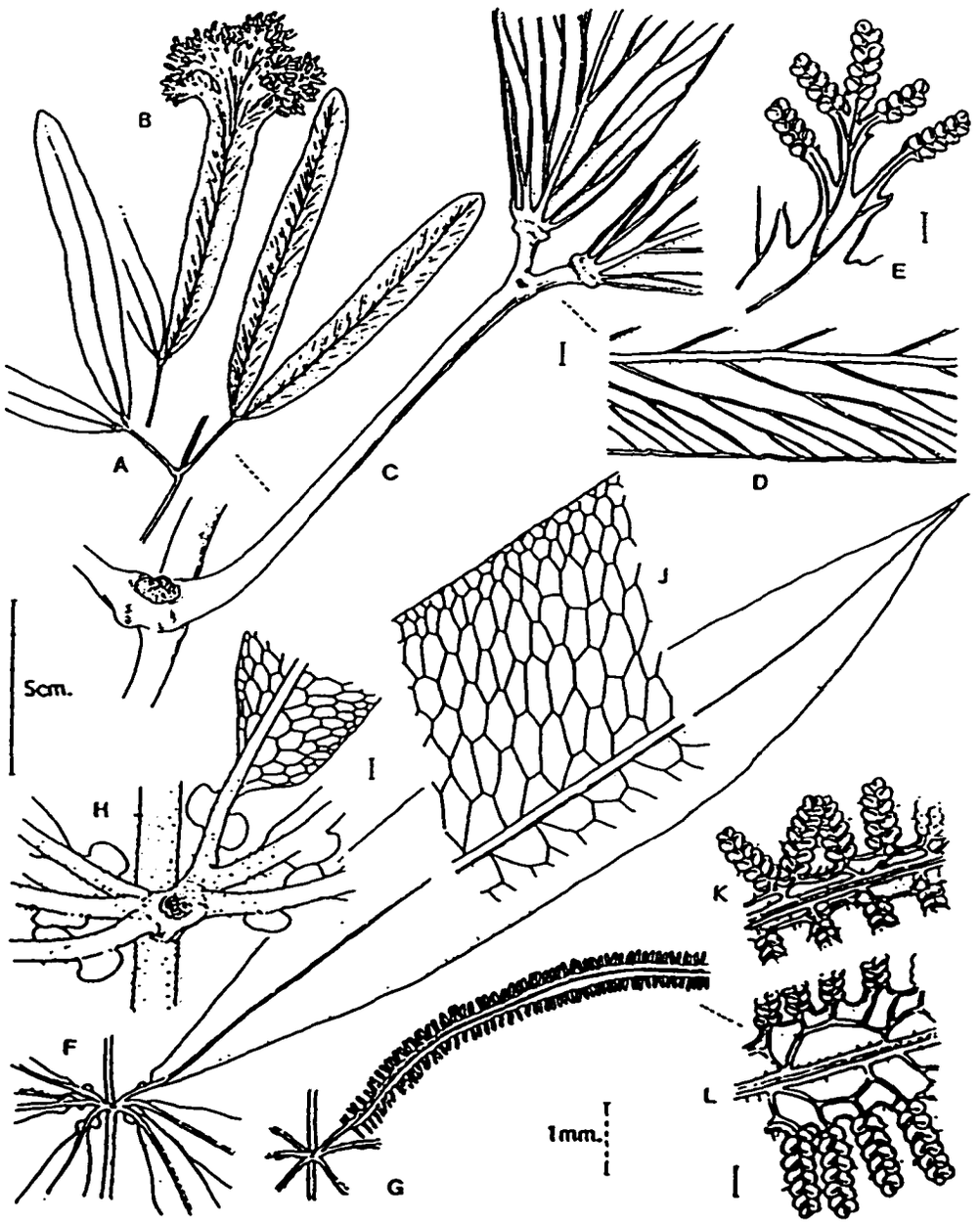


Fig. 10.28. A-E. *Lygodium hians* (*Mackee 19161*, P). A. Pinna-branch and segment; B. Fertile segment; C. Pinna-stalk, bud, branches and articulation at base of segments; D. Segment venation ending in entire margin; E. Sorophores. F-L. *Lygodium versteegii*. F. Sterile segments radiating from pinna-stalk (*Brass 13441*, MICH); G. Fertile segments (*Brass 13441*, MICH); H. Segment base and pinna-bud detail (*Gawi 25*, GH-A); J. Reticulate venation (*Gawi 25*, GH-A); K. Contracted fertile segments and sorophores (*Brass 13441*, MICH); L. Less dimorphic fertile segments, venation and sorophores (*Gawi 25*, GH-A).

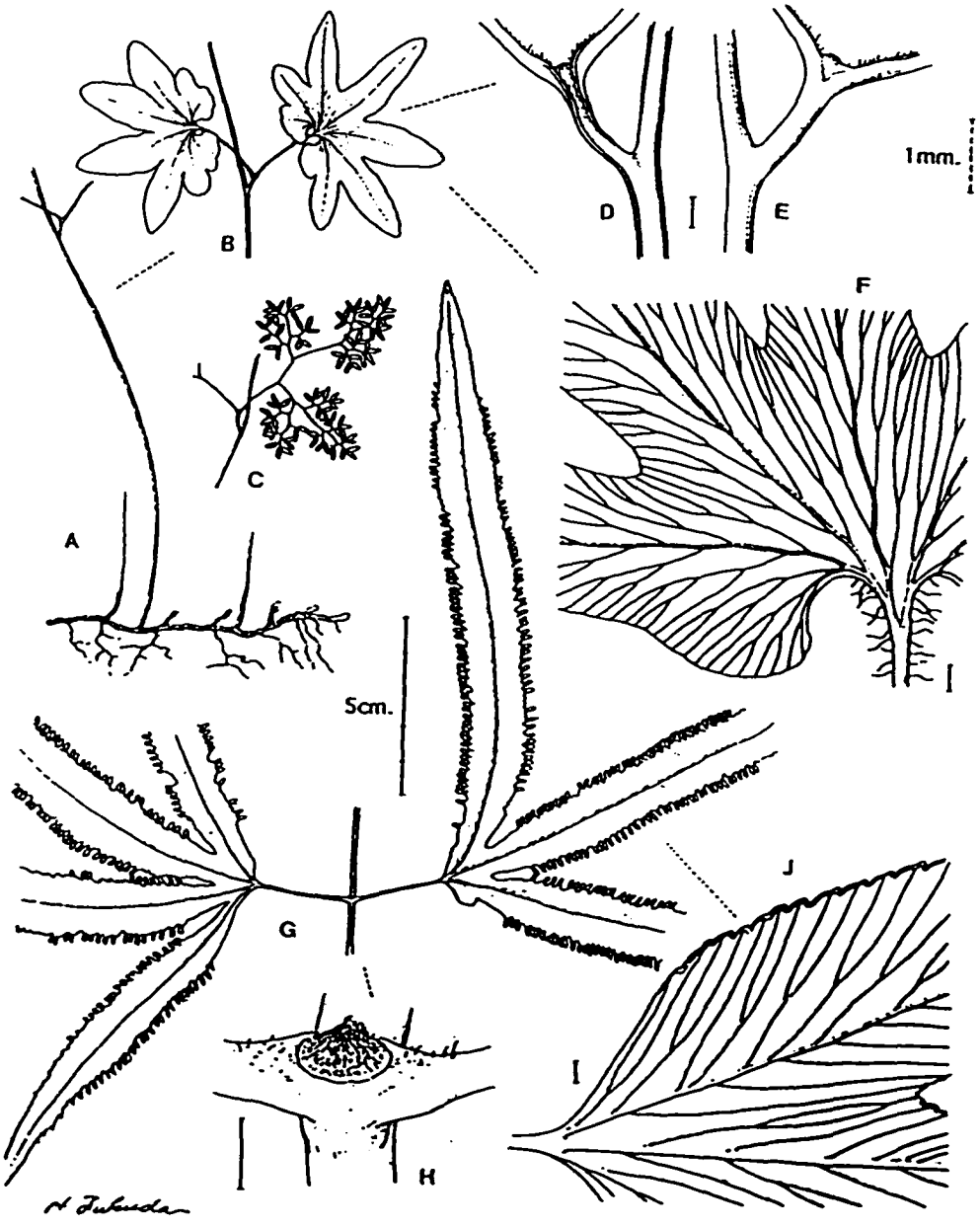


Fig. 10.29. A-F. *Lygodium palmatum*. A. Rhizome - long creeping (*Beitel et al., 74214*, NY); B. Pinna-branch and sterile segments (*Beitel et al., 74214*, NY); C. Fertile segments (*Jesup s.n.*, NY); D,E. Pinna-stalk and inconspicuous bud from both surfaces of axis (*Hill, 10441*, NY); F. Venation pattern from base of segment and detail of margin (*Jesup, s.n.*, NY). G-J. *Lygodium radiatum* (*Burch 1018*, GH). G. Pinna-branch and fertile segments; H. Pinna-stalk and pinna-bud detail; J. Venation pattern from base of segment and detail of margin.

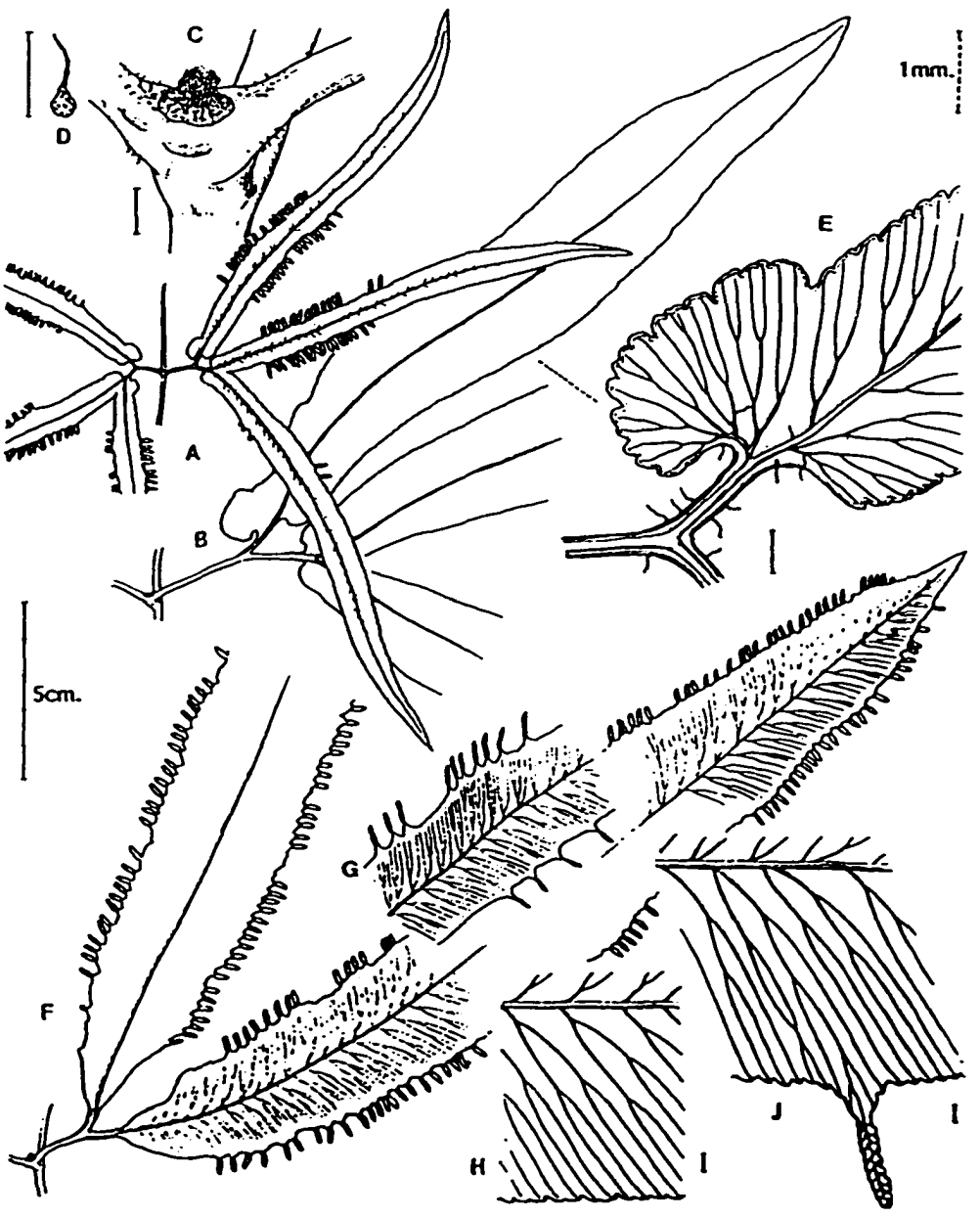


Fig. 10.30. A-E. *Lygodium auriculatum.* A. Rachis, pinna-branches and fertile segments (*Necker 316*, UC); B. "Semihastate" sterile segment (*Topping 1287*, NY); C. Pinna-stalk and pinna-bud detail (*Topping 1287*, NY); D. Swollen, multicellular-based bud hair (*Topping 1287*, NY); E. Narrowly winged pinna-branch, segment venation, and margin detail (*Necker 316*, UC). F-J. *Lygodium borneense* . F. Rachis, pinna-branches and fertile segments (*Elmer 20827*, UC); G. Venation and pedicellate sorophores (*Iwatsuki B704* UC); H. Venation and margin detail (*Iwatsuki et al., B704* UC); J. Fertile segment venation and sorophore detail (*Iwatsuki et al., B704* UC).

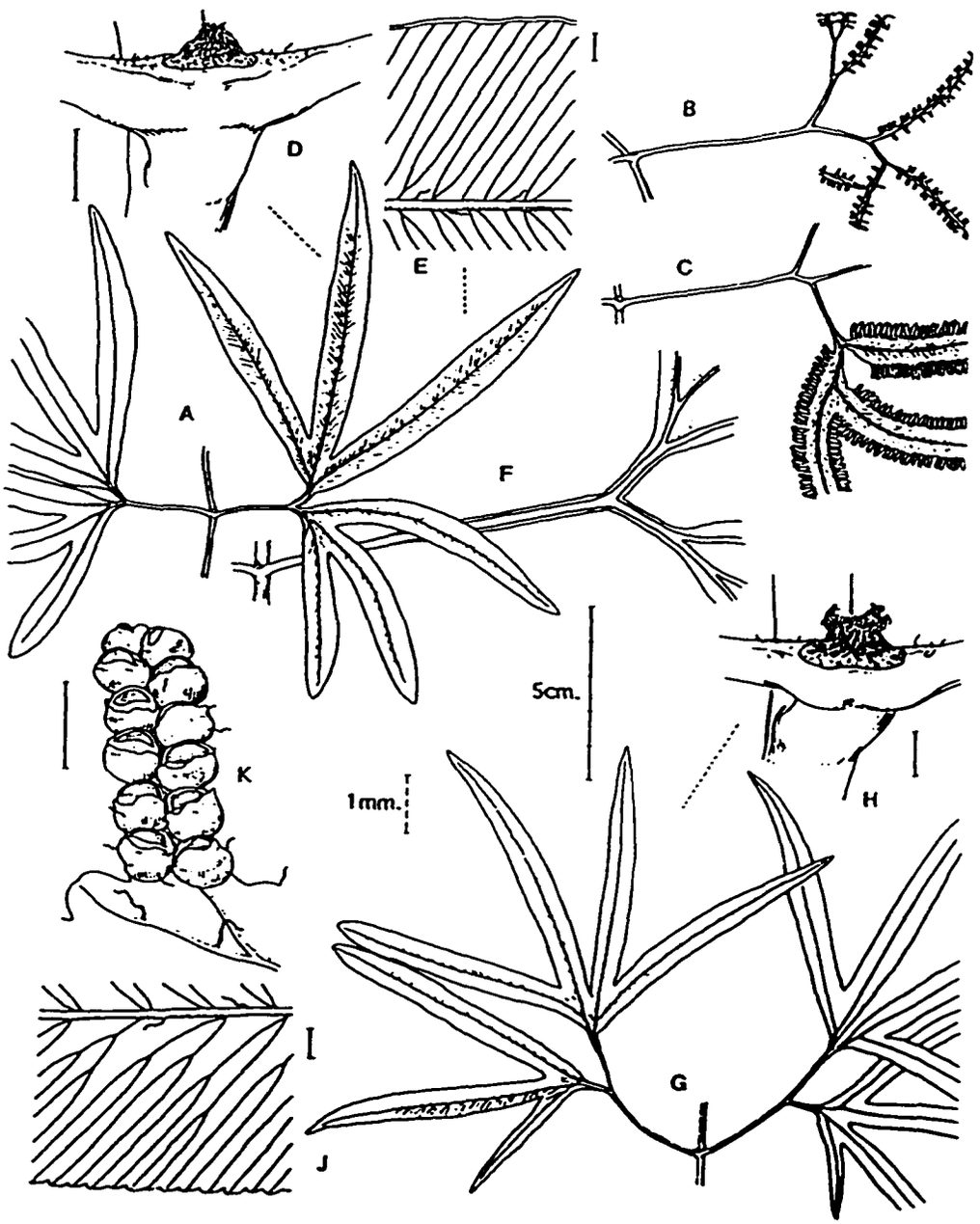


Fig. 10.31. A-F. *Lygodium circinnatum*. A. Pinna-branch and sterile segments (*Topping 1916*, NY); B. Dimorphic fertile segments (*Williams 147*, NY); C. Fertile segments, lamina less contracted (*Topping 1378*, NY); D. Pinna-stalk and sunken pinna-bud detail (*Williams 147*, NY); E. Segment venation detail with veins ending in thickened margin (*Williams 147*, NY); F. Narrowly winged pinna-branch detail(*Topping 1378*, NY). G-J. *Lygodium longifolium*. G. Pinna-branch and sterile segments (*Burkell 2766*, GH-A); H. Pinna-stalk and raised pinna-bud detail (*Burkell 2766*, GH-A); J. Segment venation ending in serrate margin (*Sinclair 10564*, GH-A); K. Sorophore and sporangia (*Zogg & Gassner 7329*, UC).

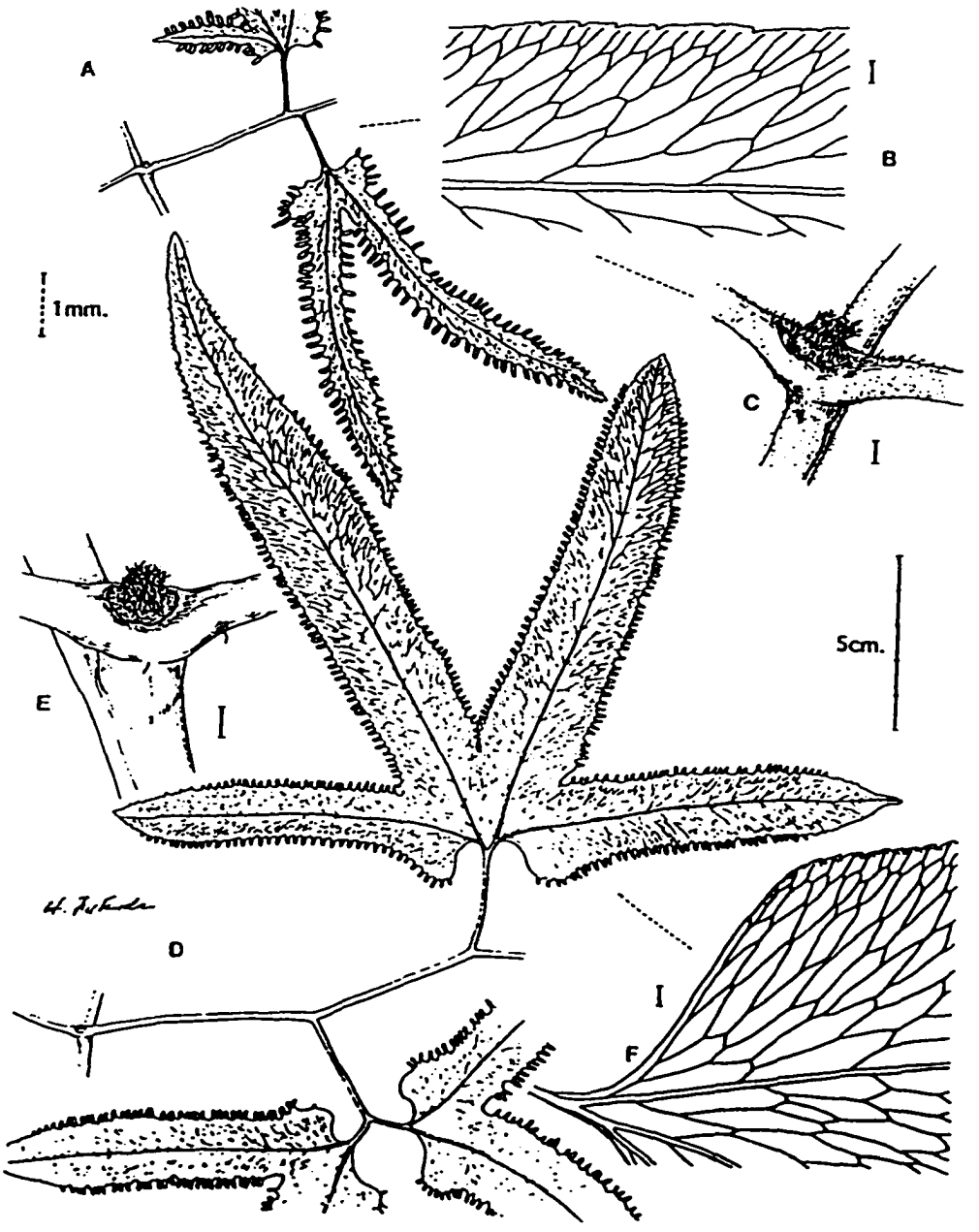


Fig. 10.32. A-C. *Lygodium merrillii.* A. Pinna-branch and fertile segment (*Ramos & Edano s.n.*, P); B. Reticulate venation detail, veins end before margin (*Topping 1318*, GH); C. Pinna-stalk and pinna-bud detail (*Copeland s.n.*, GH). D-F. *Lygodium heterodoxum.* D. Pinna-branch and fertile segments (*Hallberg 1302*, NY); E. Pinna-stalk and pinna-bud detail (*Morton 7792*, NY); F. Segment base, venation and margin detail (*Morton 7792*, NY).

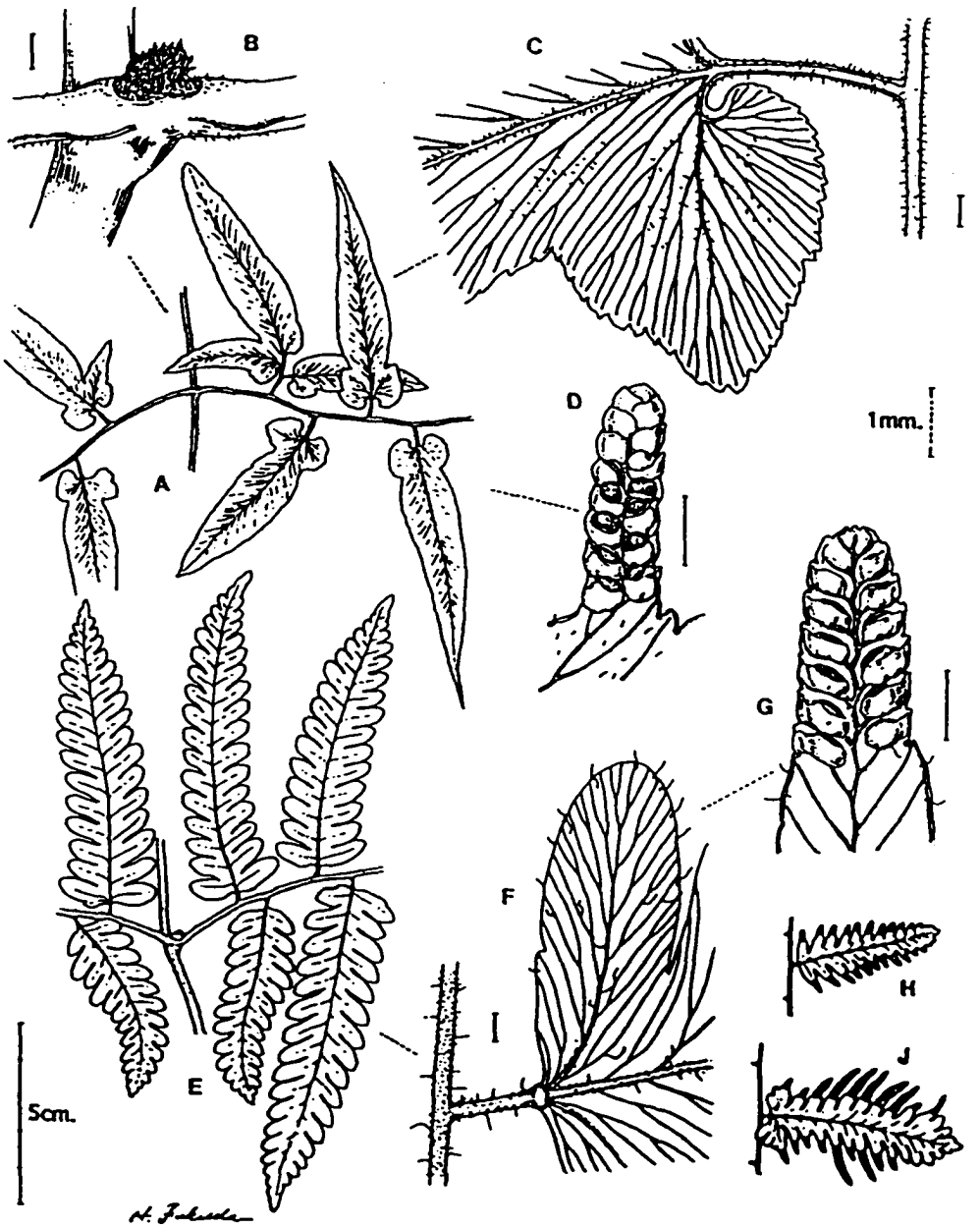


Fig. 10.33. A-D. *Lygodium flexuosum.* A. Pinna-branch and sterile segments (*Topping 1351*, GH); B. Pinna-stalk and pinna-bud detail (*Reilly s.n.*, MICH); C. Segment base, venation and margin detail (*Topping 1351*, GH); D. Sorophore (*Topping 1351*, GH). E-J. *Lygodium polystachyum* (*M. Allen 2782*, GH-A); G. (*Matthew s.n.*, K). E. Pinna-branch and pinnatifid segments; F. Segment base, articulation zone, venation detail; G. Sorophore detail; H, J. Variation in fertile segments.

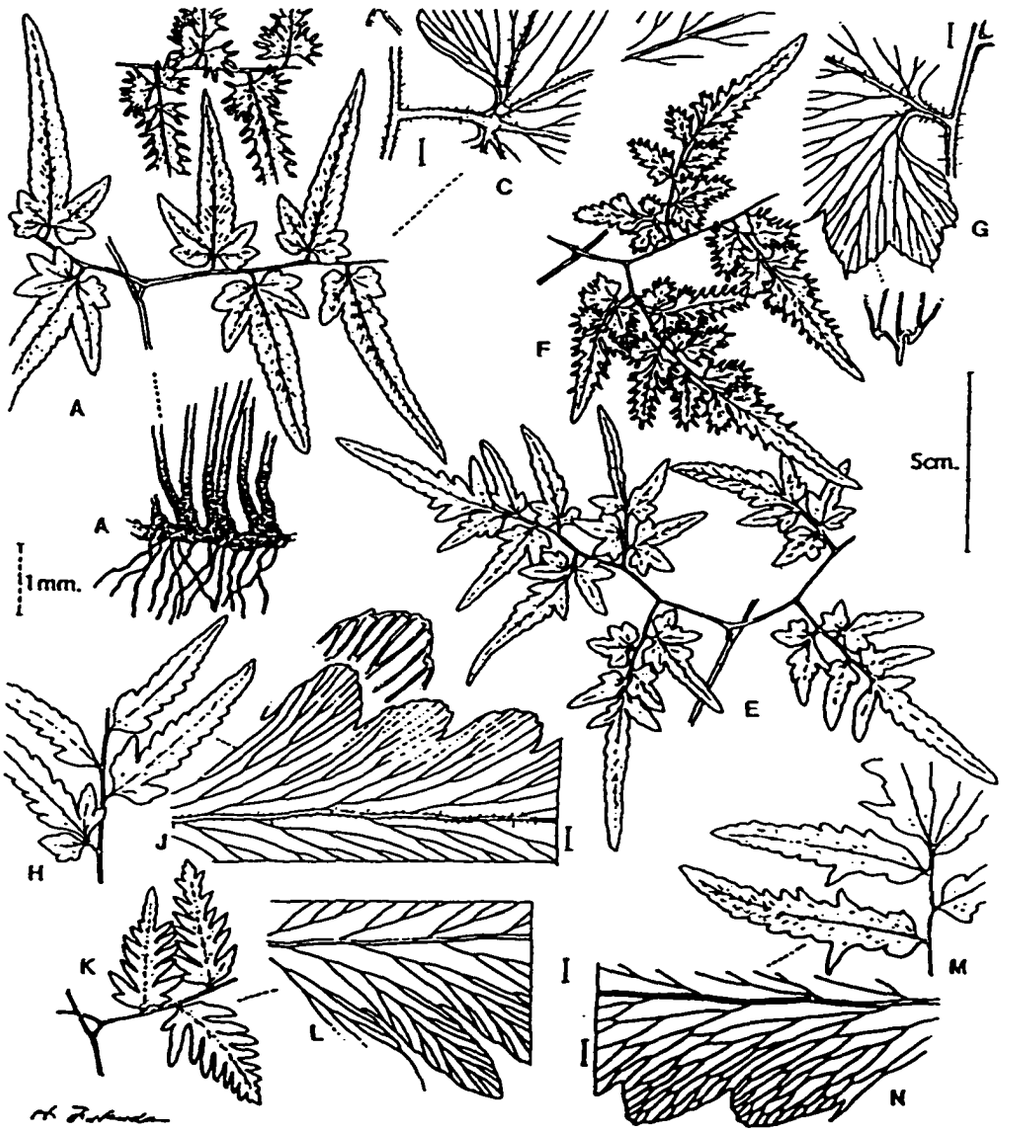


Fig. 10.34. A-D. *Lygodium venustum.* A. Short creeping rhizome and once pinnate sterile pinna-branch and segments (*Jones et al., 3033,* NY - rhizome; *Anderson 6799,* NY - pinna branch); B. Fertile segments (*Fendler 27,* NY); C. Petiole and segment base with articulation area, venation and margin detail (*Anderson 6799,* NY); D. Central lobe venation and margin detail (*Anderson 6799,* NY). E-G. *Lygodium japonicum.* E. Pinna-branch and twice pinnate sterile segments (*Brooks & Hauser 12103,* GH); F. Fertile segments (*Brooks & Hauser 12103,* GH); G. Petiole and segment base without articulation, venation and margin detail (*Ray 8386,* GH). H-L. *Lygodium kerstenii.* H. Secondary sterile pinna-branch and segments (*Taylor 3326,* GH); J. Segment venation and margin detail (*Taylor 3326,* GH); K,L. Variation in segment morphology (pinnatifid) and detail of segment venation and margin (*Humblot 1587,* UC). M-N. *Lygodium boivini* (*Boivin s.n.,* P). M. Sterile segments; N. Segment venation detail (reticulate).

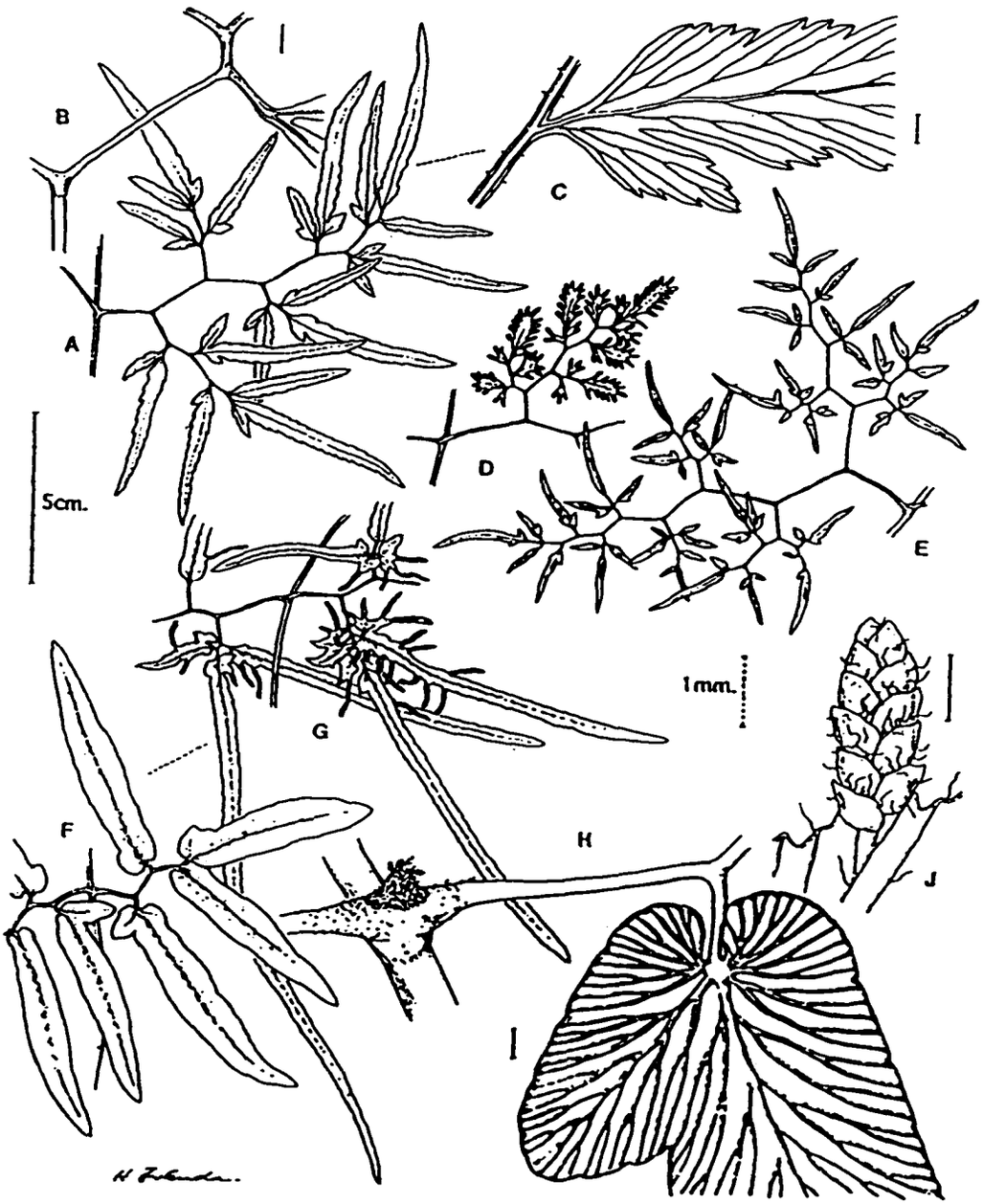


Fig. 10.35. A-E. *Lygodium oligostachyum*. A-C (*Pelaez et al., 1736*, NY). A. Pinna-branch and sterile segments; B. Branching pattern with pulvini at axis angles; C. Sterile segment base, venation and margin detail; D. Fertile segments (*Leonard 8741*, F); E. Sterile segments of a highly contracted form (*Zanoni et al., 24269*, NY). F-J. *Lygodium cubense*. F. Pinna-branch and sterile segments (*Britton et al., 7278*, F); G. Fertile segments of elongated form (*Wright 925*, MO); H. Pinna-stalk, pinna-bud and segment base, venation and margin detail (*Britton et al., 7278*, F); J. Sorophore detail (*Hioram 24323*, GH).

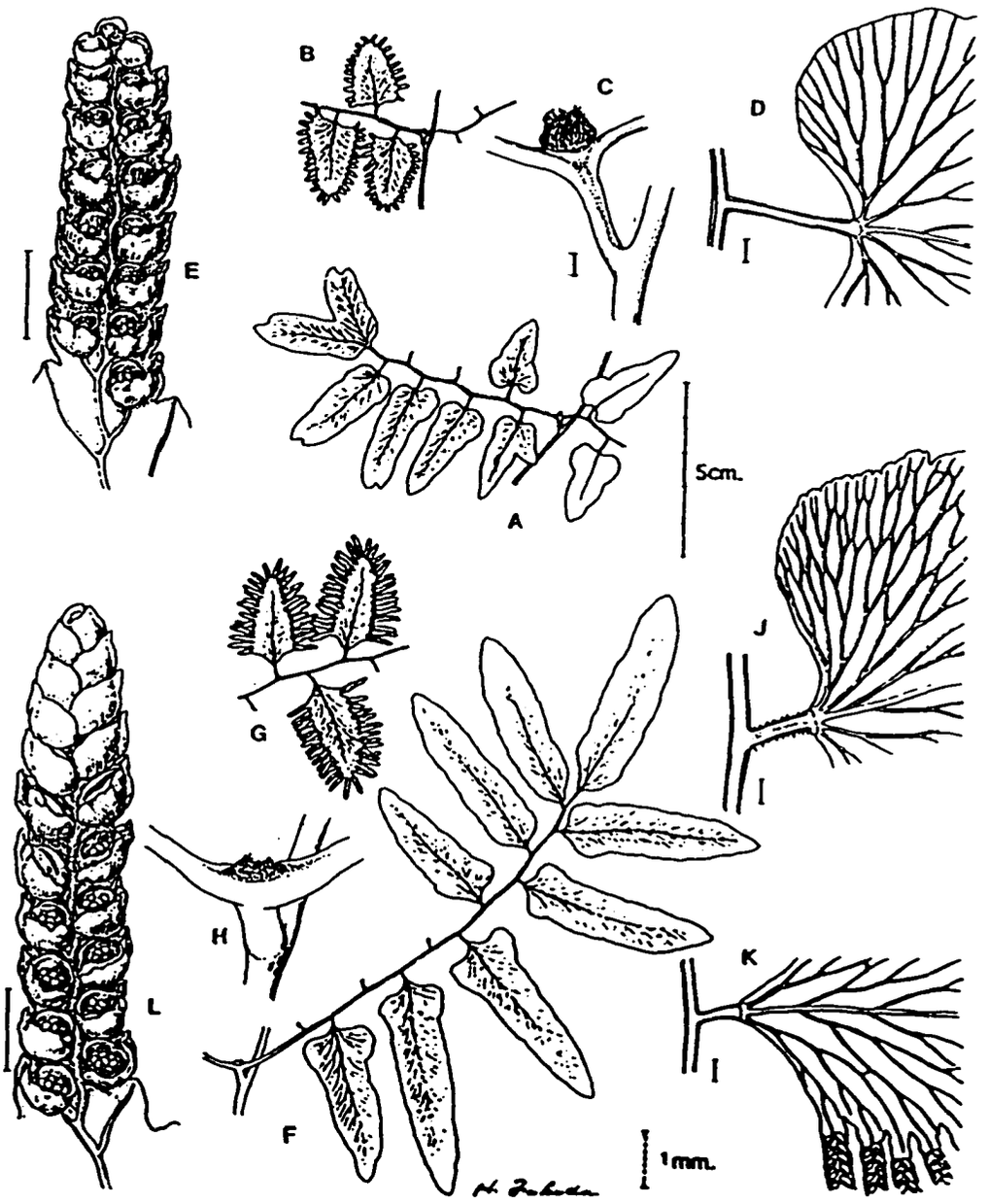


Fig. 10.36. A-E. *Lygodium microphyllum.* A. Pinna-branch and sterile segments (*Topping 1329*, MICH); B. Pinna-branch and fertile segments (Topping *1329*, MICH); C. Pinna-stalk and pinna-bud detail (*Rodin 4703*, UC); D. Segment base, articulation zone and venation detail (*Topping 1329*, MICH); E. Sorophore detail(*Rodin 4703*, UC); F-L. *Lygodium reticulatum.* F. Pinna-branch and sterile segments (*Smith 5332*, NY); G. Fertile segments (*Zogg 9117*, GH-A); H. Pinna-stalk and pinna-bud detail (*Zogg 9117*, GH-A); J. Petiole, articulation zone and venation detail (*Smith 5332*, NY); K. Petiole and fertile segment venation detail(*Zogg 9117*, GH-A); L. Venation into sorophore and sporangia (*Zogg 9117*, GH-A).

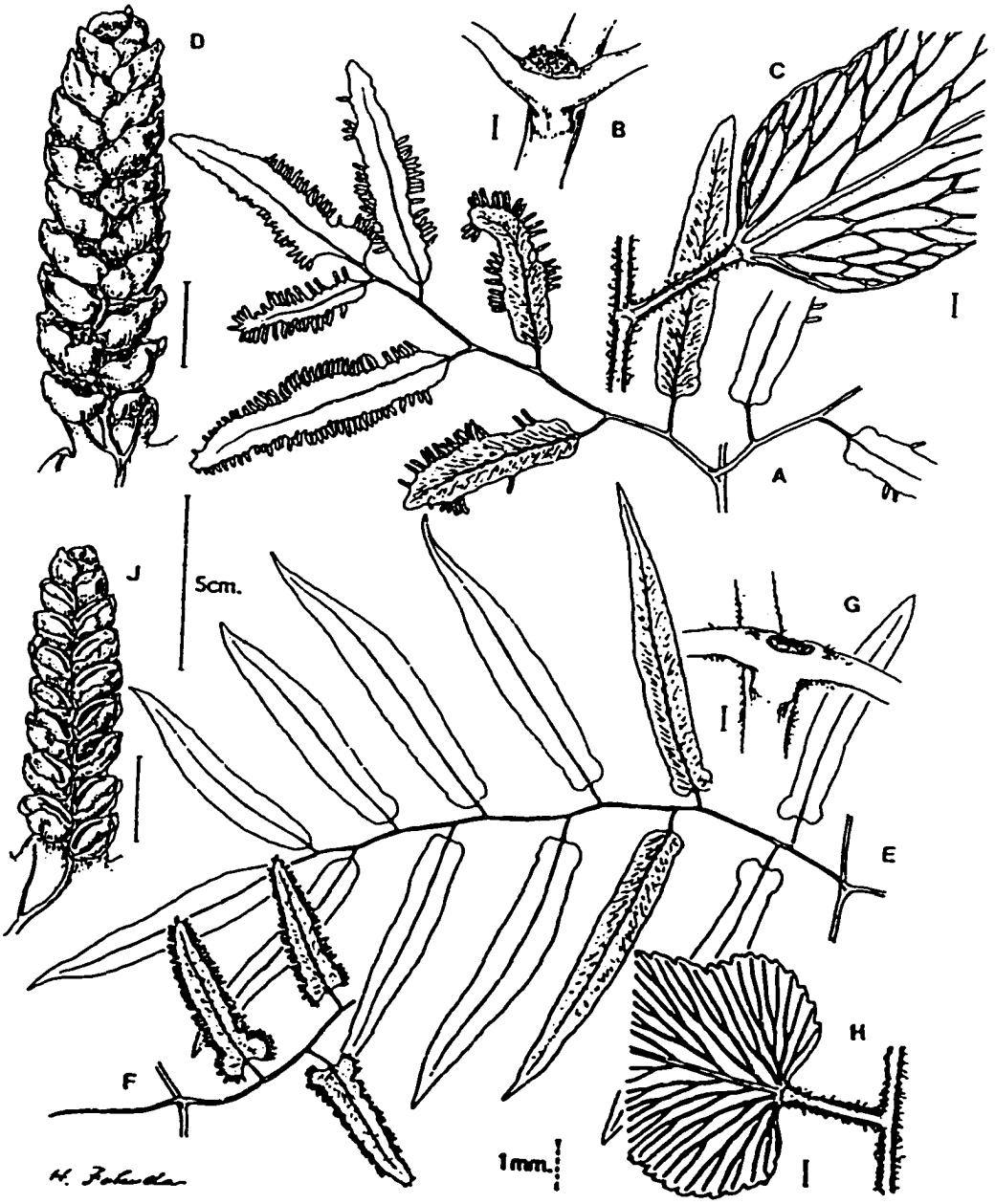


Fig. 10.37. A-D. *Lygodium lanceolatum.* A. Pinna-branch and fertile segments (*Webb 166*, F); B. Pinna-stalk and pinna-bud detail (*Miller 3300*, MO); C. Pinna-branch/petiole swelling, segment base with articulation, reticulate venation (*Webb 166*, F); D. Sorophore detail (*Miller 3300*, MO). E-H. *Lygodium salicifolium.* E. Pinna-branch and segments (*Brass 23927*, GH-A); F. Fertile segments (*Brass, 23927*, GH-A); G. Pinna-stalk and pinna-bud detail (*Spane 36232*, MICH); H. Petiole, segment base with articulation and venation (*Brass 23927*, GH-A).

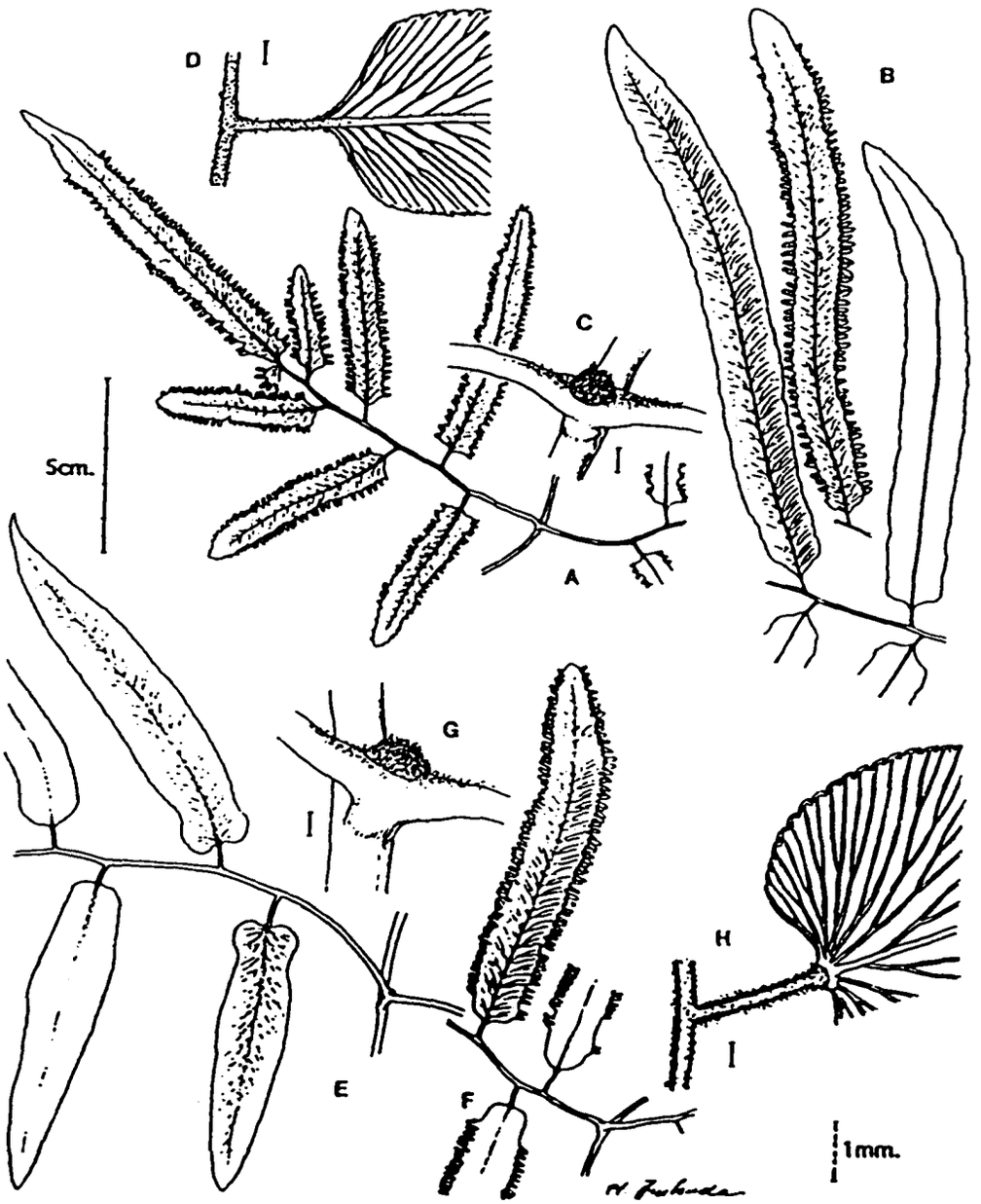


Fig. 10.38. A-D. *Lygodium smithianum.* A. Pinna-branch and fertile segments (*Letouzey 5476*, P); B. Sterile and fertile segment detail (*Fay 1229*, F); C. Pinna-stalk and pinna-bud detail (*Imhoof 1990*, MICH); D. Petiole, segment base with lack of articulation, and venation (*Imhoof 1990*, MICH). E-H. *Lygodium volubile.* E. Pinna-branch and sterile segments (*Ollgaard et al., 74803*, NY); F. Fertile segments (*Ollgaard et al., 74803*, NY); G. Pinna-stalk and pinna-bud detail (*Henkel 2575*, NY); H. Petiole, segment base with articulation and venation (*Ollgaard et al., 84903*, NY).

Appendix

The following represent the NMR and MS analysis of the compounds of *Lygodium japonicum* that have been structurally elucidated. These experiments and analyses are the results of a collaboration between Dr. Barbara Meurer-Grimes and Drs. Ruth Stark and Cherryl Tihal.

Compound A

The structure was elucidated primarily by NMR from ^1H spectra, and COSY, TOCSY, HMBC, and HMQC experiments.

The following signals were observed in the ^1H NMR spectrum, and assigned by comparison with published chemical shifts for glucose (ALDRICH library of NMR spectra) and caffeic acid (Meurer *et al.*, 1986). The ^1H NMR signals for H-7 and H-8 of the caffeoyl moiety indicate that only the (*E*)-configuration was present (formerly called *trans*-configuration in the older literature). Therefore, the interpretation of the NMR data was much easier than for the other compounds which occurred both in the (*E*)- and the (*Z*)-configuration (formerly called *cis*-configuration, see below). The chemical shift for H-1' (the anomeric proton of the glucose moiety) supports a β-1-O-linkage between the glucose and the caffeoyl moiety.

Additional support was derived from GC analysis of the compound, conduted by Dr. Feng Qiu at Oklahoma State University. The presence of glucose was confirmed as well as the C-1 linkage to the caffeoyl moiety. The ration of caffeoyl moiety to glucose was determined to be 1:1. The presence of the caffeoyl moiety was further confirmed by the UV-absorption characteristics as outlined above.

The structure of Compound A was therefore determined as β-1-O-caffeoylglucose (Fig. 8.7).

Table A1. Chemical shifts for ^{1}H and ^{13}C signals of Compound A isolated from *L. japonicum*.

^{1}H NMR chemical shifts

Moiety	Signal	Chemical shift (ppm)	J (Hz)
β-glucose	H-1'	4.82 d	(7)
	H-2'	3.50**	-
	H-3'	3.4-3.5**	-
	H-4'	3.4-3.5**	-
	H-5'	3.44**	-
	H-6a'	3.72 dd	(11)
	H-6b'	3.90 dd	(12)
caffeoyl moiety	H-2	7.08 d	(2)
	H-5	7.01 dd	(9)
	H-6	7.17 d	(8)
	H-7	7.55 d	(16)
	H-8	6.30 d	(16)

^{13}C NMR* chemical shifts

β-glucose	C-1'	103.7
	C-2'	74.7
	C-3'	77.5
	C-4'	71.2
	C-5'	78.3
	C-6'	62.3
caffeoyl moiety	C-1	128.6
	C-2	115.9
	C-3	?
	C-4	?
	C-5	122.0
	C-6	118.5
	C-7	146.2
	C-8	117.8
	C=O	170.3

* The ^{13}C signals were assigned from the HMBC and HMQC experiments. Therefore, signals corresponding to carbons that are not linked to protons could not be assigned (e.g. C-3 and C-4 of the caffeoyl moiety)
** overlapping signals

Compound C1

The structure of the isolate was fully elucidated by NMR and MS. It is evident from the NMR data that a mixture of four isomers was present. The NMR assignments are listed in Table A2, and the structures are shown in Fig. 8.6.

The interpretation of the spectra was intitially difficult due to the occurrence of several isomers leading to partial duplication of sets of signals. The first set of isomers, that can be identified easily is due to the presence of (*E*)- and (*Z*)-isomers of the caffeoyl moiety.

The chemical shifts for H-7 and H-8, as well as for C-7 and C-8 will be affected by the isometry. Adjacent signals of the glucose moiety (e.g. H-4') or the aromatic ring (H-2, H-5, and H-6, as well as C-1 to C-6) may also show slightly modified chemical shifts as seen in the Tables above.

In addition, conjugates of caffeic acid with both α- and β-glucose were present, which can be seen from the different chemical shifts for H-1' of the glucose moieties. All combinations of (*E*)- and (*Z*)-isomers with α- and β-glucose were detected, e.g. four sets of signals each corresponding to a different isomer of caffeoylglucose.

The presence of both α- and β- glucose in the Compound C1 isolate was confirmed by GC. This also confirmed a ratio of 1:1 for the aromatic and the sugar moieties of the molecule. In addition, GC also provided first evidence for the 4-O-linkage between the glucose moiety and the caffeoyl moiety. The position of the linkage is also corroborated by the chemical shift observed for H-4' in the ^{1}H NMR spectrum at 4.85 ppm.

Table A2. ^{1}H chemical shifts for Compound C1 from *Lygodium japonicum*.

Compound/ Moiety	Assignment	(*E*)-isomer ppm		(*Z*)-isomer ppm
caffeoyl-α-glucose				
α-glucose	H-1'	5.17	H-1'	5.16
	H-2'	3.50	H-2'	3.48
	H-3'	3.92	H-3'	3.86
	H-4'	4.85	H-4'	~4.85
	H-5'	4.00	H-5'	~4.00
	H-6a'	3.51	H-6a'	~3.51
	H-6b'	3.60	H-6b'	~3.60
caffeoyl	H-2	7.06	H-2	7.46
	H-5	6.79	H-5	6.74
	H-6	6.96	H-6	7.09
	H-7	7.60	H-7	6.83
	H-8	6.31	H-8	5.80
caffeoyl-β-glucose				
β-glucose				
	H-1'	4.55	H-1'	4.53
	H-2'	3.26	H-2'	3.24
	H-3'	3.61	H-3'	3.62
	H-4'	4.84	H-4'	4.84
	H-5'	3.54	H-5'	3.61
	H-6a'	3.51	H-6a'	~3.51
	H-6b'	3.60	H-6b'	~3.60
caffeoyl	H-2	7.06	H-2	7.46
	H-5	6.79	H-5	6.74
	H-6	6.96	H-6	7.09
	H-7	7.60	H-7	6.83
	H-8	6.28	H-8	5.79

Table A3. ^{13}C chemical shifts for Compound C1 from *Lygodium japonicum*. (assigned by HMBC and HMQC)

Compound/ Moiety		(*E*)-isomer ppm	(*Z*)-isomer ppm	
caffeoyl-α-glucose				
α-glucose	C-1'	93.86		
	C-2'	73.86	chemical shifts not	
	C-3'	71.12/76.68	affected	
	C-4'	72.92		
	C-5'	71.21		
	C-6'	~62.42		
caffeoyl	C-1	127.95	C-1	128.24/128.34
	C-2	115.23	C-2	118.76
	C-3	147.1	C-3	145.85
	C-4	149.9		
	C-5	116.57		
	C-6	123.09	C-6	125.50
	C-7	147.60	C-7	148.72
	C-8	115.01	C-8	116.22
	C-9	169.01	C-9	167.75
caffeoyl-β-glucose				
β-glucose	C-1'	98.36		
	C-2'	76.43		
	C-3'	~62.42	chemical shifts not	
	C-4'	72.92	affected	
	C-5'	76.16		
	C-6'	~62.42		
	C-1	127.95	C-1	128.24/128.34
	C-2	115.23	C-2	118.76
	C-3	147.1	C-3	145.85
	C-4	149.90		
	C-5	116.57		
	C-6	123.09	C-6	125.50
	C-7	147.73	C-7	148.72
	C-8	114.73	C-8	116.13
	C-9	169.01	C-9	167.75

The connectivities between protons and carbons were established by two-dimensional TOCSY, COSY, HMBC, and HMQC experiments.

Mass spectrometry confirmed the proposed structures. Caffeoylglucose esters have an expected molecular weight of MW = 342, and major product ions are expected for a glucosyl moiety (162 amu) and for the caffeoyl moiety (164 amu). The electrospray ionization spectra (ESI-MS) were obtained as full mass scans and MS/MS experiments both in the positive and the negative ion mode. The major signals are summarized in Table A4.

The MS experiments confirmed the expected molecular weight of the compound of MS = 342, and several expected daughter ions, such as for the caffeoyl and the glucosyl moieties. The ions corresponding to [2M + Na +1]+ at *m/z* 707 in the positive ion mode and the ion corresponding to [2M]- at *m/z* 684 in the negative ion mode were identified as artifacts. Fragmentation of these ions in the MS/MS mode did not yield any product ions, or only very low ion counts. This is typical for artifact ions in the electrospray mass spectrometers.

Based on the NMR, MS, UV and hydrolysis results, Compound C1 was identified as a mixture of four isomers of caffeoylglucose.

Compound D3

The ^{1}H NMR spectra and the ^{13}C NMR spectra revealed some similarities between Compound C1 and D3. The signal assignments are shown in Table A5. The ^{1}H NMR spectrum showed that both α- and β-glucose are present as in C1. Also present are the characteristic (*Z*) and (*E*)-isomers of the *p*-coumaric acid moiety (see corresponding description for caffeic acid in Compound C1). These can be easily identified by the characteristic chemical shifts for the protons in 7 and 8 positions at 5.7 ppm (H-7 of (*Z*)-isomer) and 6.7 ppm (H-8 of the (*E*)-isomer), and at 6.35 ppm (H-7 for the (*E*) isomer) and 7.6 ppm (H-8ί for the (*E*)-isomer).

Table A4. Mass spectra of Compound C1 from *Lygodium japonicum.*

Experiment	*m/z*	Assignment
positive ion mode:		
full MS	163	[glucosyl + 1]$^+$
	365	[M + Na + 1]$^+$
	707	[2M + Na + 1]$^+$
MS/MS on *m/z* 707	365	[M + Na + 1]$^+$
MS/MS/MS on *m/z* 707-365	347	[M + Na - OH + 1]$^+$
	185	[glucosyl + Na]$^+$
negative ion mode:		
full MS	161	[glucosyl - 1]$^-$
	341	[M-1]$^-$
	684	[2M]$^-$
MS/MS on *m/z* 341	323	[M - OH - 1]$^-$
	179	[caffeic acid - 1]$^-$ or [glucose - 1]$^-$
	161	[glucosyl - 1]$^-$

Table A5. ^{1}H NMR chemical shifts for Compound D3 from *Lygodium japonicum*.

	(*E*) isomer		(*Z*) isomer	
Moiety		ppm		ppm
α-glucose	H-1'	5.11	H-1'	5.12
	H-2'	3.43	H-2'	3.45
	H-3'	3.82	H-3'	3.82
	H-4'	4.80	H-4'	4.80
	H-5'	3.96	H-5'	3.96
	H-6'a	3.49	H-6'a	3.49
	H-6'b	3.58	H-6'b	3.58
β-glucose	H-1'	4.47	H-1'	4.51
	H-2'	3.21	H-2'	3.21
	H-3'	3.58	H-3'	3.52
	H-4'	4.80	H-4'	4.80
	H-5'	3.88	H-5'	3.88
	H-6'a	3.49	H-6'a	3.49
	H-6'b	3.58	H-6'b	3.58
p-coumaroyl				
	H-2	6.68	H-2	6.72
	H-3	7.43	H-3	7.63
	H-5	7.43	H-5	7.63
	H-6	6.78	H-6	6.72
	H-7	7.63	H-7	6.86
	H-8	6.32	H-8	5.78

Table A6. ^{13}C NMR chemical shifts for Compound D3.

Moiety	(*E*)-isomer	ppm	(*Z*)-isomer	ppm
a-glucose	C-1'	93.27	C-1'	93.27
	C-2'	73.35	C-2'	73.35
	C-3'	72.00	C-3'	72.00
	C-4'	71.86	C-4'	71.86
	C-5'	obscured	C-5'	obscured
	C-6'	61.93	C-6'	61.93
b-glucose	C-1'	97.70	C-1'	97.77
	C-2'	75.87	C-2'	75.87
	C-3'	75.26	C-3'	obscured
	C-4'	71.86	C-4'	71.86
	C-5'	obscured	C-5'	obscured
	C-6'	61.93	C-6'	61.93
p-coumaroyl				
	C-1		C-1	
	C-2	116.33	C-2	115.24
	C-3	130.69	C-3	146.63
	C-4		C-4	
	C-5	130.69	C-5	146.63
	C-6	116.33	C-6	115.24
	C-7	133.27	C-7	145.52
	C-8	114.30	C-8	115.67
	C-9		C-9	

The ^{13}C chemical shifts were assigned from HMBC and HMQC experiments, therefore, the list of signals is not complete because carbons not carrying a proton are not always assigned.

A total of four anomeric protons are seen for the α- and β-glucose moieties. Other signals could be assigned to the α- or β-glucose moieties using the COSY and TOCSY correlations. This indicates that, as in C1, all possible combinations of α- and β- glucose with the (*E*)- and (*Z*)-isomers of the *p*-coumaroyl moiety are present in the isolate.

The glucose moiety is linked to the aromatic moiety in 4-position, as in C1, which can be seen from the chemical shifts for H-4'. This was also confirmed by GC-MS, which also revealed a 1:1 ratio for the sugar and the aromatic moieties. Therefore, the structure of 4-O-*p*-coumaroyl glucose was proposed.

The proposed structure was confirmed by mass spectrometry in the positive and negative mode. The expected molecular weight for p-coumaroyl glucose is 326, and fragments for the p-coumaric acid moiety are expected at 164 and 146 amu, and for glucose at 180 and 162 amu. The observed parent and product ions and their assignment are listed in Table A7.

The mass spectra and fragmentation paths of D3 confirmed the structure proposal derived from the NMR spectra. MS/MS experiments on the ions observed at *m/z* 675, $[2M+Na+1]^+$, and *m/z* 651, $[2M-1]^-$, did not yield any product ions indicating that they are indeed artifacts. Therefore, the isolate Compound D3 was determined to be a mixture of four isomers of 4-O-*p*-coumaroyl glucose.

Table A7. ESI-mass spectrometry results for Compound D3 from *Lygodium japonicum*.

Experiment	*m/z*	Assignment
positive ion mode		
full MS	675	$[2M+Na+1]^+$
	147	$[p\text{-coumaroyl} + 1]^+$
negative mode		
full MS	651	$[2M-1]^-$
	325	[M-1]-
	145	$[p\text{-coumaroyl} - 1]^-$
	117	$[p\text{-coumaroyl} - C{=}O - 1]^-$
MS/MS on *m/z* 325	187	$[p\text{-coumaroyl} + HOAc - 1]^-$
	307	$[p\text{-coumaroyl} - OH - 1]^-$

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IMAGE EVALUATION
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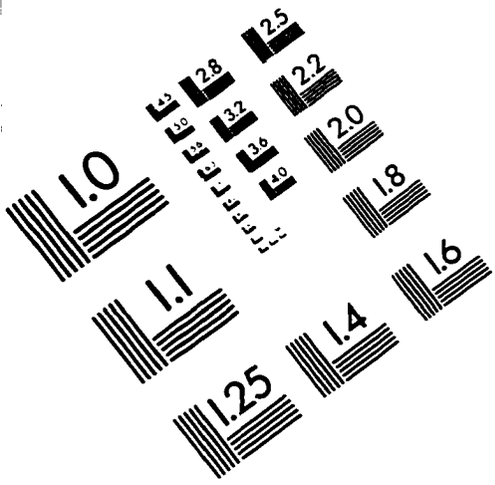

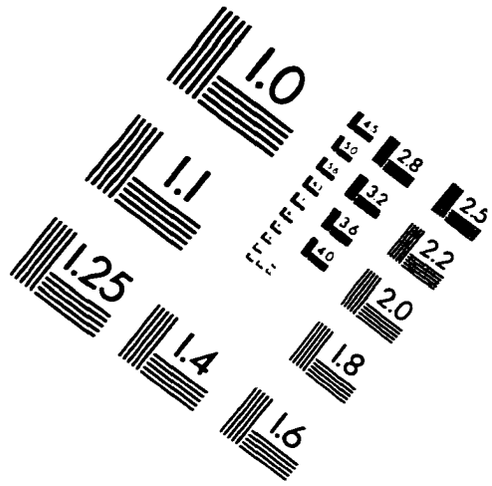

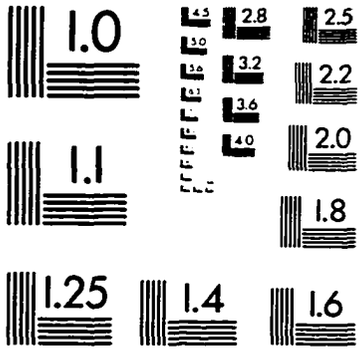

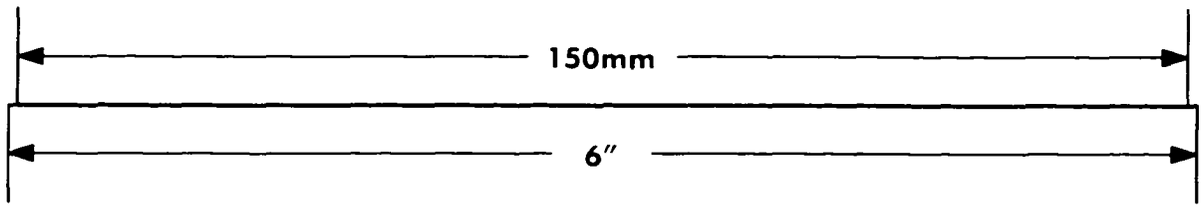

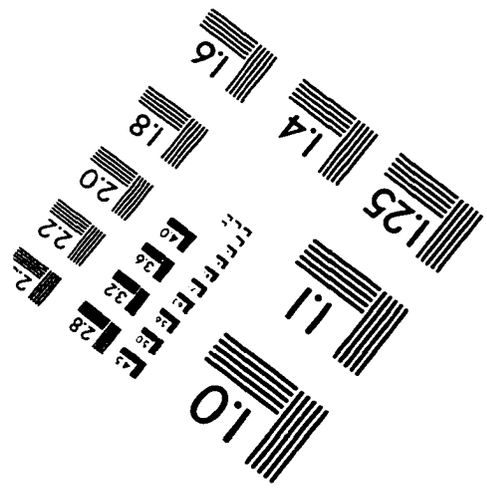

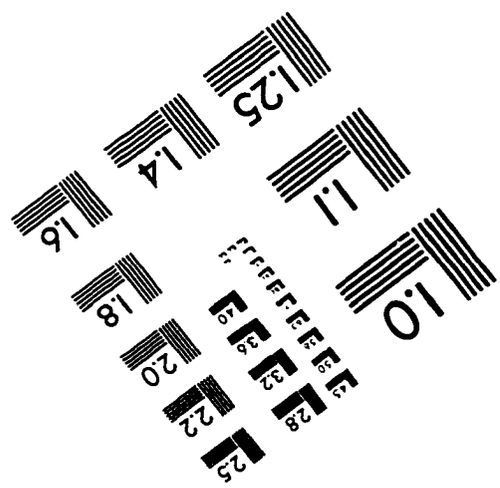

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