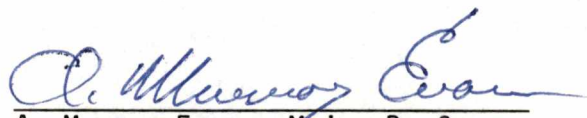
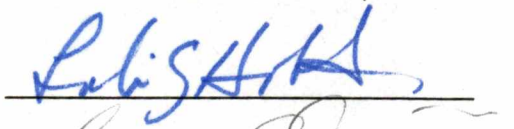
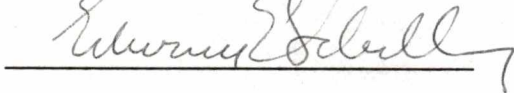


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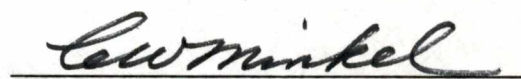
I am submitting herewith a thesis written by Andrea Leigh Sessions entitled "A Systematic Study of the Diplazium lonchophyllum Complex." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Botany.


A. Murray Evans, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:


The Graduate School

4

A SYSTEMATIC STUDY OF THE DIPLAZIUM LONCHOPHYLLUM COMPLEX

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Andrea Leigh Sessions

June 1984

DEDICATION

This work is dedicated,
with love and thanks,
to
Nancy and Bill Sessions

ACKNOWLEDGMENTS

The author wishes to acknowledge the contributions of those people who made this work possible. Appreciation is extended to Dr. A. Murray Evans for professional guidance and provocative suggestions throughout this endeavor.

Drs. Leslie Hickok and Edward Schilling provided helpful and stimulating comments during all phases of the study.

For suggesting this interesting research project and for providing me with live plants collected in Mexico, I thank Dr. John Mickel.

Drs. Bruce McAlpin, David Lellinger, and Harold Robinson have been a continuing source of inspiration.

And for their constant friendship, assistance, and encouragement throughout this study, I wish to thank all my fellow graduate students. But my deepest thanks and appreciation go to Marty Zenni and Jeremy Zucker. Without their support, tolerance, encouragement, and help, this would not have been possible.

I thank Marilyn Caponetti for typing the manuscript.

I thank the curators and directors of the following herbaria for loans of specimens of Diplazium:

California Academy of Science (CAS)

Field Museum of Natural History (F)

Michigan State University (MSC)

Missouri Botanical Garden (MO)

New York Botanical Garden (NY)

United States National Herbarium (US)

University of Tennessee (TENN)

And finally, I thank the Sigma Xi Research Society for
a grant in aid in support of this.

ABSTRACT

A systematic study was undertaken on Diplazium lonchophyllum and other members of the New World tropical D. lonchophyllum complex. Morphological data analyzed by numerical methods and data from gametophytes, spores, stomates, cytology, and distribution were used in assessing taxonomic relationships.

Cluster analysis and principal coordinates analysis were performed on 150 specimens for 25 morphological characters. Results indicated that the specimens could be divided into eight groups which corresponded to species as eventually recognized. Factor analysis revealed that at least four characters which had not been used previously to delineate taxa in this complex were important in separating groups in principal coordinates analysis. Numerical analysis confirmed that the disjunct populations in Louisiana are D. lonchophyllum and do not deserve special recognition. This analysis also resulted in the splitting of D. cristatum into D. cristatum and D. arboreum.

Gametophytes of 12 plants of 1 species and 4 hybrids were studied to observe general growth and morphology. A female to hermaphroditic developmental pattern was common to all samples except one which was initially male. Gametophytes of three collections had distinctive shapes. Gametophytes of one collection exhibited a positive response to antheridiogen, and one exhibited a reverse response to antheridiogen, producing more females and

fewer males and hermaphrodites. All samples displayed considerable intra- and inter-populational variability in size, morphology, and sexual development.

Limited results were obtained from studies of spore lengths, spore morphology using SEM, stomate patterns, frond flavonoid chemistry, and cytology. Diplazium werckleanum had distinctive spores and stomate patterns. No flavonoids were detected. Meiotic counts of $n > 70$ for D. lonchophyllum from Louisiana indicate it is probably a tetraploid.

This study has shown that members in the species pairs which previously were confusing can be easily distinguished using a combination of stable morphological characters. These pairs are D. lonchophyllum/cristatum and D. lonchophyllum/subsilvaticum. However, further studies are suggested in the complex especially involving D. lonchophyllum/franconis, D. cristatum/arboreum, and D. tablazianum/subsilvaticum.

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CHAPTER I

INTRODUCTION

Diplazium is a large pantropical genus of ferns with 300 to 400 species, 100 of these found in the New World. Previous work with Diplazium has been conducted mainly at the genus or family level (Sledge, 1962; Ching, 1964; Sledge, 1973). Some authors have combined Diplazium with the closely related genus Athyrium (Copeland, 1947; Holttum, 1954), and many species were originally described under Asplenium. However, the genus can be reliably separated from Athyrium and Asplenium by chromosome number, spore morphology, rhizome scale, indusium, sporangium and sorus characters (Sledge, 1962; Sledge, 1973; Smith, 1976; Stolze, 1981).

Studies at the species level exist only in regional floras or as isolated treatments addressing particular questions such as cytology or gametophyte morphology. None have seriously attempted to resolve species complexes but all point out the plasticity of characters used to define species, and the need for further work is suggested. In addition, the existence of polyploidy in Diplazium has been well-documented ($X=41$, $2X - 6X$ counts have been reported), and numerous polyploid and hybrid complexes are known or thought to exist in Malaysian and tropical American groups (Holttum, 1954; Manton and Sledge, 1954; Sledge, 1962; Mickel, Wagner, and Chen, 1966; Smith and Mickel, 1977; Smith, 1981; Stolze,

1981). These polyploid complexes have consistently been a problem for taxonomists because of the degree and range of variability exhibited by their members. This study involves such a polyploid complex and concentrates on D. lonchophyllum and other closely related species in the D. lonchophyllum complex.

The number of taxa comprising the D. lonchophyllum complex is debatable. A conservative treatment would include six species: D. werckleanum Christ, D. cristatum (Desr.) Alston, D. subsilvaticum Christ, D. lonchophyllum Kunze, D. drepanolobium Smith, and D. franconis Liebm. However, each of these species except D. werckleanum and D. drepanolobium has been split into at least two additional species (Smith, 1981; Stolze, 1981; Lellinger, pers. comm.) bringing to a total of 12 the possible number of species in this complex. In the present study, eight taxa were delimited; D. arboreum (Central America, South America, Cuba, Trinidad), D. cristatum (West Indies), D. drepanolobium (Mexico), D. franconis (Mexico, Central America), D. lonchophyllum (Louisiana, Mexico, Central America), D. subsilvaticum (Central America), D. tablazianum (Central America), and D. werckleanum (Mexico, Central America) (Figure I-1).

The taxa within the D. lonchophyllum complex have traditionally been separated by a combination of frond features, including frond length, pinna size and degree of dissection, segment shape, relative length of basal segments, attachment of the basal acroscopic pinnule or segment, and prominence of the costae and

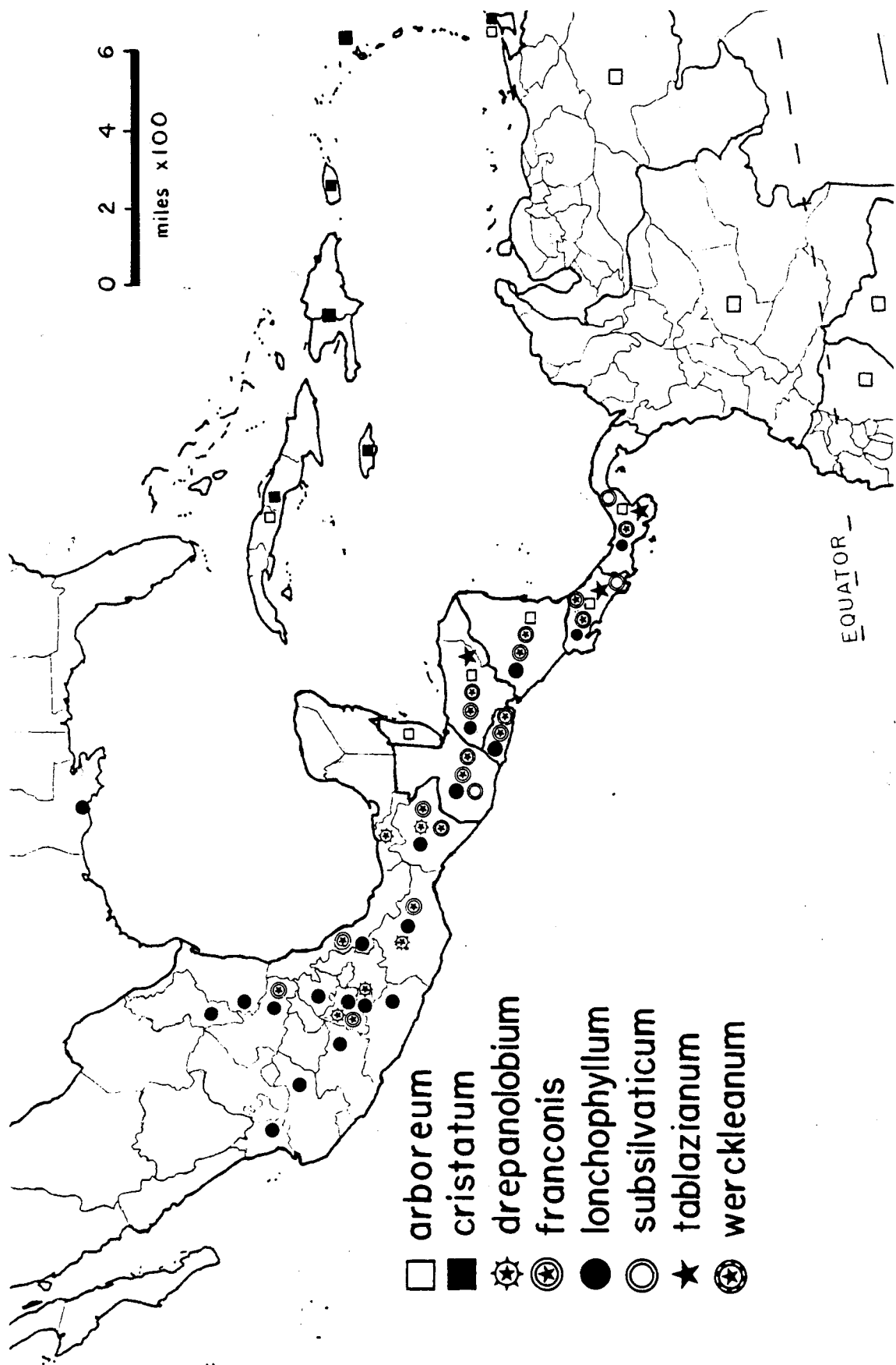


Figure I-1. Distribution of Species in the *Diplazium lonchophyllum* Complex.

costules. Variation within species is considerable, and the variation of one may overlap others. Not only is there a continuum in frond characters from simply pinnate D. werckleanum to nearly tri-pinnate D. franconis, but fronds with immature and therefore less dissected morphologies are often precociously fertile. Traditional frond characters by themselves do not seem to be a useful taxonomic character as Sledge (1962) noted for Ceylon species.

Diplazium lonchophyllum is especially problematic because in size, degree of dissection, and attachment of basal acroscopic pinnules, its frond morphology is intermediate among the taxa of this complex. A combination of characters which does not rely so heavily on degree of dissection is needed to adequately separate it from the other taxa.

The Louisiana population of D. lonchophyllum is of special interest because it represents a northern disjunction of nearly 600 miles. Previously found on Avery Island in Iberia Parish (Brown and Correll, 1942; Reese and Thieret, 1966), the plants are now known to exist only in ravines on Weeks Island a few miles away. It is not known to have grown wild or in cultivation nearby such that it may have migrated into these ravines. The population of several hundred plants in all stages of development appears quite uniform morphologically. While it is slightly different from "typical" Mexican material, it falls within the wide range of variability for D. lonchophyllum.

Inadequate keys and an unsettled taxonomy indicate the need for a critical examination of D. lonchophyllum and other members of this complex. In the present study, data from morphology of sporophytes, spores, and gametophytes, distribution, ecology, and cytology were analyzed using multivariate statistical techniques in order to understand and more critically circumscribe these taxa throughout their range. This study was intended to provide a foundation for further studies and to suggest useful areas to explore in greater depth in a complete biosystematic investigation of the entire complex. Except for several plants in the University of Tennessee greenhouse, all data were collected from herbarium specimens.

CHAPTER II

NUMERICAL ANALYSIS OF MORPHOLOGY

Taxonomy deals with the variation, classification, and relationships between organisms. Relationships between plant groups have traditionally been assessed based on morphological characters. Groups having more features in common with each other than with other groups are considered to constitute natural groups (Duncan and Baum, 1981). However, ultimate decisions concerning degree of similarity and taxonomic rank are left to the particular investigator. Intuition and subjectivity will likely play a part in decision-making thus challenging the scientific tenets of objectivity and repeatability.

The field of numerical taxonomy or numerical phenetics has arisen in an attempt to make taxonomy more objective, repeatable, and predictive. As with traditional taxonomy, numerical phenetics results in classifications based on patterns of phenetic similarity (Sneath and Sokal, 1973; Duncan and Baum, 1981). Debate persists over whether or not numerical taxonomy has reached its goals of objectivity and repeatability and whether one method of analysis is better than another (Duncan and Baum, 1981; Ivimey-Cook, 1969; Sneath and Sokal, 1973). However, computer assisted numerical methods do enable simultaneous evaluation of large amounts of data and require uniformity and consistency in methodology throughout data collection, analysis, and interpretation.

The D. lonchophyllum complex consists of eight taxa which when seen in their "typical" forms, are not difficult to distinguish or to describe using only a few characters. However, individual specimens frequently display a combination of features which does not fit a typical form. This creates a continuum in variation from one taxon to the next. In the present study, numerical taxonomic methods were employed in order to define and characterize morphological groups in the complex.

Materials and Methods

In a numerical taxonomic investigation, character states of the specimens or OTUs (Operational Taxonomic Units) under study are observed and recorded in a data matrix. From this matrix, a resemblance or similarity matrix is computed by pair-wise comparisons of all OTUs. Information about the relationships among OTUs is stored in the similarity matrix and can be analyzed for taxonomic structure using statistical procedures.

In this study, numerical analysis was performed on 150 OTUs. An OTU was an individual herbarium specimen. In order to sample from the entire morphological and geographical range of the complex, approximately 750 specimens were first grouped into piles of similar morphological types disregarding previous identifications on the sheets. These piles were then subdivided geographically, and the most complete specimens were selected for analysis.

A list was made of characters used previously in the classification of the D. lonchophyllum complex. To this list were added

other attributes which were seen to vary between the taxa. After a trial run, the list was amended, and the final characters used are listed in Table II-1. Figure II-1 illustrates several characters and the ways in which they were measured. Ideally, characters from all phases of a plant's life cycle should be examined (Sneath and Sokal, 1973). In the present study, this was not possible because of the reliance on herbarium specimens. Therefore, only characters of the sporophytic phase were measured.

Two separate analyses were performed. The first analysis examined all the taxa (Appendix A). The second analyzed OTUs of D. cristatum, D. arboreum, D. lonchophyllum, D. tablazianum, and D. subsilvaticum (Appendix A).

Morphological data were analyzed by standard methods (Sneath and Sokal, 1973). Gower's coefficient of similarity was used because it allows use of both quantitative and qualitative data (Gower, 1971). The resulting similarity matrix was analyzed for taxonomic structure using clustering and ordination techniques, a combination thought to be most effective in deriving classifications (Boyce, 1969; Sneath and Sokal, 1973; Clifford and Stevenson, 1975; Duncan and Baum, 1981). Cluster analysis is better at revealing relationships among similar OTUs, whereas ordination more faithfully reflects relationships between larger groups (Cole, 1969; Sneath and Sokal, 1973). In the present study, UPGMA, Unweighted Pair-Group Using Arithmetic Means, was used in clustering, and PCA, Principal Coordinate Analysis, was used for

Table II-1. List of Characters Scored in Morphological Analysis.

Characters
Frond - length - stipe length:lamina length Pinnae (measured on basal pinnae unless stated otherwise) - number of pinnae pairs - width - blade length:width - petiolule length - petiolule length:lamina length - angle of first acroscopic vein divergence from pinna midrib - angle of first basisopic vein divergence from pinna midrib - angle of median pinna base - first basisopic vein closer to first (0), or second (1) acroscopic vein - first acroscopic segment shorter (0), or longer/equal to others (1) - attachment of first acroscopic segment to pinna: segment broadest at point of attachment (0), segment broadest above point of attachment but not stalked (1), segment stalked (2) - pinna dissection: less than 1/2 the segments pinnatifid to midrib (0), one half pinnatifid to less than 1/3 pinnate (1), at least 1/3 pinnate - length of free portion of third acroscopic segment - length:width of free portion of third acroscopic segment - ultimate segments falcate (1), or not (0) - number of acroscopic costules - sorus length (longest sorus on median acroscopic segment of middle pinna) - costae prominent (1), or not (0) Frond Apex - length - length:width - angle of divergence of basal segment from frond rachis - apex length:frond lamina length Stipe Base - scales present (1), or not (0)

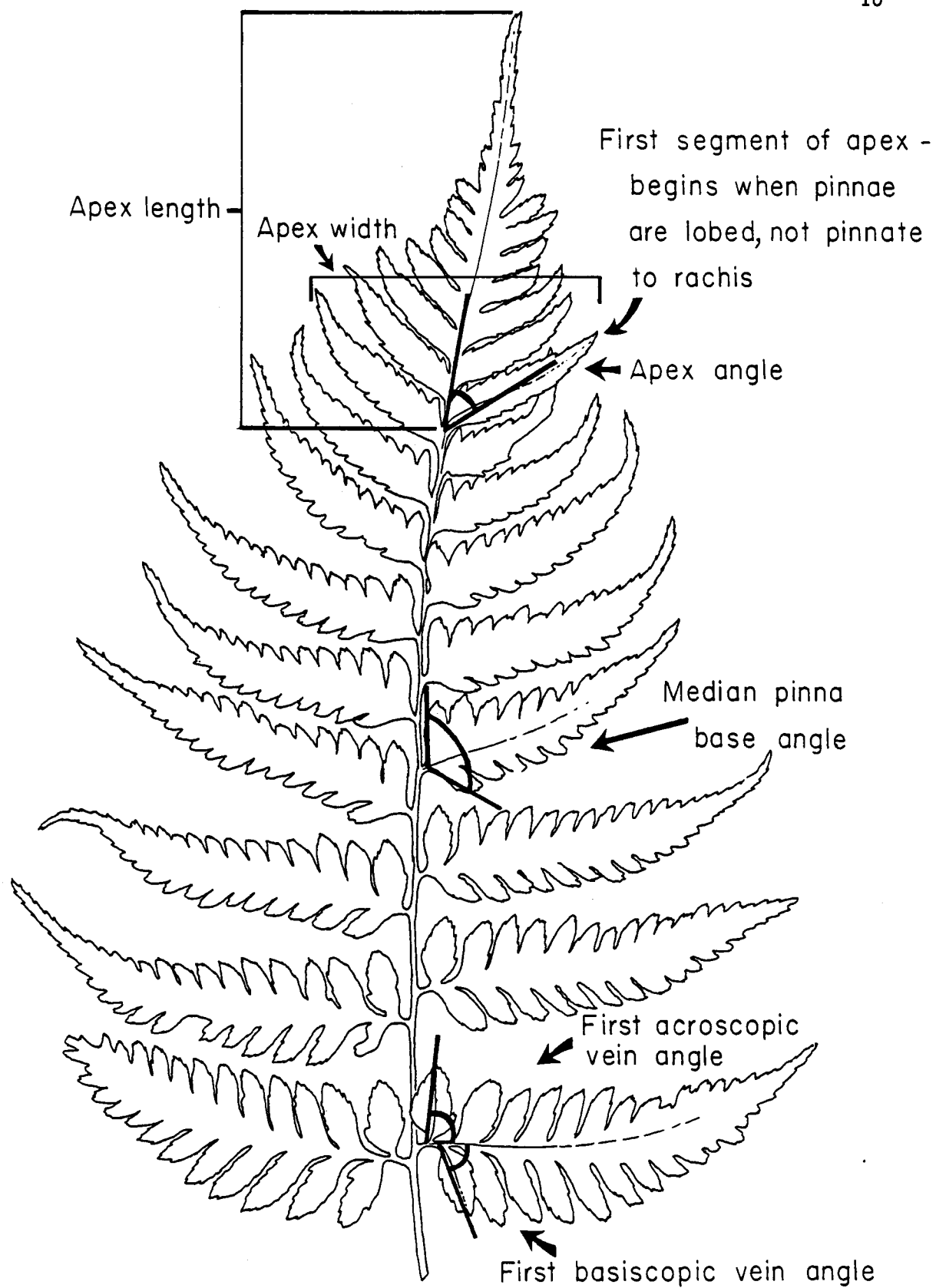


Figure II-1. Illustration of Several Frond Characters.

ordination. Factor analysis was performed in order to detect characters primarily responsible for the separation of OTUs along the principal axes. Analyses were done on the DEC-10 computer at the UT Computer Center using SAS and SPSS (Statistical Package for the Social Sciences) programs and using computer programs for numerical taxonomy written by Dr. E. Schilling (pers. comm.).

Results and Discussion

The phenogram resulting from cluster analysis of 150 OTUs representing all taxa in the D. lonchophyllum complex is shown in Figure II-2. Except for the cluster of D. lonchophyllum OTUs 21 through 121, there is a good correlation between clusters and taxa. This cluster is discussed more fully under the analysis of D. lonchophyllum at the end of this chapter.

The graph of principal coordinates analysis of the 150 OTUs is shown in Figure II-3. Clusters of taxa overlap somewhat when OTUs are plotted on the first two principal axes. However, plotting OTUs on the first and third axes resulted in better separation of D. werckleanum and D. cristatum, and of D. drepanolobium and D. lonchophyllum (Figure II-4) although it resulted in less separation between D. lonchophyllum and D. subsilvaticum.

Factor analysis performed on the 150 OTUs revealed that the first three principal axes account for 55% of the total variation among OTUs (31%, 12%, and 12%, respectively). Characters important in the separation of groups along the first axis include: amount

Figure II-2. Phenogram Derived from UPGMA Cluster Analysis of 150 OTUs for 25 Morphological Characters. Gower's coefficient of similarity is scale at top. Numbers indicate OTUs. OTUs clustering outside of their taxon clusters are denoted by the first letter of their specific epithet. A letter and a question mark indicate a probable hybrid involving the species abbreviated by the letter.

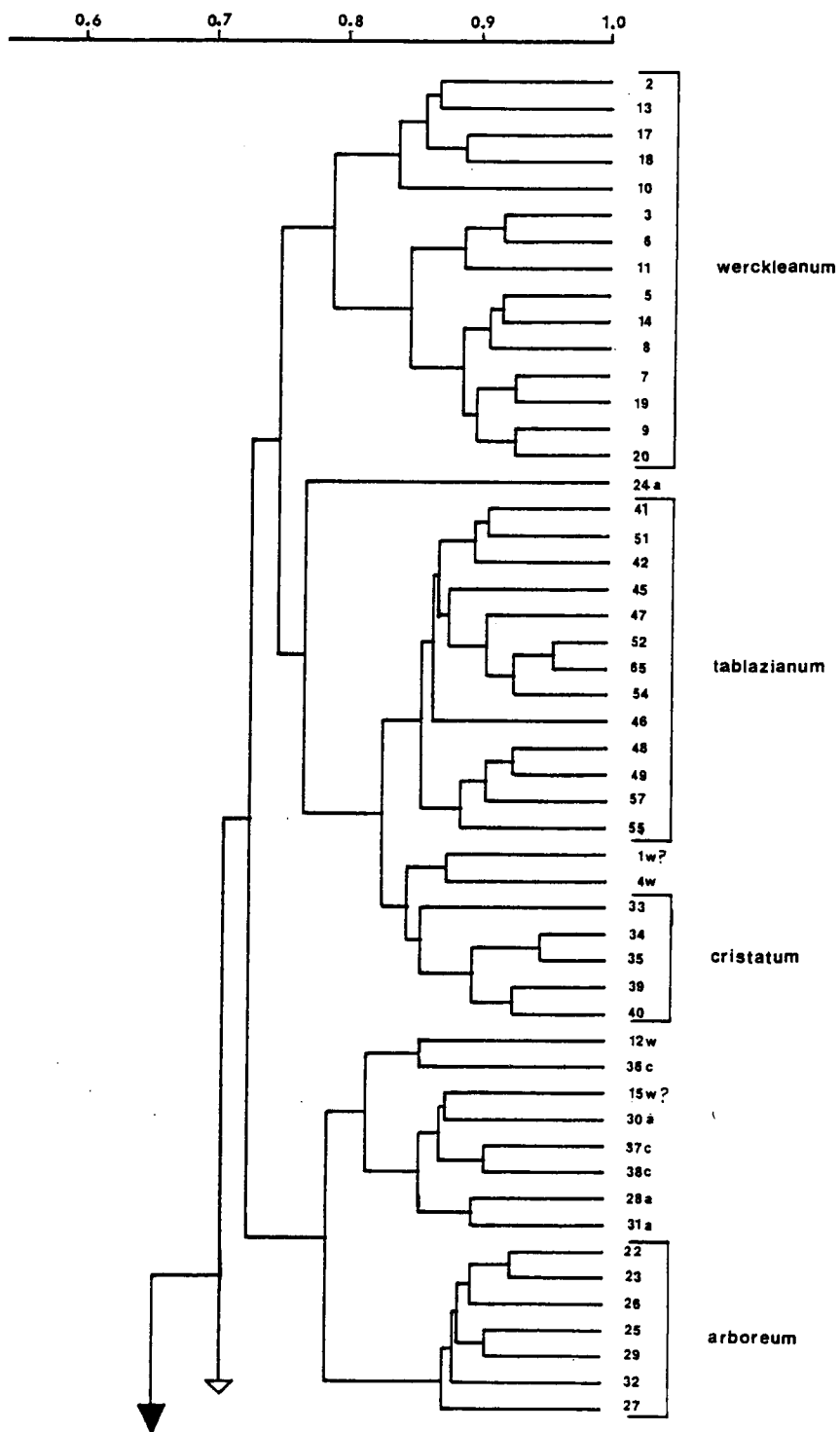


Figure II-2

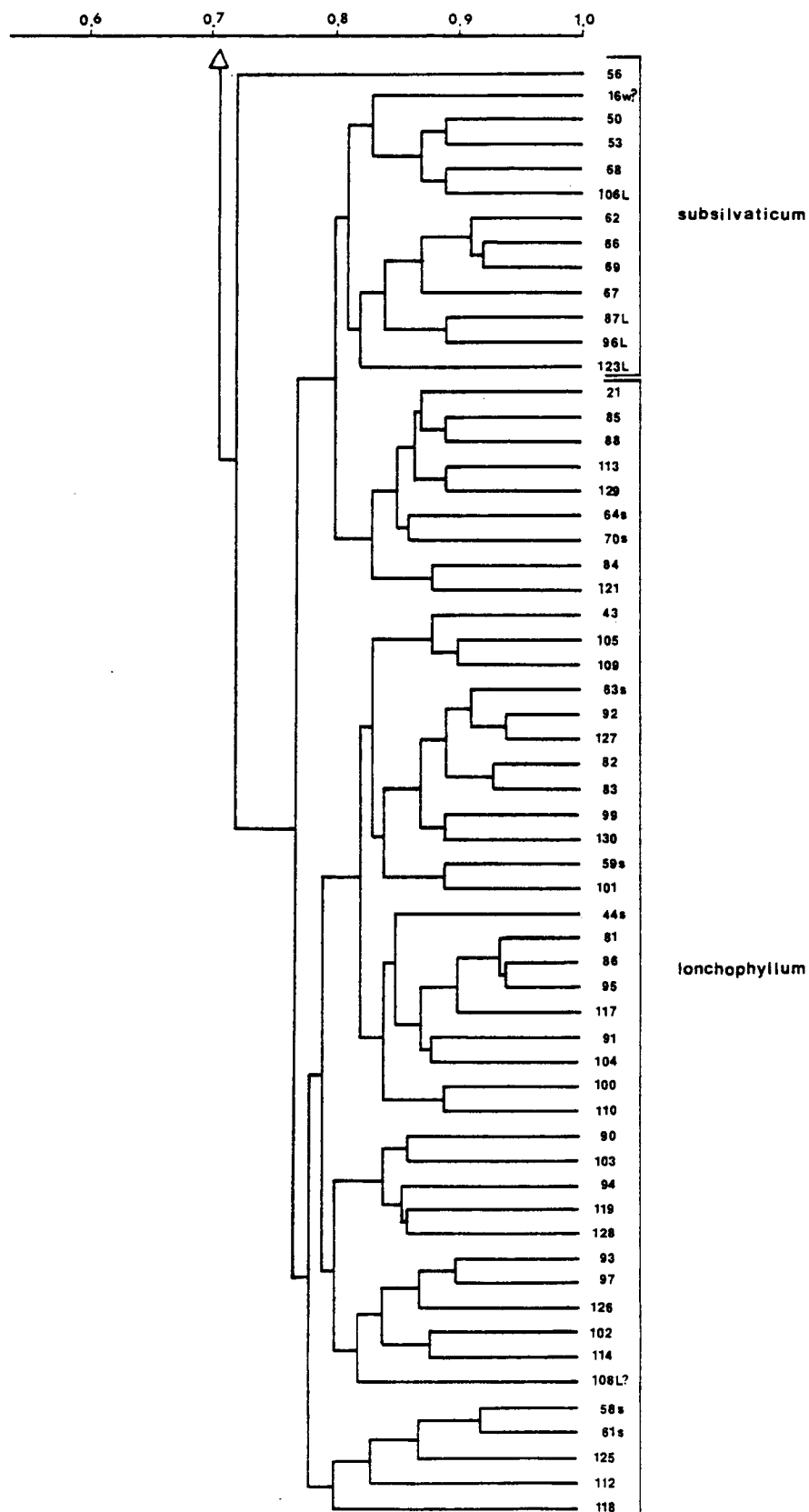


Figure II-2 (Continued)

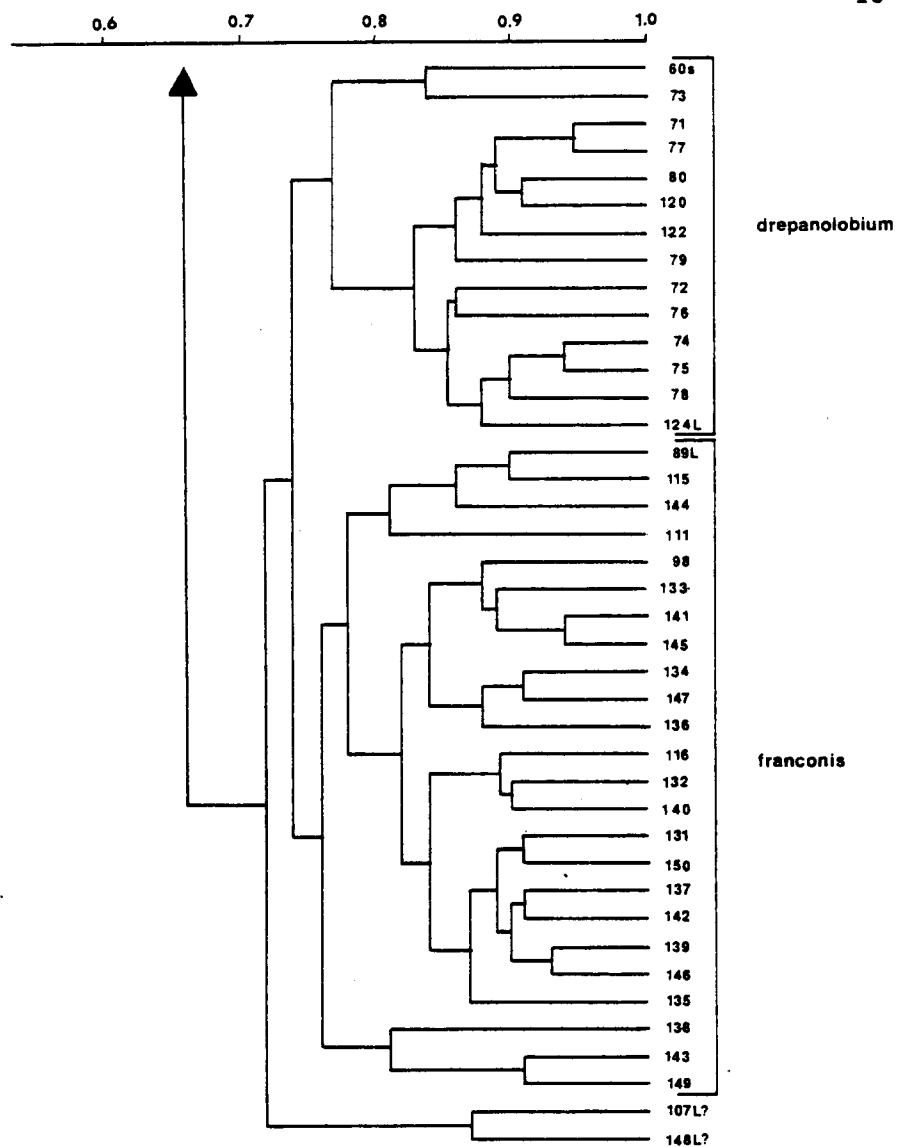


Figure II-2 (Continued)

Figure II-3. Graph of 150 OTUs on the First Two Axes Determined by Principal Coordinate Analysis. Species are indicated by the following symbols:

- D. arboreum
- D. cristatum
- ⊛ D. drepanolobium
- ⊙ D. franconis
- D. lonchophyllum
- D. subsilvaticum
- ★ D. tablazianum
- ⊛ D. werckleanum

? probable hybrid involving the species
next to it

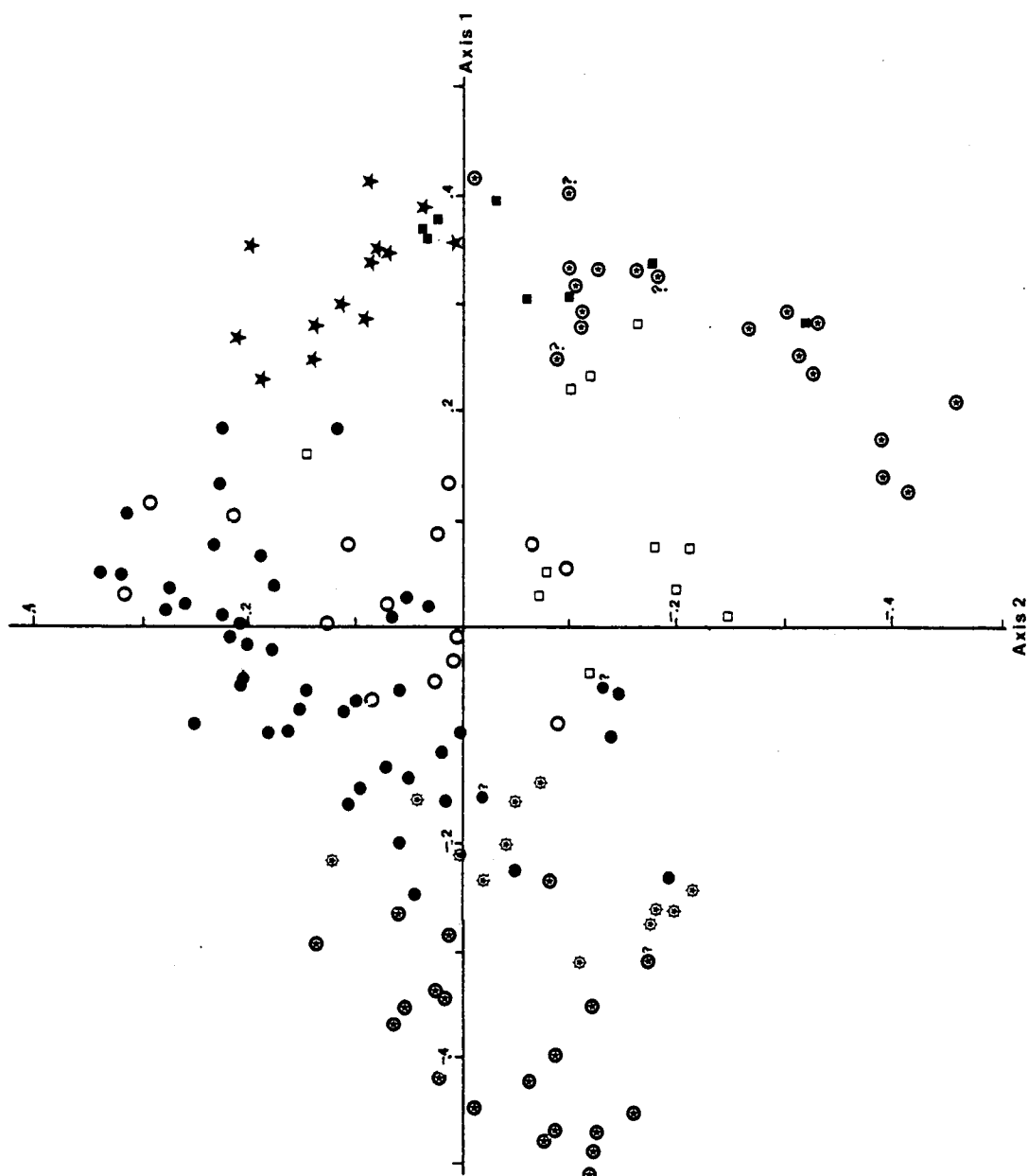


Figure II-3

Figure II-4. Graph of 150 OTUs on the First and Third Axes Determined by Principal Coordinate Analysis. Species are indicated by the following symbols:

□ D. arboreum

■ D. cristatum

✱ D. drepanolobium

⊙ D. franconis

● D. lonchophyllum

○ D. subsilvaticum

★ D. tablazianum

⊙ D. werckleanum

? probable hybrid involving the species
next to it

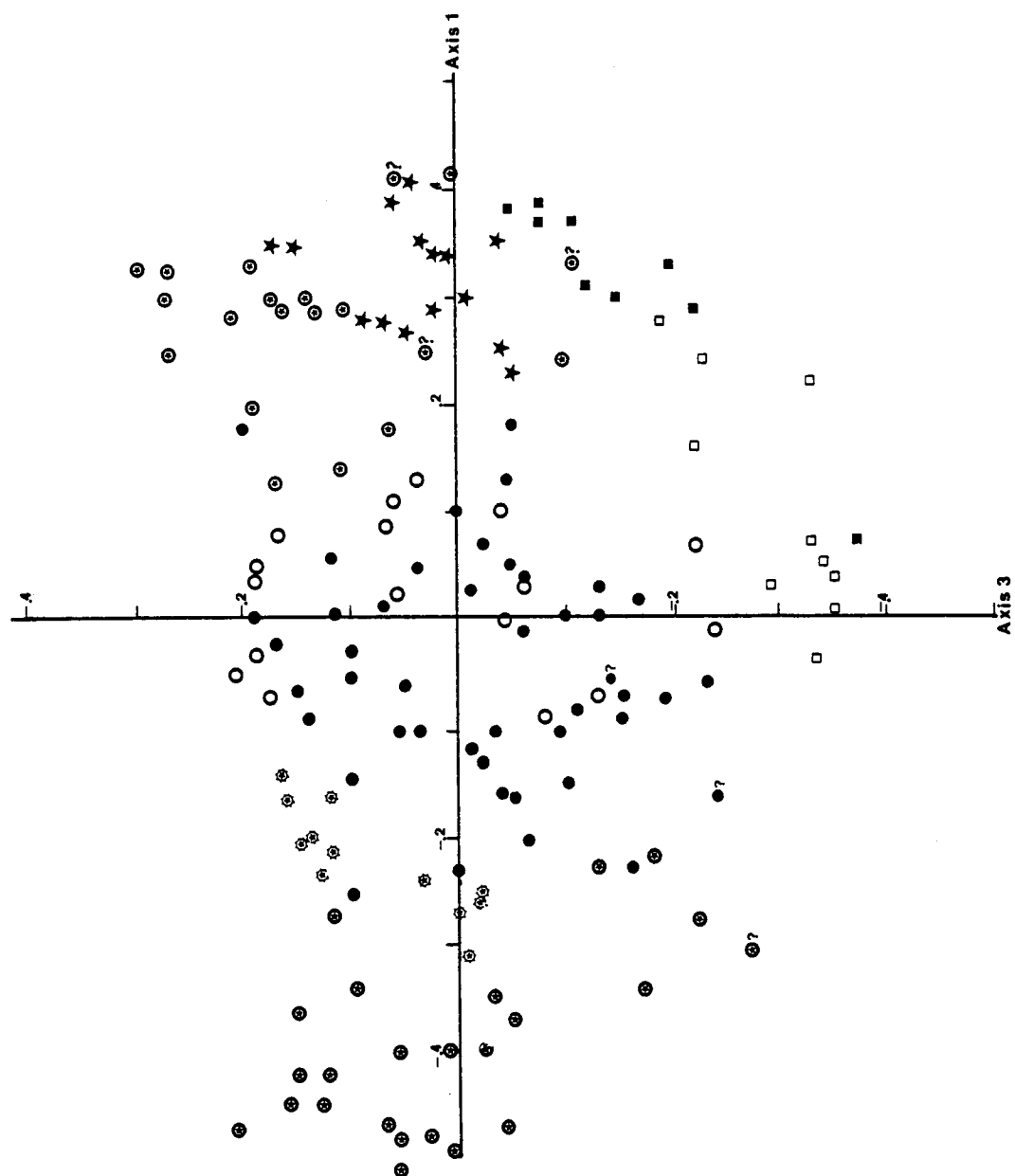


Figure II-4

of dissection, length of pinna lobes or pinnules, length:width of pinna lobes, pinna width, apex length:frond lamina length, and the type of attachment of the first acroscopic segment to the pinna. Characters responsible for group separation along the second axis include: petiolule length, angle of divergence of the first acroscopic vein, frond length, and length:width of the pinna blade. Along the third axis, frond apex length:width, apex angle, number of costules per pinna, and petiolule proportions were important.

In the PCA graph for the first two axes (Figure II-3), there is a continuum of taxa clusters along axis one from D. franconis (twice pinnate-pinnatifid, longest pinnules, and widest pinnae) to clusters of D. werckleanum and D. tablazianum (least dissected, shortest free lobes, narrowest pinnae). The second axis mainly separates D. lonchophyllum and D. werckleanum based on first acroscopic vein angles (widest in D. lonchophyllum and narrowest in D. werckleanum) and petiolule length.

Since OTUs of D. franconis, D. drepanolobium and D. werckleanum generally formed distinct clusters in PCA and cluster analysis, they were removed from the data set, and a second analysis was run on the remaining 100 OTUs to see how the three species had effected clustering of the others. The resulting phenogram shows a good correlation between clusters and taxa (Figure II-5). However, there are several OTUs which cluster outside of their taxon cluster. OTUs 1-77 and 1-78 are large aberrant forms with aborted spores and are suspected hybrids involving D. lonchophyllum,

Figure II-5. Phenogram Derived from UPGMA Cluster Analysis of 100 OTUs for 25 Morphological Characters. Gower's coefficient of similarity is scale at top. Numbers indicate OTUs. OTUs clustering outside of their taxon clusters are denoted by the first letter of their specific epithet. A letter and a question mark indicate a probable hybrid involving the species abbreviated by the letter.

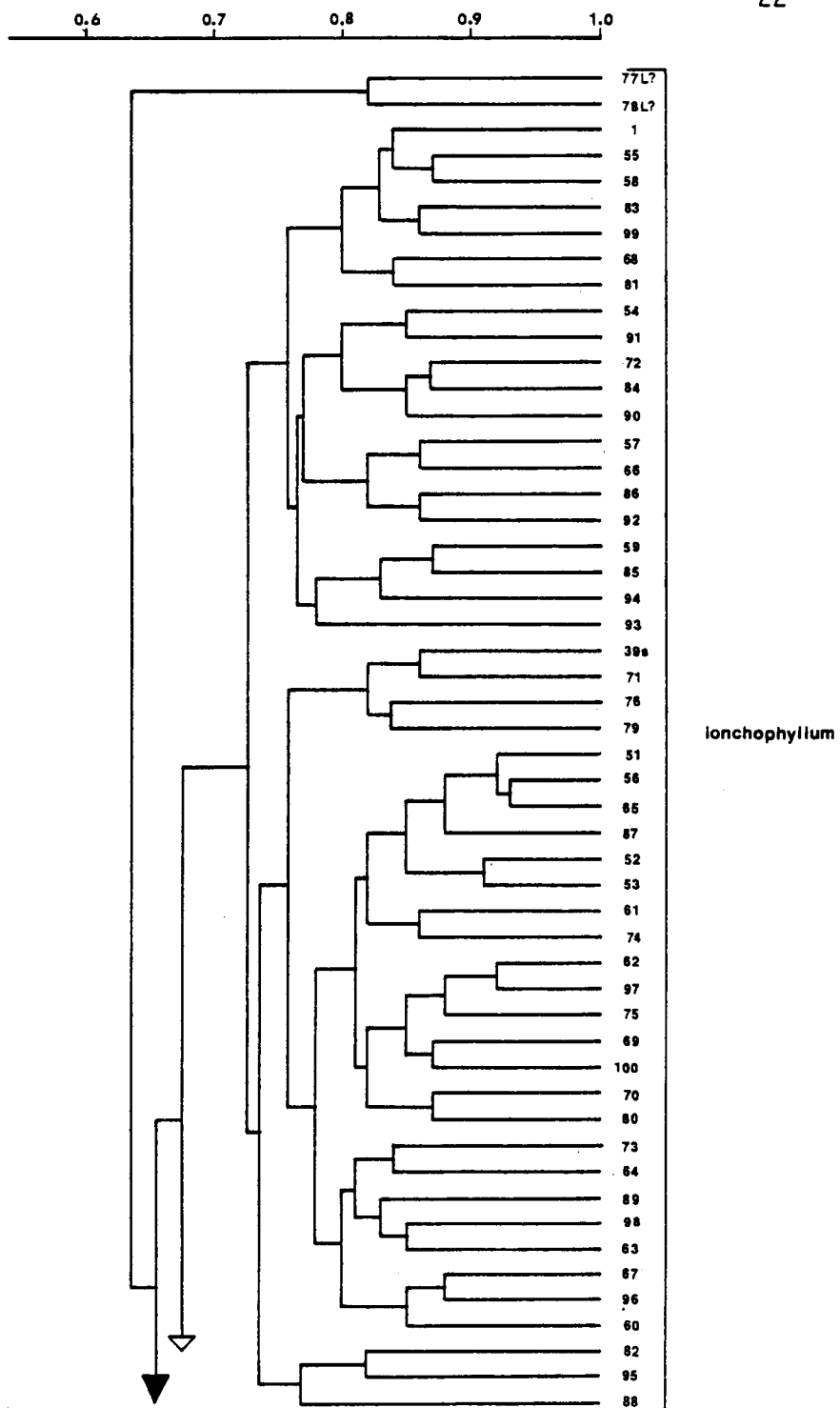


Figure II-5

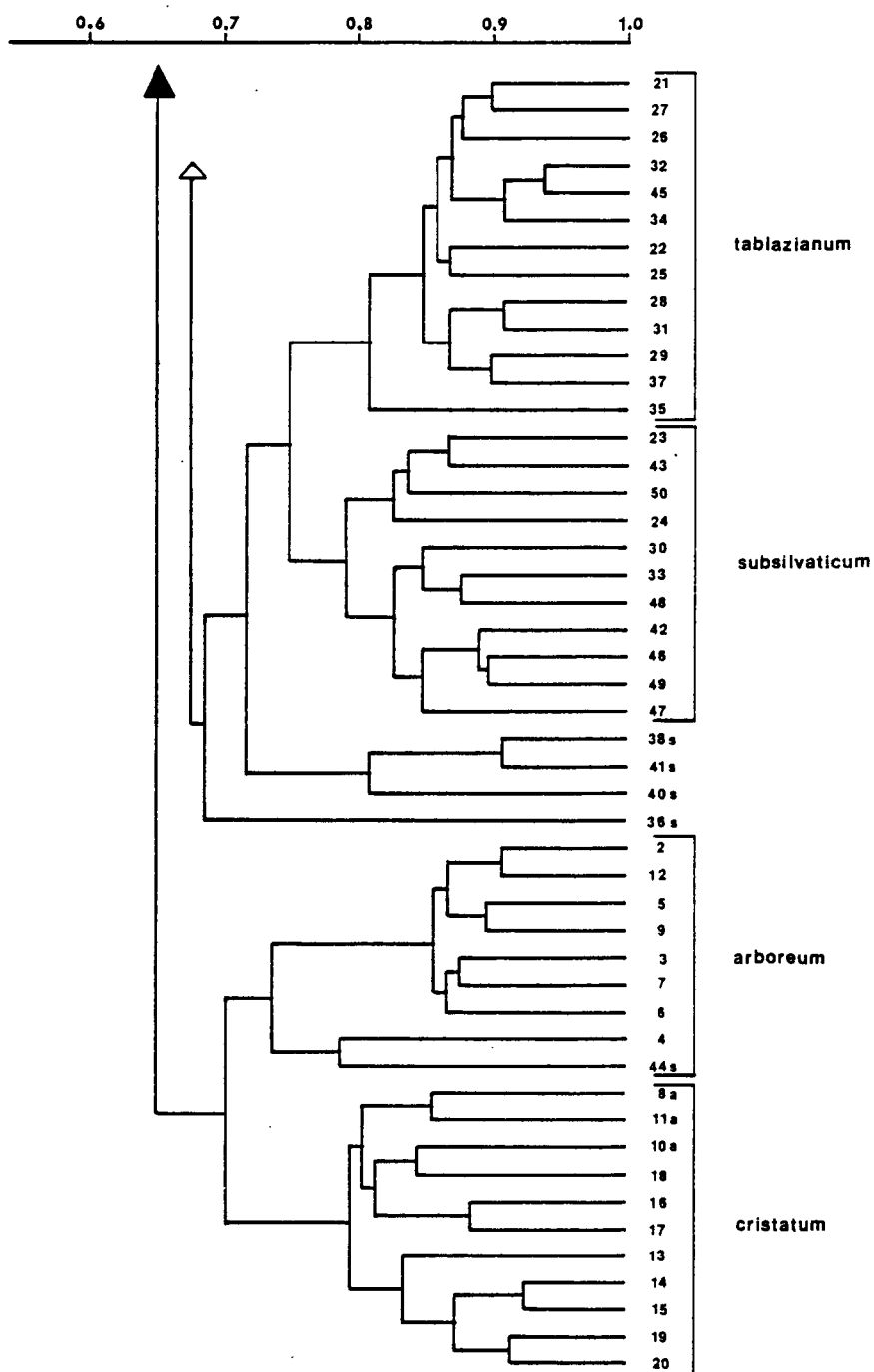


Figure II-5 (Continued)

D. franconis, or D. arboreum. The four OTUs of D. subsilvaticum which cluster outside of the subsilvaticum/tablazianum group are especially large and dissected. OTUs of D. cristatum and D. arboreum which cluster as a group have morphologies intermediate to the two taxa. The aberrant cristatums may be especially vigorous forms, and the aberrant arboreums may be juveniles.

The graph of the first two principal axes from PCA of the 100 OTUs shows some overlap between OTUs of D. subsilvaticum and D. lonchophyllum (Figure II-6), but when OTUs are plotted on the first and third axes, they are clearly delimited (Figure II-7). Factor analysis of the 100 OTU set showed that generally the same characters as in the 150 OTU set were responsible for group separation. The first axis (23% of the variation among OTUs) separates groups primarily on dissection, size, and shape of pinnae, and frond length. The second axis (17%) uses all four frond apex characters and number of pinnae pairs. Characters of petiolules are important on the third axis (11%).

A number of OTUs did not cluster with other members of their taxon in cluster analysis or in PCA, especially in the larger 150 OTU set covering the entire complex. An examination of the particular character states showed that when compared to mean and modal values for the taxa to which they were presumed to belong, many of the values for the aberrant OTUs were atypical. Atypical character values often were for important characters, but ultimately, the particular combination of all the character values determined

Figure II-6. Graph of 100 OTUs on the First Two Axes Determined by Principal Coordinate Analysis. Species are indicated by the following symbols:

□ D. arboreum

■ D. cristatum

⊠ D. drepanolobium

⊙ D. franconis

● D. lonchophyllum

○ D. subsilvaticum

★ D. tablazianum

⊛ D. werckleanum

? probable hybrid involving the species
next to it

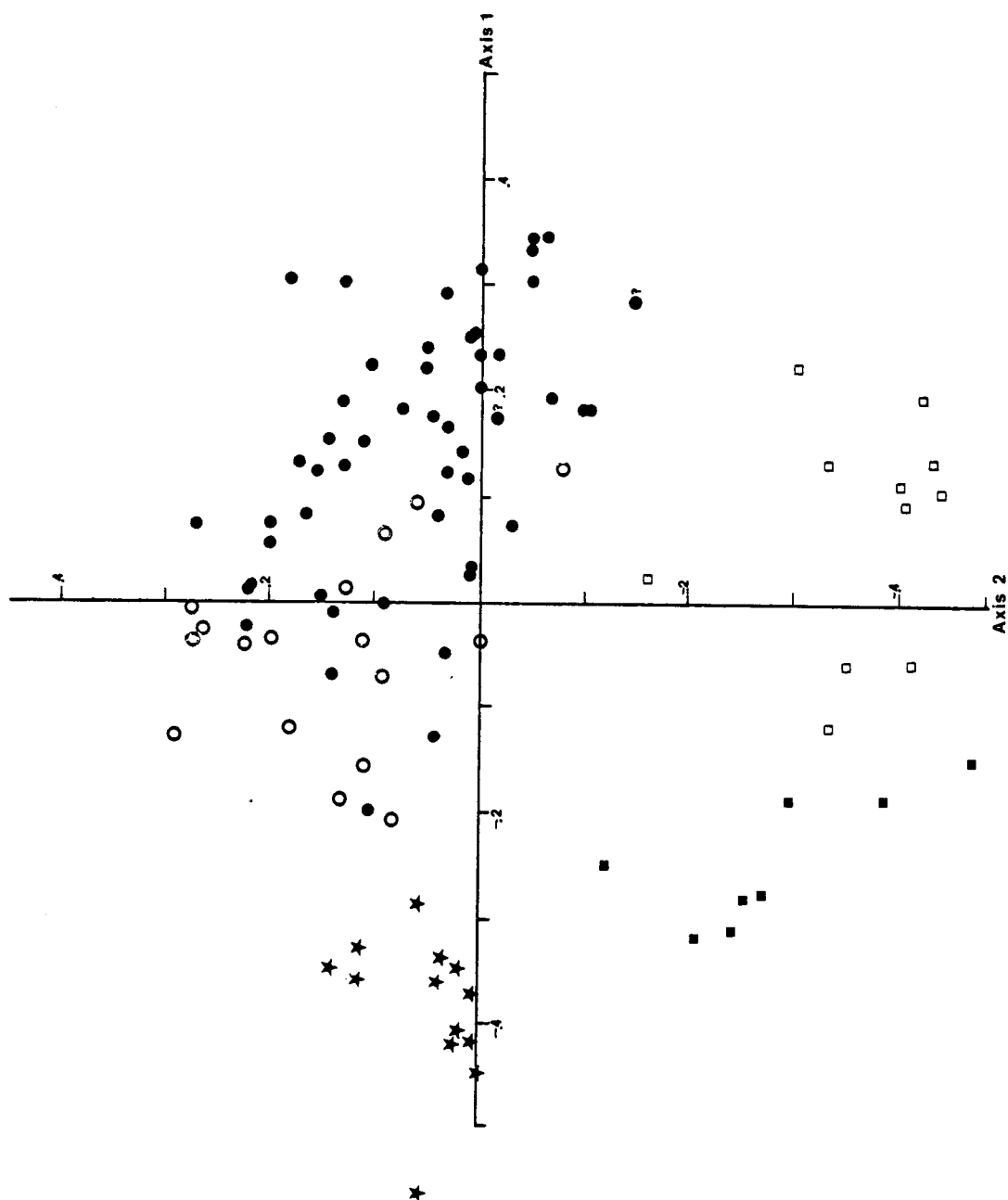


Figure II-6

Figure II-7. Graph of 100 OTUs on the First and Third Axes Determined by Principal Coordinate Analysis. Species are indicated by the following symbols:

□ D. arboreum

■ D. cristatum

⊛ D. drepanolobium

⊙ D. franconis

● D. lonchophyllum

○ D. subsilvaticum

★ D. tablazianum

⊙★ D. werckleanum

? probable hybrid involving the species
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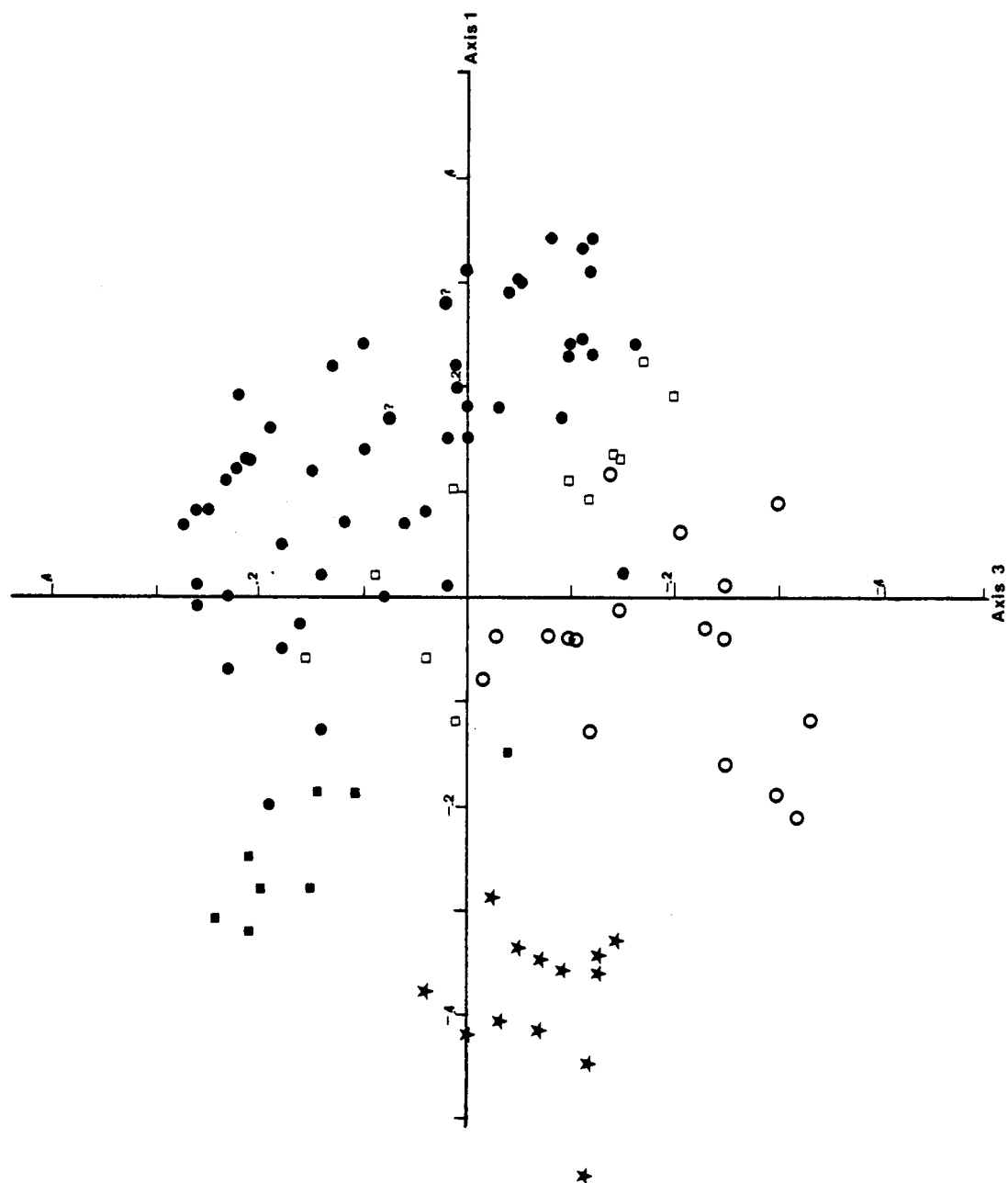


Figure II-7

placement of individual OTUs in the phenogram and in the ordinations. However, after examining character state values and considering results from cluster analysis and PCA, aberrant or atypical OTUs were eventually assigned to taxa based on geographical distribution and intuitive judgment.

The placement of an OTU outside of its expected taxon cluster may reveal hybridity. Schilling and Heiser (1976), Barber (1982) and Allred and Gould (1983) found that hybrids often cluster with one parent in phenograms but in ordinations, fall somewhere between parental types. In the present study of 150 OTUs, of the 6 OTUs with aborted spores, 5 clustered with a suspected parent and in ordination fell between possible parents based on intermediate morphology and geographical distribution (OTUs w-1, w-15, l-107, l-108, l-148). The remaining OTU with aborted spores, w-16 clustered with D. subsilvaticum in cluster analysis, and with D. werckleanum in PCA (see further discussion under D. werckleanum.)

Based on examination of the phenograms, PCA graphs, and significant characters according to factor analysis, it is apparent that there are at least eight distinct morphological groups in the D. lonchophyllum complex. Following are discussions of each of the taxa in relation to cluster analysis and PCA.

1. D. werckleanum

In both cluster analysis and PCA, OTUs of D. werckleanum cluster as a fairly discrete group. It is the only member of the D. lonchophyllum complex to be simply pinnate. In fact, for nearly

half of the characters scored in this analysis, the means of the character state values for D. werckleanum were character state extremes in the complex. For instance, D. werckleanum has: the longest stipe to blade length ratio, the greatest pinna length to width ratio, narrowest pinnae, narrowest pinna base angles, shortest pinna lobes which are widest in relation to their length if and when they occur, and the longest sori. Additional characters contributing to D. werckleanum clusters are few pinna pairs, long and proportionately long petiolules, and frond apex proportionately broad and occupying much of the frond lamina. Figure II-2 (page 12) shows five D. werckleanum specimens to cluster outside of the main group. Three of these, w-1, w-15, and w-16, have aborted spores and slightly lobed pinnae. The other two are atypical for the taxon in several characters. OTU placement in cluster analysis and in PCA suggest w-15 may be a hybrid involving D. arboreum. Numerical methods do not clarify the identification of w-1 and w-16 which aside from their placement in the phenogram and their aborted spores, appear to be fairly typical D. werckleanum. There may be some physiological explanation other than hybridity which resulted in spore abortion.

2. D. arboreum

Traditionally, distinguishing between D. arboreum and D. lonchophyllum has been problematic in this complex. Authorities have relied on degree of pinna dissection and length of pinna lobes as the primary means of differentiating these taxa. In this study,

however, cluster analysis and PCA show that the two taxa are quite distinct. The frond apex of D. arboreum is longer and is proportionately the broadest in the complex versus a shorter narrower apex in D. lonchophyllum. The apex occupies 1/4 to almost 1/2 the lamina length in D. arboreum versus less than 1/3 in D. lonchophyllum. The frond apex angle and median pinnae angles are also widest in D. arboreum. It is primarily these frond apex characters that effectively separate D. arboreum (and D. cristatum) from other groups in PCA (Figures II-6, 7). In addition, D. arboreum has few pinna pairs (only D. cristatum has fewer). While the range in pinna lobe lengths overlaps considerably in D. arboreum and D. lonchophyllum, in the latter they are proportionately much longer. Juvenile specimens of D. lonchophyllum should still be clearly distinguishable from D. arboreum regardless of dissection because of the frond apex characters.

Although modern authors synonymize D. arboreum under D. cristatum (Smith, 1981; Stolze, 1981), this study has shown that D. arboreum and D. cristatum are distinct taxa. In cluster analysis and PCA, they form two distinct and one intermediate cluster. The distinct clusters are composed of OTUs either from the West Indies (D. cristatum) or from Central America (D. arboreum) while the intermediate group contains OTUs from both areas. In Figure II-2 (page 12), D. cristatum clusters closely with D. tablazianum and D. werckleanum. D. arboreum and the intermediates cluster outside of this group.

Diplazium cristatum has shorter fronds, fewer and narrower pinnae, and shorter petiolules than does D. arboreum. Pinnae are lobed less than half way to the costa, and the lobes are as wide as long. Diplazium arboreum is pinnatifid and has longer pinna lobes. Characters of the apex separate the two forms from other taxa in the complex, but the apex length:lamina length is proportionately much larger in D. cristatum than in D. arboreum. In addition, the median pinna base angle is nearly twice as wide in D. arboreum. Character states are consistent within these two taxa, and for significant characters there is little or no overlapping in the ranges. Thus it would seem that there are consistent differences between these taxa, and these are correlated with distribution.

OTUs falling between clusters of D. arboreum and D. cristatum possess some attributes typical of or approaching those of the other geographical type. The intermediate Central American D. arboreum OTUs may be less dissected and smaller because of environmental or developmental conditions. The D. cristatum intermediates from the West Indies may be especially vigorous. All specimens appear to be fully fertile. It is difficult to say whether the clusters resulting from cluster analysis and PCA are artifacts of the small sample size or whether clusters of the two taxa would be as distinct if the sample size were increased. Until further tests are conducted to determine the basis for the observed differences, the West Indies form is here considered to be D. cristatum, a species distinct from, but closely related to, D. arboreum which extends into South America.

3. D. cristatum

Typical forms of D. cristatum cluster closely in both phenograms and in all ordinations. Diplazium cristatum is characterized by having short fronds with few pairs of lobed pinnae. Pinna bases are broad, the frond apex is long, and it occupies about 1/2 of the lamina. As discussed under D. arboreum, the two species differ in a number of characters, although intermediate forms are apparent. In Figure II-2 (page 12) the placement of the D. cristatum cluster close to D. tablazianum is primarily a result of size and degree of dissection. However, in Figure II-5 (page 21) where the total range of variation over all the 100 OTUs sampled is not as great as in the 150 OTU set, D. cristatum shows closer affinities to D. arboreum, and this appears to reflect similarities of their apices, pinna base angles, few pinna pairs, and a common presence of stipe base scales.

4. D. tablazianum

In all phenograms and PCAs, D. tablazianum clusters are a distinct entity. The most obvious features distinguishing it from other taxa in the complex are its lack of stipe base scales, short fronds, narrow shallowly lobed pinnae, and its short apex which may be twice as long as wide. Other researchers have sometimes considered anything with lobed to pinnatifid pinnae to be D. arboreum (D. cristatum when D. arboreum is synonymized under it). However, D. tablazianum lacks stipe base scales, has many more pinna pairs, and its apex is much narrower, smaller, and less

spreading than that of D. arboreum. In addition, D. tablazianum is restricted to higher elevations in Costa Rica and Panama, while D. arboreum is widespread at lower elevations throughout Central America.

5. D. subsilvaticum

Diplazium subsilvaticum is quite similar to D. lonchophyllum in overall size, degree of pinna dissection, pinna base angles, and features of the apex. This is why in cluster analysis and PCA of 150 OTUs, the two taxa show considerable overlapping. However, in the phenogram (Figure II-5, page 21) and PCA of the first and third axes (Figure II-7, page 27) in the 100 OTU set, the two taxa are well separated. The features separating them are the glabrous stipe bases and very long petiolules in proportion to the pinna length of D. subsilvaticum. In addition, D. subsilvaticum is restricted to Costa Rica and Panama while D. lonchophyllum rarely occurs there.

In all analyses, D. subsilvaticum and D. tablazianum are distinct although they share in being the only taxa in the complex to have glabrous stipe bases. However, the characters by which they differ with little or no overlapping are all quantitative characters: frond length, pinna width, degree of pinna dissection, length of pinnules, and number of costules. It is possible that these characters are environmentally or developmentally variable. The characters for which their values overlap mostly are ratios and angles (i.e., shape) and may therefore be more useful

taxonomically: stipe length:lamina length, length:width pinna blade, petiolule length:pinna blade length, all pinna base and apex angles, number of pinna pairs, sorus and apex length, apex length:width, and apex length:lamina length. However, since cluster analysis and PCA separate the two groups despite overlapping and a continuum in character values, at the present time, I consider the two to be distinct entities divisible on the basis of size and dissection. These taxa certainly deserve careful study in controlled environments and in the field (both are restricted to Costa Rica and Panama) to help determine whether maturation, vigor, and/or ecology may be effecting phenotypes, a possibility I suspect. On several sheets of D. subsilvaticum which contained several fronds from a single rhizome, I did observe immature fronds resembling D. tablazianum. Also, sorus placement and number per segment (characters used to describe the two taxa previously) were seen to vary according to maturity.

6. D. lonchophyllum

As shown in cluster analysis, PCA, and factor analysis, D. lonchophyllum occupies a central or intermediate position in the D. lonchophyllum complex between the large 2- or 3-pinnate D. franconis and the smaller simply pinnate or pinnate and lobed D. werckleanum and D. tablazianum. None of its mean character state values are extremes for the complex.

The large cluster of D. lonchophyllum OTUs in both phenograms (Figures II-2, page 12, and II-5, page 21) can be subdivided

into several smaller groups. A factor analysis performed separately on D. lonchophyllum showed size, dissection, and apex characters to be important in cluster formation. While this species is variable phenetically, its variation is continuous internally, and D. lonchophyllum OTUs are, for the most part, more similar to themselves than to the other taxa. However, in Figure II-2 (page 12) D. lonchophyllum OTUs 21 through 121 cluster more closely with D. subsilvaticum than with D. lonchophyllum. Examination of the individual OTUs showed this was because in overall size and degree of dissection, they were more similar to D. subsilvaticum. The presence of stipe base scales and proportionately shorter petiolules and the fact that they were collected outside of Costa Rica and Panama where D. lonchophyllum very rarely occurs, clearly indicates, however, that these specimens are D. lonchophyllum and not D. subsilvaticum.

Diplazium lonchophyllum OTUs from Louisiana clustered throughout the large D. lonchophyllum cluster. This study has shown them to fall well within the range of variability for D. lonchophyllum.

In PCA (Figure II-3, page 16), there is some overlapping of D. lonchophyllum clusters with D. subsilvaticum which has been discussed above, and there is also some overlap with D. drepanolobium. Diplazium lonchophyllum is generally smaller and has fewer pinna pairs and costules per pinna. Also, D. drepanolobium never has any free or stalked pinnules, and its pinnules are always falcate.

7. D. drepanolobium

OTUs eventually assigned to D. drepanolobium form discrete clusters in both cluster analysis and PCA (Figures II-2, page 12; II-3, page 16; II-4, page 18). They cluster near D. franconis because of their long fronds, many pinnae pairs, wide pinnae, and proportionately long pinnules. Basal pinna dissection in D. drepanolobium is intermediate--pinnatifid to pinnate. The apex of D. drepanolobium is 2-1/2 times longer than broad making it proportionately the narrowest apex type in the complex. The characters which make this taxon unique are the strongly falcate and acute pinnules which are always sessile to the pinna. In cluster analysis (Figure II-2, page 12), OTUs 1-124 and s-60 clustered with D. drepanolobium. Examination of the original data showed that the two OTUs are similar to D. drepanolobium only in overall size, dissection, apex features, and pinna base angles. OTU 1-124 does not have acute falcate pinnules, and OTU s-60 has a glabrous stipe base and was collected outside of the range of D. drepanolobium.

8. D. franconis

The OTUs of D. franconis form a distinct group in cluster analysis and PCA (Figures II-2, page 12; II-3, page 16; II-4, page 18). For 13 of the 25 characters scored in the present study, the mean character state values of D. franconis were extremes. These characters include longest fronds with the greatest number of pinnae, widest most dissected pinnae, longest petiolules, longest

pinnules, shortest sori, and shortest frond apex. In some cases, a very robust D. lonchophyllum or an immature D. franconis may be difficult to identify. Frond apex length and apex length:lamina length combined with degree of dissection and attachment of the basal acroscopic pinnule should effectively separate the two taxa.

In conclusion, numerical analysis of the D. lonchophyllum complex suggests that eight morphological groups are present. Each taxon as eventually recognized, occupies a general area on the PCA graphs but some overlapping is evident. While most OTUs were readily assigned to a taxon correlated with a cluster, some, exhibiting phenetic intermediacy, cluster with or between different taxa in cluster analysis and PCA. These (if their spores are aborted) are presumed to be hybrids. Diplazium arboreum is here recognized as distinct from D. cristatum, but further studies (including a type study), are necessary to confirm this. Further studies may also suggest combining D. tablazianum and D. subsilvaticum as one species. The plants from Louisiana fall well within the range of variability for D. lonchophyllum.

CHAPTER III

GAMETOPHYTE STUDIES

Extensive work on fern gametophytes as experimental material has revealed frequent interspecific (Atkinson, 1967; Miller, 1968; Näf, 1979) as well as intraspecific (Pray, 1968; Lloyd and Gregg, 1975; Cousens, 1979) differences in gametophyte morphology, ontogeny, and sexual expression. In the study by Atkinson (1967), gametophytes of four species of Diplazium were studied to determine whether there were any morphological characteristics of the prothallus that might support placing one of the species into another genus. She found there were interspecific distinctions involving germination pattern; gametophyte size, shape, and thickness; rate of maturation and gametangial development; and presence or absence of papillae. However, the species she examined were not closely related and were of varying ploidy levels. Such differences may not be evident between closely related taxa or taxa at the same ploidy level.

A program was initiated to study general gametophyte development and morphology of the available members of the D. lonchophyllum complex. The purpose was to describe gametophyte development and to determine whether the gametophytes had distinctive characteristics as Atkinson (1967) had found in other species of Diplazium. If gametophyte populations showed distinct intraspecific characteristics, the gametophyte generation could be

sampled for additional attributes to be used in biosystematic investigations. In addition, isolate cultures were established and tested for apogamous production of sporophytes, and three samples were tested for antheridiogen activity.

Materials and Methods

Fertile pinnules were collected from mature plants in the greenhouse. Voucher specimens are on deposit at TENN and are listed in Table III-1. These represented four different collections from the Louisiana population of D. lonchophyllum, four D. lonchophyllum from Mexico, and four plants that were suspected hybrids involving D. lonchophyllum or D. franconis from Mexico. The pinnules were rinsed with distilled water and air dried in glassine envelopes to release spores. Fresh spores were sterilized in a 5% Chlorox solution, centrifuged, resuspended in sterile water, and pipetted onto sterile 15 x 100 mm Petri dishes containing Knudson's medium solidified with 0.8% Bacto Agar (pH 5.5). Sowing by pipette resulted in a rather dense sowing. Spore availability varied between plants and over time resulting in unequal sample sizes and therefore required several sowings throughout the year. However, each spore type was collected and sown two to four times on a minimum of five plates per sowing, resulting in 10 to 20 replicate sowings. Uniform cultural conditions were maintained throughout the observation period by growing the cultures in continuous lighting 45-55 cm. below cool white fluorescent bulbs (23 Wm^{-2}) at $25^{\circ}\text{C} \pm 2^{\circ}$.

Table III-1. Voucher Specimens for Gametophyte Studies and Live Plants in Greenhouse.

Species	Identification Number and Source	Voucher ^a
<u>D. lonchophyllum</u>	1129a. Oaxaca, Mexico:	Sessions 33
	1130. Oaxaca, Mexico:	Sessions 35
	1142. Oaxaca, Mexico:	Sessions 36
	1141. Oaxaca, Mexico:	Sessions 37
	47. Iberia Parish, Louisiana:	Sessions 47
	F2. Iberia Parish, Louisiana:	Sessions 60
	824. Iberia Parish, Louisiana:	Sessions 61
	W3. Iberia Parish, Louisiana:	Sessions 68
<u>Hybrids</u>		
<u>D. lonchophyllum</u> x?	Vol. Origin unknown:	Sessions 51
<u>D. franconis</u> x?	1129b. Oaxaca, Mexico:	Sessions 34
	1145. Oaxaca, Mexico:	Sessions 52
	1128. Oaxaca, Mexico:	Sessions 53

^aAll vouchers on deposit at TENN.

Gametophytes were observed in mass cultures for up to 120 days. Periodic observations (usually every four or five days) were made until at least 20% were cordate or until day 40, whichever came first. At this time, samples for recording sex expression were removed from the culture dishes and mounted on slides in acetocarmine and Hoyer's solution to enable staining and counting of gametangia. Fifteen gametophytes per spore batch were removed at random at weekly intervals. On days 45, 60, 75, and 90, the percentage of gametophytes exhibiting a particular sex was calculated. Data for multiple sowings was pooled and mean values taken to arrive at final sex expression values at 15 day intervals. On day 60, mean widths of female gametophytes were calculated. All sampling and transfers were done under sterile conditions in a laminar flow hood.

Gametophytes were observed for sporophyte production. As soon as cultures contained both sexes, one or two dishes per spore batch were watered with aerated sterile distilled water at various times of the day for a period of one week. As this introduced contamination and/or caused rapid death of the gametophytes, watering to encourage sperm release and fertilization was not continued. Gametophytes were also tested for the apogamous production of sporophytes. As soon as the prothalli became cordate but before becoming sexual, 20 gametophytes per collection were removed from mass cultures and placed individually in 15 x 50 mm petri dishes containing sterile Knudson's medium. Water was

withheld from the dishes so fertilization could not occur. After 200 days, isolated gametophytes were scored for apogamous sporophyte production.

Lastly, three of the collections were tested for antheridiogen activity. Crude antheridiogen extracts were made by scraping off gametophytes of five culture dishes of each collection containing fully mature sexual prothalli. The bare plates were then frozen and thawed and the solid agar removed leaving liquid extract which presumably contained antheridiogen. (Miller, 1968). Liquid extracts were incorporated into the new sterile medium at approximately 30% of the aqueous portion. Because it was not known whether sterilization by autoclaving would deactivate or destroy the antheridiogens, suction filtration sterilization using Whatman No. 2 paper was performed on 1/2 of the media, and sterilization by autoclaving was performed on the rest. Spores from the same plant were then sown on the extract containing medium, and gametophyte ontogeny observed.

Results and Discussion

Spores from 12 plants were collected, but four of these had aborted or malformed spores. Very few spores germinated from these four plants, and those that did died shortly after producing one or two rhizoids with irregular growth. These four--numbers 1128, 1129b, 1145, and "Vol"--are presumed hybrids involving D. franconis and D. lonchophyllum. There are no data for these

plants. The eight that germinated successfully were D. lonchophyllum from Mexico, numbers 1129a, 1130, 1141, and 1142 and D. lonchophyllum from Louisiana, numbers 47, F2, W3, and 824.

Gametophyte development for all spore collections followed a similar pattern. Four to six days after sowing, the spore wall split and the protoplast divided into a visible rhizoid initial and a basal cell. The basal cell then divided to initiate a filamentous or protonemal stage composed of one or two (rarely four) cells before two dimensional or planate growth began. Two dimensional growth was initiated 10 to 15 days after sowing. Continued divisions of distal cells resulted in the typical cordate thallus within 20 to 30 days. Between 35 and 40 days, the first archegonia appeared. With the exception of D. lonchophyllum number 1130 which was initially male, all gametophytes were initially female. They became hermaphroditic slowly over time. Male gametophytes were never in abundance, with the exception of 1130. Rhizoids were colorless or very pale tan, becoming numerous with age. By 100 days, a massive central cushion had developed making slide preparations difficult. Gametophytes were broadly cordate and up to 1 cm. wide. All cultures except 1130 occasionally had irregular basal cell divisions resulting in spatulate, elongate, or globose basal vegetative outgrowths. In addition, although the general growth pattern was consistent, there was much variation within cultures as well as between sowings.

On day 60, the widths of female gametophytes were measured from prepared slides. Table III-2 shows the mean width and standard deviation of females in samples where at least 15 and up to 60 counts were available. It is apparent that much variation in size within spore collections exists.

The gametophytes of three plants had distinctive shapes. (Figure III-1). Early two dimensional growth in D. lonchophyllum 1141 produced a long narrow thallus (approximately two to four cells wide and up to 10 long) before expanding into the broader cordate portion giving the gametophytes an elongate cordate appearance. Diplazium lonchophyllum 1130 was characterized by vigorous growth and broadly cordate to reniform gametophytes with an open U-shaped apical notch and spreading wings, or a closed notch and overlapping wings. Sometimes these gametophytes resembled two circles fused by narrow tangents. The gametophytes of Louisiana D. lonchophyllum 47 were distinctive in being very broadly cordate with their wings touching or overlapping thus closing the notch. Meristematic cells were abundant in the notch area and along the margins distally forming a characteristic circular pattern of cells. The other Louisiana samples never quite showed this trait, but their growth was never as vigorous either.

Perhaps the most interesting aspect of gametophyte growth was sex expression and particularly the atypical pattern displayed by sample 1130. The general trend as mentioned was from female to hermaphrodite except in sample 1130 which was the only one

Table III-2. Mean Width (mm) of Female Gametophytes on Day 60.^a

Species and Accession Number	$\bar{X}$ Width (mm)
<u>D. lonchophyllum</u>	
1129a	2.91 (1.03)
1141	1.74 (0.35)
1142	3.19 (1.32)
47	4.53 (2.44)

^aValues calculated for samples having a minimum of 15 female gametophytes. Numbers in parentheses are standard deviations.

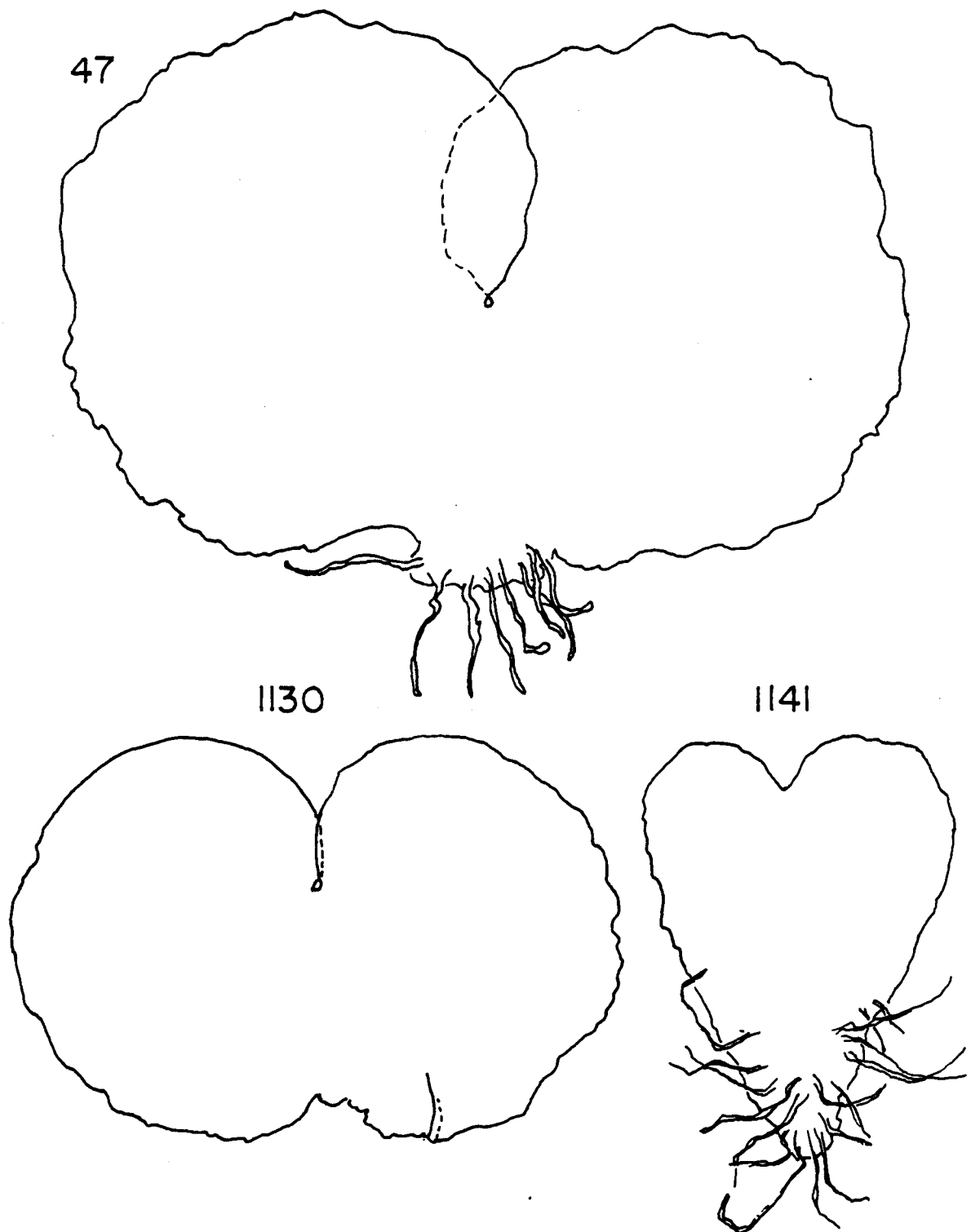


Figure III-1. Gametophyte Shapes for Diplazium lonchophyllum Numbers 47, 1130, and 1141. 30X.

to have a very high initial percent of male gametophytes. On day 35, nearly 90% of the randomly scored prothalli were male, all others being neuter. Between 45 and 60 days, the percent of hermaphrodites surpassed the males and generally remained high, but the number of males always exceeded that of females. This pattern was consistent throughout replicate sowings. It was not possible to determine whether males or neuters became bisexual. Atkinson (1967) observed a similar situation in D. stellato-pilosum with gametophytes first being male and gradually becoming bisexual. Figure III-2 depicts sex expression values for 8 spore batches or treatments over time. Figure III-2C clearly shows the abundance of males and lack of females in sample 1130.

An initial high percent of males may indicate the presence of an antheridiogen system (Miller, 1968; Näf, 1979). At present, antheridiogens are thought to be hormone or pheromone-like substances secreted by cells in the meristem area of cordate gametophytes (Näf, 1958; Miller, 1968; Näf, Nakanishi, and Endo, 1975; Näf, 1979) which diffuse through the culture medium and effectively induce the formation of antheridia on smaller or non-cordate gametophytes. The gametophytes that secrete antheridiogens are themselves insensitive to it (Näf, 1958; Miller, 1968; Näf et al., 1975; Näf, 1979). The result is a population initially composed of female and male gametophytes which presumably would facilitate intergametophytic mating or crossing (Voeller, 1971; Cousens, 1979; Näf, 1979). In time both male and female gametophytes become

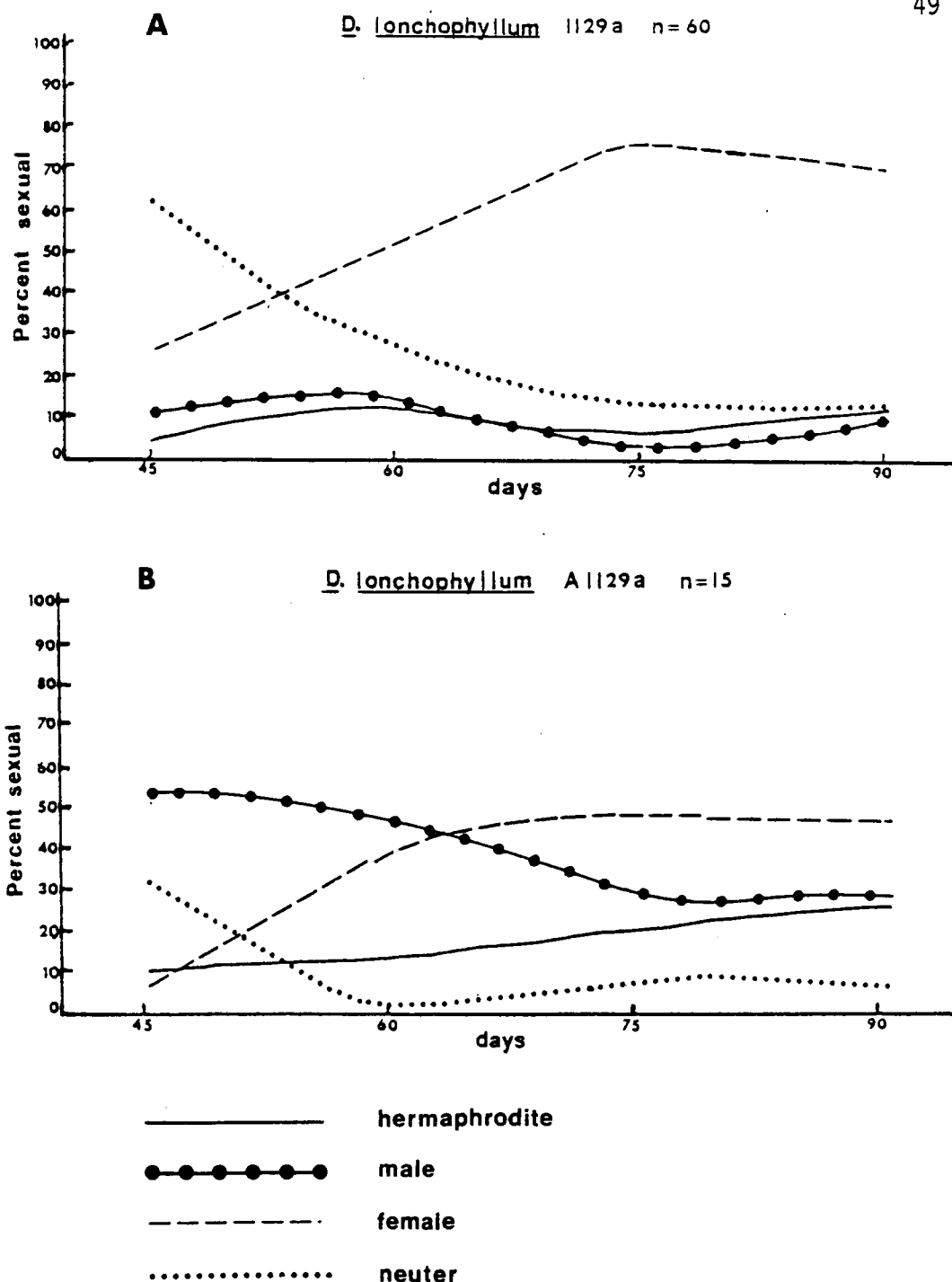


Figure III-2. Mean Percent Sex-expression Values of Gametophytes at Fifteen Day Intervals. n=number of gametophytes examined. "A" preceding gametophyte sample number indicates spores sown on antheridiogen containing medium. A. D. lonchophyllum 1129a. B. D. lonchophyllum A1129a. C. D. lonchophyllum 1130. D. D. lonchophyllum A1130. E. D. lonchophyllum 1141. F. D. lonchophyllum 1142. G. D. lonchophyllum A1142. H. D. lonchophyllum 47.

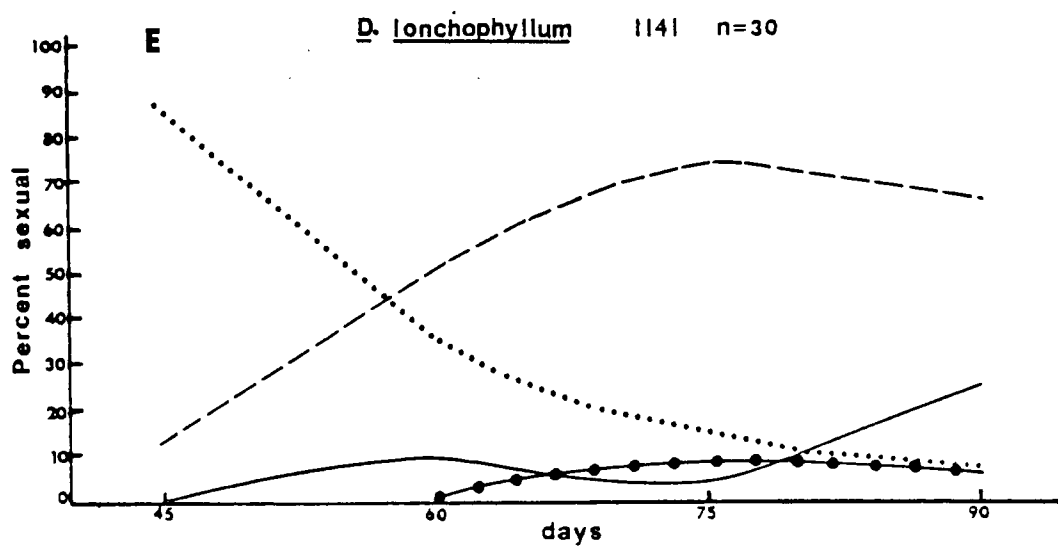
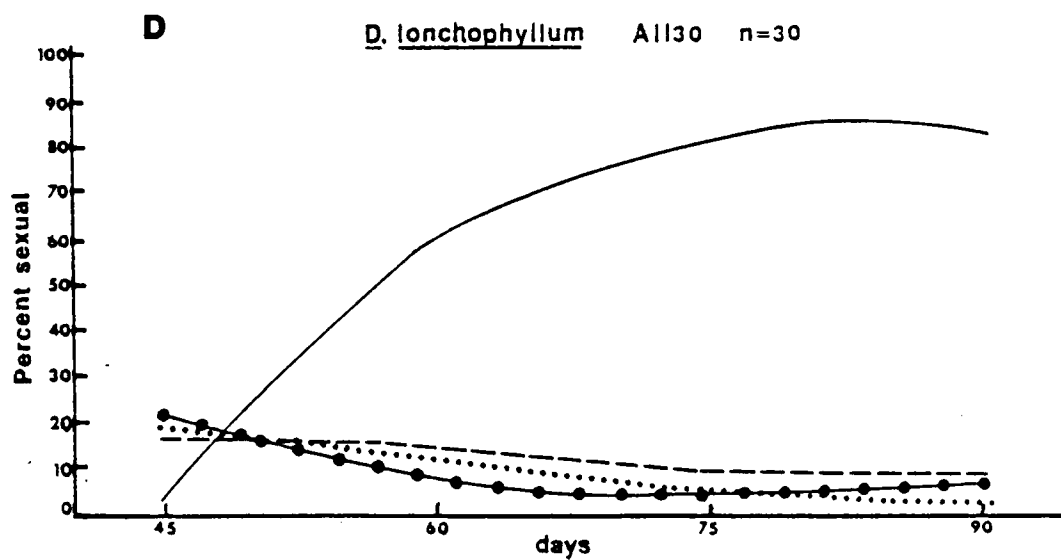
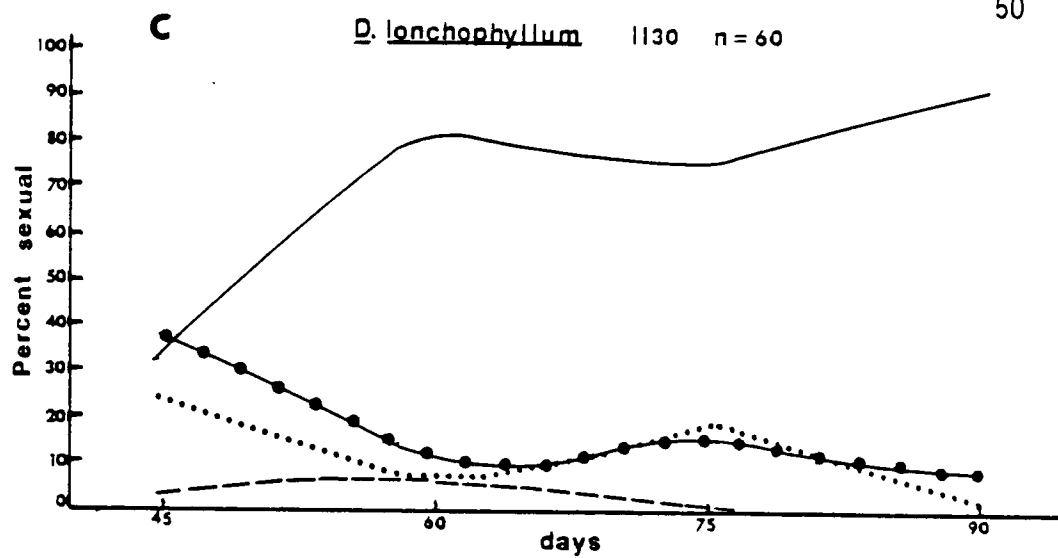


Figure III-2 (Continued)

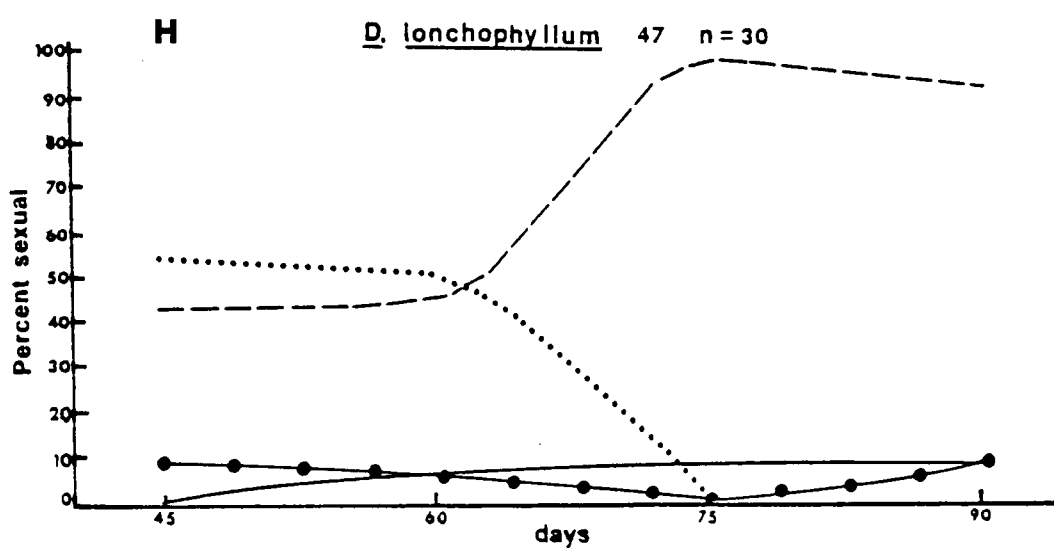
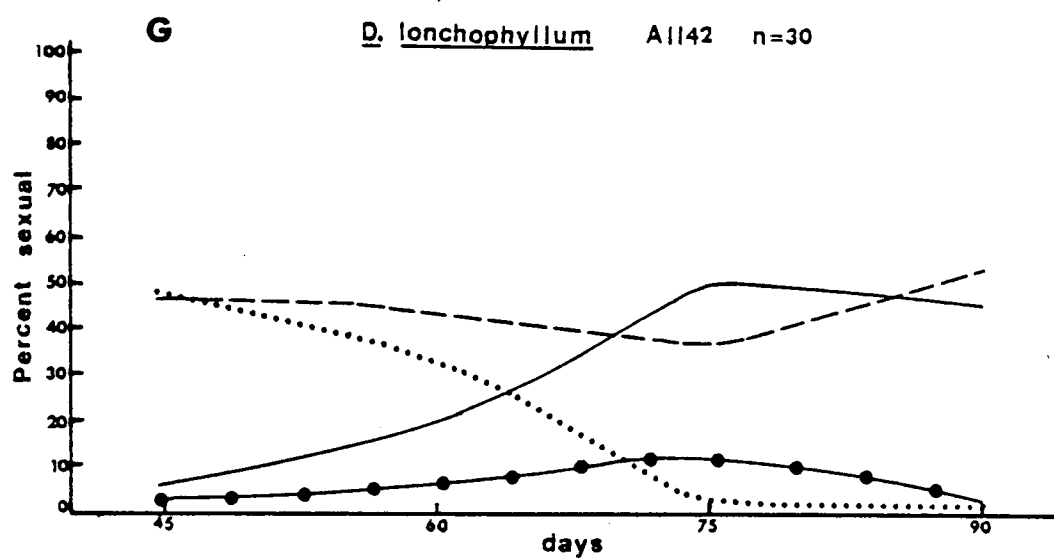
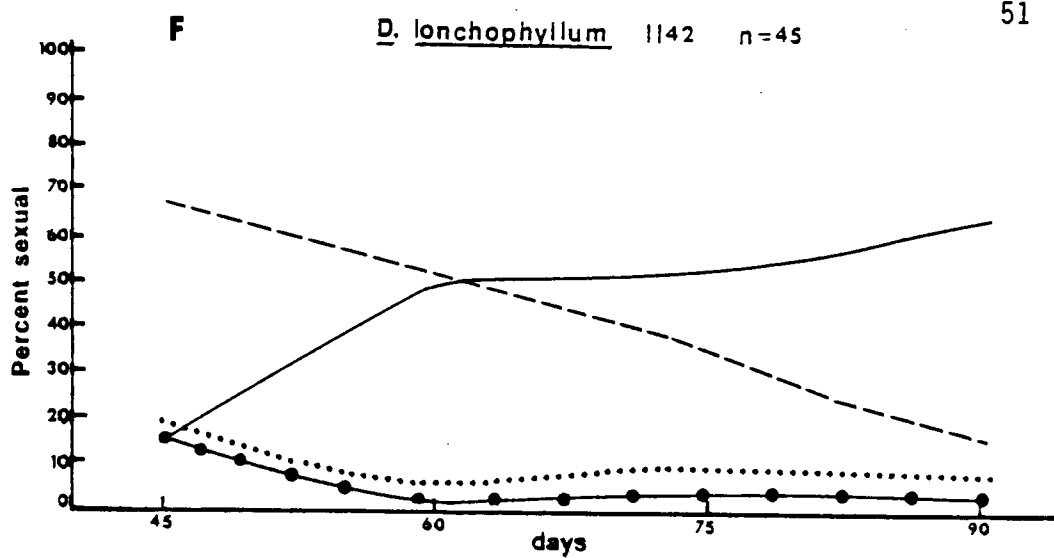


Figure III-2 (Continued)

hermaphroditic allowing intragametophytic selfing. The action of a specific proven antheridiogen on other taxa varies. The antheridiogen from Pteridium aquilinum is effective on species throughout the Polypodiaceae sensu lato; antheridiogens from Lygodium and Anemia are effective only within the Schizeaceae; and others are effective only on themselves as in Onoclea sensibilis (Miller, 1968; Näf, 1979) and Ceratopteris (Hickok, 1983).

When gametophytes of 1130 were grown on medium containing antheridiogen extract, the expected response to antheridiogen was not observed (Figure III-2D). The percent of females increased while that of males and bisexuals decreased. Increased females due to "archegoniogens" are not known in ferns. In addition, the average gametophyte size on extract-containing medium was greater than on plain, and the number of antheridia per gametophyte was smaller. This is exactly the opposite of what was expected, and the same response occurred when the experiment was repeated. Plate contamination was not a problem. Although 1130 did not react positively to filter-sterilized or autoclaved antheridiogen extract-containing medium, it cannot be concluded that it does not have an antheridiogen system. At present, the cause of its unusual response is unknown and warrants further study.

Gametophytes of D. lonchophyllum 1129a were also tested for antheridiogen response. On plain medium, sex expression was normal--initially female followed by bisexuals and males (Figure III-2A). On medium containing antheridiogen extract,

however, there initially was a greater percent of males which then gradually tapered off as females and hermaphrodites increased (Figure III-2B). Not only did the percentage of males increase and females decrease, but the average number of antheridia per gametophyte also increased on extract-containing medium. These results support the conclusion that 1129a has an antheridiogen system. Whether response depends on the higher concentration of antheridiogen in the extract-containing medium or on heat activation by autoclaving as in Onoclea sensibilis (Näf, 1979) is not known. The gametophytes of 1142 also showed an increase in males when grown on extract-containing medium (Figure III-2F,G), but the increase was slight and too late to be considered a definite response to antheridiogen.

The salient feature of the gametophyte study was the variation in size, shape, pattern and timing of sex expression, and antheridiogen response. This is especially interesting since all plants used as viable spore sources are considered to be morphological forms of D. lonchophyllum. Recent work has revealed that interspecific as well as intraspecific variation among gametophyte populations is common, and that variations in gametophytes are not necessarily correlated with sporophytic differences distinguishing taxonomic varieties (Pray, 1968; Klekowski, 1972; Lloyd and Gregg, 1975; Cousens, 1979). Even when different populations are fully interfertile, statistically different patterns of sex expression, early ontogeny, and degree of self-compatibility between

populations has been shown (Pray, 1968; Klekowski, 1972; Lloyd and Gregg, 1975; Cousens, 1979). Schedlbauer and Klekowski (1972) have also shown intraspecific variation in gametophyte response to Ceratopteris antheridiogens.

It has long been known that cultural conditions influence sexuality in gametophytes--generally unfavorable and crowded conditions promoting maleness (Klekowski and Lloyd, 1968; Miller, 1968; Pray, 1968; Cousens and Horner, 1970; Tryon and Vitale, 1977; Bell, 1979; Cousens, 1979). In the present investigation, spores were sown at approximately uniform densities, and gametophytes were grown under uniform cultural conditions. Some variation due to sampling error and the effect of pooling data from several sowings may be expected, but the major differences between the shapes, for instance, of D. lonchophyllum 1130, 47, and 1141, should be considered stable morphological differences. However, it is important to stress that a study of gametophytes from one collection may lead to erroneous conclusions.

Mating and apogamy experiments yielded no sporelings. In the isolate cultures, after 204 days, no sporelings had formed, i.e., sporeling production by apogamy did not occur. When mass cultures were watered to induce sperm release and fertilization, no sperm were ever observed, no fertilizations occurred, and all cultures subsequently died. In natural populations of D. lonchophyllum in Louisiana, large numbers of gametophytes bearing young sporelings were observed beneath fertile plants

indicating that fertilization occurs readily in nature. (It is unlikely that the population is apogamous.) It is interesting to note that when spores of 1129a, 1130, 1141, and 1142 were sown septically on sterilized soil in clay pots on the lab bench, sporelings were formed in abundance when watered from above. Further tests could be conducted culturing gametophytes on soil.

To summarize, spores of 12 plants were sown on sterile medium. Four plants had aborted spores and are presumed to be hybrids. The others were slightly different phenotypical forms of D. lonchophyllum. Gametophytes were cultured for four months under uniform conditions. Within five weeks, gametophytes had progressed through a brief protonemal stage of one to four cells, to a two-dimensional stage often characterized by irregular basal divisions, to a fully cordate sexual stage with a notch meristem. A protogynous to bisexual pattern was common to all samples except 1130 which was initially male. One sample, 1129a, demonstrated a positive response to antheridiogen, and one, 1130, demonstrated a reverse response by an increased number of females. Gametophyte shape was characteristic for three of the spore collections (1130, 1141, and 47). No sporelings were observed in mating or apogamy experiments, although sporelings were produced on non-controlled pot-grown gametophytes. Although gametophyte response varied greatly among and between sowings, the shape of three samples and sexual sequence of one showed that if sufficient populations were examined, these may be useful biological characters for assessing taxonomic boundaries within the D. lonchophyllum complex.

CHAPTER IV

ADDITIONAL SYSTEMATIC CHARACTERS

A taxonomic study cannot rely totally on macroscopic morphological features on herbarium specimens to summarize dynamic relationships present in a group especially when the organisms are phenotypically variable. Microscopic features as well as field characteristics of the organisms contribute to an overall understanding. In addition to the characters that were scored in the numerical analysis, several other features were considered with the hope that they may provide useful information. Spore lengths, spore morphology using SEM, stomate patterns, frond flavonoid chemistry, and cytology were surveyed to observe intra- and interspecific characteristics. Plants were also grown in the greenhouse to observe general growth.

Spores

The use of spore morphology as a character for elucidating systematic relationships in ferns has become very popular in the past 50 years (Wood, 1973; Wagner, 1974). Spores of Diplazium have been examined by light microscopy for species in Ceylon (Sledge, 1962), Japan and India (Erdtman, 1957; Nayar and Devi, 1963; Nayar, 1964), and Mexico and Central America (Smith, 1976). The authors report interspecific distinctions (except Smith who noted intergeneric differences) in spore shape, size, and

ornamentation for some but not all species examined. Sledge (1962) considered spore characters especially to be of the greatest value in identification. No scanning electron microscope (SEM) studies of Diplazium spores are known.

The great resolution and depth of focus provided by the SEM allow taxonomists to see critical features of spore morphology. In the present study, spores were obtained from dried herbarium specimens and affixed to standard 3/4" aluminum stubs with double-faced cellophane tape. Specimens were coated under vacuum with vaporized gold:palladium (60:40) and viewed on an ETEC Autoscan scanning electron microscope. Photographs were taken with Polaroid Type 655 Positive/Negative film. Spore lengths were measured under a light microscope from permanent slides made by mounting spores in silicone oil and ringing the coverslip with clear nail polish. Vouchers for spore studies are listed in Table IV-1.

The spores in the D. lonchophyllum complex are monolete, bilateral, and plano-convex laterally. A loosely folded or winged perispore forms undulating and casually anastomosing ridges (rugulae) over the spore surface (Figure IV-1). In D. werckleanum, the perine ridges rise sharply and abruptly from the exine surface in contrast to less distinct rugulae in the other taxa. All taxa showed variation in the degree of exine ornamentation. On a single stub, spore surfaces between and on rugulae, were smooth to muricate to papillose to very finely reticulate-papillose between the larger ridges. This may reflect developmental stages of the spores.

Table IV-1. Spore Study Vouchers.

Species	Subject	Voucher
<u>D. arboreum</u>	SEM and length	COSTA RICA: Cartago; <u>Maxon</u> 183(US); HONDURAS: Atlantida; <u>Standley</u> 54206(F).
	SEM	PANAMA: Panama; <u>Witherspoon</u> 8395(MO).
<u>D. cristatum</u>	SEM and length	GUADELOUPE: <u>Proctor</u> 20116(US); JAMAICA: <u>Maxon</u> 9331(US).
<u>D. drepanolobium</u>	SEM and length	MEXICO: Chiapas; <u>Breedlove</u> 38162(CAS),
	length	<u>Breedlove</u> 32852(CAS). MEXICO: Tabasco; <u>Cowan</u> 3162(CAS).
<u>D. franconis</u>	SEM and length	MEXICO: Chiapas; <u>Breedlove</u> 22670(CAS).
	SEM	EL SALVADOR: Chalatenango; <u>Seiler</u> 336(F); MEXICO: Chiapas; <u>Breedlove</u> 42520(CAS); Oaxaca; <u>Sessions</u> 34(TENN), <u>Sessions</u> 52(TENN), <u>Sessions</u> 53(TENN).
	length	GUATEMALA: San Marcos; <u>Williams et al.</u> 23179(US); MEXICO: Vera Cruz; <u>Ventura</u> 266(F).
<u>D. lonchophyllum</u>	SEM and length	GUATEMALA: Santa Rosa; <u>Standley</u> 79142(US); MEXICO: Chiapas; <u>Breedlove</u> , <u>Smith</u> 21624(CAS), <u>Breedlove</u> 33658(CAS); UNITED STATES: Louisiana, Iberia Parish; <u>Sessions</u> 46(TENN), <u>Sessions</u> 47 (TENN), <u>Sessions</u> 61(TENN).
	SEM	EL SALVADOR: San Salvador; <u>Seiler</u> 218(F); HONDURAS: Comayagua; <u>Molina</u> 18100(US); MEXICO: Oaxaca; <u>Hallberg</u> 1448(NY), <u>Sessions</u> 33(TENN), <u>Sessions</u> 35(TENN), <u>Sessions</u> 36(TENN), <u>Sessions</u> 37(TENN); ORIGIN UNKNOWN: <u>Sessions</u> 51(TENN).
	length	MEXICO: Oaxaca; <u>Hallberg</u> 1396(NY).
<u>D. subsilvaticum</u>	SEM and length	COSTA RICA: Alajuela, Puntarenas, Guanacaste; <u>Dryer</u> 236(F).
	SEM	PANAMA: Chiriqui; <u>Allen</u> 1348(F).
	length	COSTA RICA: San José <u>Skutch</u> 4735(US); PANAMA: Chiriqui; <u>Seibert</u> 249(MO).
<u>D. tablazianum</u>	SEM and length	COSTA RICA: Heredia; <u>Standley</u> , <u>Valerio</u> 49242(US).
	SEM	COSTA RICA: Alajuela, Puntarenas, Guanacaste; <u>Dryer</u> , 379(F).
<u>D. werckleanum</u>	SEM and length	GUATEMALA: El Quiche; <u>Molina</u> 30301(MO); MEXICO: Chiapas; <u>Breedlove</u> 34693(MO).

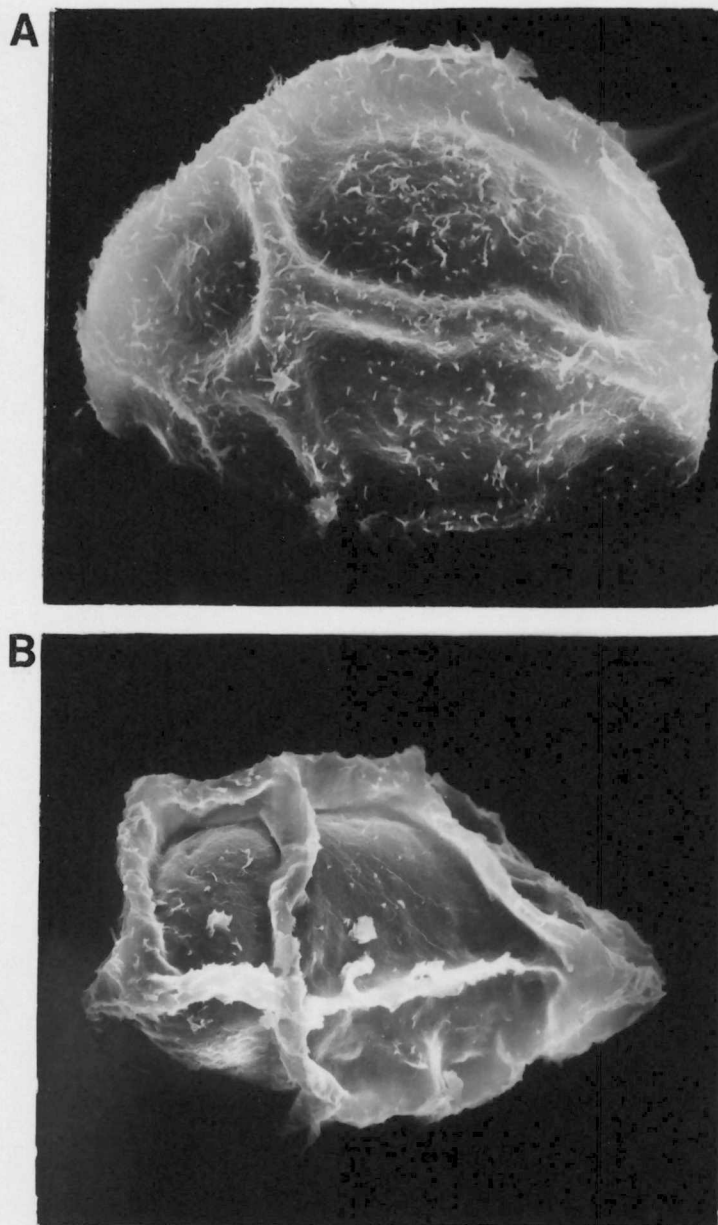


Figure IV-1. Scanning Electron Micrographs of Diplazium Spores. A. D. lonchophyllum (Sessions 46). Lateral view, 1400X. B. D. werckleanum (Breedlove 34693). Lateral view, 960X.

The spore surface of D. werckleanum is sparsely minutely tuberculate to smooth. Sledge (1962), Nayar and Devi (1964) and Smith (1976) report similar spore morphologies in Ceylonese, Indian, and Mexican Diplazium, respectively. Smith (1976) found variation in perispore morphology but did not state whether it was intra- or interspecific. Spores of suspected hybrids from the greenhouse were irregular in shape, size, and ornamentation. There was a marked tendency towards perine elaboration which is typical of aborted spores (Wagner, 1974).

Spore lengths (including the perispore) were measured with the light microscope. Analysis of variance of spore lengths revealed length was significant at the .05 level of probability for groups of taxa but not between individual taxa (Table IV-2). Mean spore length ranged between 65.91 μ in D. werckleanum which also had the most extensively developed perine, and 44.07 μ in D. franconis. Spore lengths varied significantly between D. lonchophyllum from Mexico/Central America (53.95 μ) and Louisiana (47.19 μ) and between D. cristatum and D. arboreum lending further weight to the separation of the latter two taxa. However, variation within most species was considerable, and the variation of each overlapped others, so that spore size in itself is not a useful taxonomic character for distinguishing species. Whether this variation is genetical, developmental, or due to sampling, is not known (A. R. Tryon, 1971; Hennipman, 1977).

Table IV-2. Analysis of Variance of Spore Lengths.^a

Grouping	Mean (μ)	Species
A	65.91	<u>D. werckleanum</u>
B	53.95	<u>D. lonchophyllum</u> (Mex., Cen. Amer.)
B	53.69	<u>D. cristatum</u>
C	49.79	<u>D. arboreum</u>
C	47.19	<u>D. lonchophyllum</u> (Louisiana)
C	47.06	<u>D. subsilvaticum</u>
C	45.24	<u>D. drepanolobium</u>
D	44.33	<u>D. tablazianum</u>
D	44.07	<u>D. franconis</u>

^aGroups with the same letter are not significantly different at the .05 level.

Spore morphology and length may be effective characters in distinguishing taxa in the D. lonchophyllum complex from those outside of it. Studies on tropical American species outside of the D. lonchophyllum complex remain to be done. However, within the complex, morphological similarity probably attests to the close relationship among the taxa. The large size, smooth spore surface and more prominent ridges of D. werckleanum, in conjunction with other characters, may indicate a more distant relationship of this species to others in the complex.

Stomates

While stomatal architecture has generally been found to be most useful at higher levels of taxonomy (Radford, Dickison, Massey, and Bell, 1974), examination of stomates has been fruitful at the species level especially when different ploidy levels are involved (Van Cotthem, 1973; Stace, 1980). In the present study, dried pinnules were removed from herbarium specimens and cleared in NaOH (5%). After rinsing in distilled water and bleaching in sodium hypochlorite (0.5%), cell walls were stained using alternations of tannic acid (1%) and ferric chloride (3%). Pinnules were then taken through a gradual dehydration series up to absolute alcohol. Permanent slides were made with Diaphane (see Table IV-3 for a list of specimens examined). Epidermal and subsidiary cell shapes and guard cell lengths were examined for consistent intraspecific patterns.

Table IV-3. Stomate Study Vouchers.

Species	Voucher
<u>D. arboreum</u>	HONDURAS: Atlántida; <u>Standley</u> 54206(US). PANAMA: Panama; <u>Witherspoon</u> 8395(MO).
<u>D. cristatum</u>	HAITI: <u>Ekman</u> 10570(US). JAMAICA: <u>Maxon</u> 9331(US).
<u>D. drepanolobium</u>	MEXICO: Chiapas; <u>Breedlove</u> 32852(CAS); Oaxaca; <u>Wendt et al.</u> 276(US).
<u>D. franconis</u>	COSTA RICA: Heredia; <u>Stolze</u> 1576(US). HONDURAS: Comayagua; <u>Yuncker et al.</u> 5920(MO). MEXICO: San Luis Potosi; <u>Pennell</u> 17969(US). NICARAGUA: Matagalpa; <u>Williams et al.</u> 23841(F).
<u>D. lonchophyllum</u>	HONDURAS: Comayagua; <u>Williams, Molina</u> 12526(F). UNITED STATES: Louisiana, Iberia Parish; <u>Sessions</u> 17(TENN).
<u>D. subsilvaticum</u>	COSTA RICA: Alajuela; <u>Lellinger</u> 1642(US); San José; <u>Skutch</u> 4755(MO), <u>Stolze</u> 1427(F).
<u>D. tablazianum</u>	COSTA RICA: Heredia; <u>Standley, Valerio</u> 49242(US). PANAMA: Chiriqui; <u>Croat</u> 15803(MO).
<u>D. werckleanum</u>	GUATEMALA: Alta Verapaz; <u>Williams et al.</u> 42092(US); El Quiche; <u>Molina</u> 30301(F). HONDURAS: Morazan; <u>Molina</u> 17018(F). MEXICO: Chiapas; <u>Breedlove</u> 35217(MO), <u>Purpus</u> 352(US). NICARAGUA: Matagalpa; <u>Williams et al.</u> 24973(F).

All taxa had very sinuous jig-saw puzzle-like epidermal cells which were more intricately patterned on the abaxial surface of the leaf than on the adaxial surface. Stomates were hypostomatic (only on abaxial surface) and varied in size and number per unit area depending on their location on the pinnule--fewer and smaller distally and towards margins. Diplazium werckleanum was the only species possessing fairly well defined subsidiary cells, the pattern being polocytic (Figure IV-2). The arrangement of the others was mixed polocytic and anomocytic (sensu Van Cotthem, 1973). Diplazium werckleanum had the largest guard cells ($\bar{x} = 53.39 \mu$) and the fewest per square mm (70), while D. drepanolobium ($\bar{x} = 36.1, 140/\text{mm}^2$), D. franconis ($\bar{x} 39.62, 100/\text{mm}^2$), and D. subsilvaticum ($\bar{x} 40.5, 140/\text{mm}^2$) all had small and numerous stomates. Guard cell length was more or less negatively correlated to number per unit area. Again, as in spore sizes, the variation within samples was great and overlapped variation of other samples. To determine any statistical or taxonomic significance, many more samples would have to be measured. It is not possible to make inferences regarding ploidy levels from stomate sizes as not enough reference chromosome numbers were available. However, the larger spores and stomates of D. werckleanum may prove with further research to be correlated to its tetraploid cytology as reported in the literature.

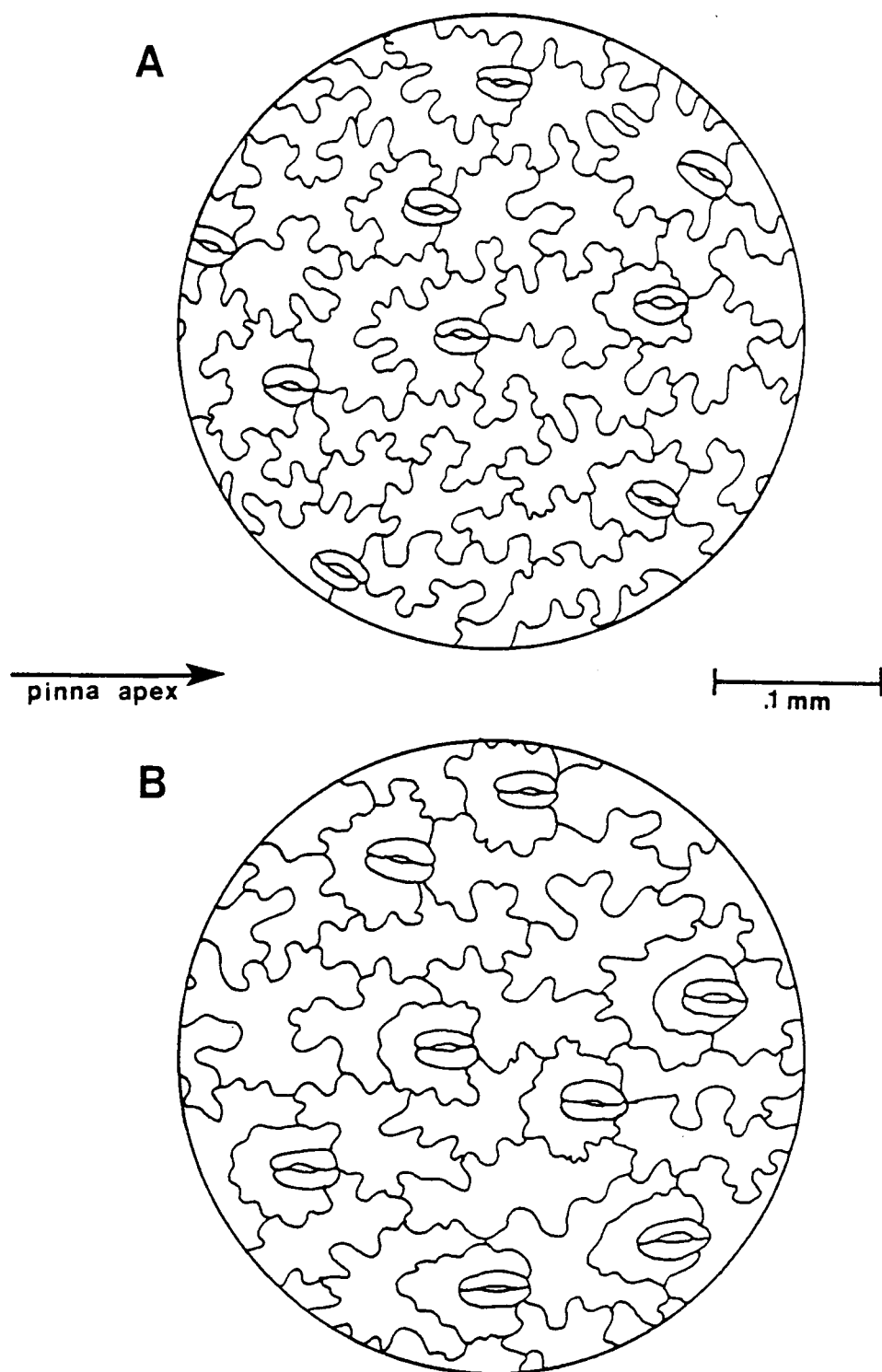


Figure IV-2. Stomate and Epidermal Cell Patterns.
A. *D. lonchophyllum* (Williams 12526). B. *D. werckleanum* (Breedlove 35217). Both 100X.

Flavonoid Chemistry

The usefulness of flavonoids in plant systematics is well known. Species may exhibit species-specific flavonoid profiles, and interspecific hybridization may be detected due to the additive nature of flavonoid compounds (Smith and Levin, 1963; Cooper-Driver, 1980; Swain, 1980). Hiraoka (1978) surveyed four Japanese species of Diplazium for leaf flavonoids and found kaempferol 3-glucoside and quercetin 3-glucoside (common among ferns) with no variation among the species. Using standard phytochemical procedures (Mabry, Markham, and Thomas, 1970), my own attempts to extract frond flavonoids from field collected D. lonchophyllum indicated there were no flavonoids present.

Cytology

Cytological investigations of species within the D. lonchophyllum complex have revealed polyploidy and suggested hybridization. Walker (1966) reported D. cristatum as 4X from Jamaica. Mickel et al. (1966) and Smith and Mickel (1977) reported as diploids D. cristatum (= D. arboreum?) and D. tablazianum from Costa Rica; as tetraploids D. werckleanum and D. franconis from Mexico, and; as both 2X and 4X D. lonchophyllum from Mexico. In addition, they made counts of plants with 41 bivalents and 75 univalents, and others with 90 bivalents and 93 univalents. Pairing behavior and aborted spores suggested higher ploidy levels and hybridization. Counts for D. subsilvaticum and D. drepanolobium

have not been reported. Undoubtedly, polyploidy and hybridization play a role in obscuring species limits in this group.

In the present study, a cytological investigation was undertaken for two reasons. One was to determine chromosome numbers of individual plants of D. lonchophyllum and suspected hybrids in the greenhouse (Table III-1, page 41). The other was to observe chromosome pairing behavior during meiosis. Chromosome behavior and the degree of univalent, multivalent, and bivalent formation during meiosis may be useful in assessing chromosomal homologies and parental ploidy levels (Manton, 1950; Stebbins, 1945; de Wet and Harlan, 1972). Fresh pinnae of the appropriate age were fixed in both Carnoy's and Newcomer's Solutions. Unfortunately, the chromosomes of the plants sampled had a great propensity to stick together. Pretreatments in saturated PDB, 10% HCl, and 70% alcohol did not help in spreading. No good squashes were obtained; however, it was apparent that none of the material is diploid. Counts above $n=70$ were obtained for Louisiana plants indicating they are probably tetraploids. No meiotic irregularities were observed (univalents, multivalents, or lagging chromosomes), even in the suspected hybrids. It is likely that the methods used were inadequate to detect them.

Greenhouse Culture

Greenhouse culture of several plants of D. lonchophyllum and suspected hybrids revealed morphological variation over time and suggested particular features to observe under natural

conditions. In particular, sterile fronds were always less dissected and had larger and wider pinnae than fertile ones. Segments on sterile fronds were more often imbricate versus distant on fertile fronds. A single plant often had mature fronds both with and without stalked pinnules. Sori also varied on a single plant, the length and number of sori per segment differing on different fronds. Cultural conditions effected growth. These plants definitely were intolerant of too much light or lack of humidity, conditions which resulted in tip and margin burning, pale green color, and brittleness. In conjunction with this, frond color and aspect or orientation and rachis succulence may be useful in the field. One plant of D. lonchophyllum 1129a was root prolific. This has not been reported before in this group.

Summary

Although major emphasis was placed on numerical morphological studies and gametophyte studies, surveys were conducted in SEM spore ornamentation, spore lengths, stomatal patterns, cytology, and flavonoid chemistry. Limited results were obtained. However, the larger spore and stomate size of D. werckleanum and the larger spore size of D. cristatum may correlate with their tetraploid cytologies. Several other taxa have been reported at the 4X level without corresponding large spores or stomates so any conclusions need to await further studies.

CHAPTER V

TAXONOMY*

Diplazium Swartz, J. Bot. (Schrader) 1800(2)61 "1801" [1802].

Key to the Species of the Diplazium lonchophyllum Complex

- a. Basal pinnae entire to simply lobed for less than half their length, no free pinnules present.
 - b. Apex length approximately 1/2 that of frond lamina; 8 or fewer pinna pairs; basal petiolules less than 0.40 cm. long. 1. D. cristatum
 - b. Apex length less than 1/3 of frond lamina length; more than 8 pinna pairs; basal petiolules more than 0.35 cm.
 - c. Stipe less than 3/4 as long as blade; basal pinnae simply lobed; stipe base scales absent. 2. D. tablazianum
 - c. Stipe 3/4 to as long as blade; basal pinnae entire to pinnatifid; stipe base scales abundant.
 - d. Pinnae less than 3.0 cm. wide, entire or shallowly lobed, 4-6 times longer than broad. 3. D. werckleanum
 - d. Pinnae more than 3.0 cm. wide, lobed to pinnatifid, 3-4 times longer than broad. 4. D. arboreum
- a. Basal pinnae lobed for more than 1/2 their length to pinnate or bipinnate; free pinnules present or not.

*A definitive type study has not been done. The names used here are those generally in use except where noted. It is realized that an in-depth type study involving the whole genus needs to be performed before names in this complex can be stabilized. In addition, species descriptions are based on specimens used in the numerical analysis.

- e. Less than 11 pinnae pairs; median pinna base angle wider than 110° ; frond apex as long as wide 4. D. arboreum
- e. 11 or more pinnae pairs; median pinna base angle less than 110° ; frond apex at least 1-1/4 times as long as wide.
 - f. Proximal pinnules on basal pinnae stalked, basal pinnae pinnate or bipinnate for at least 1/3 their length; frond apex less than 1/6 the blade length 5. D. franconis
 - f. Proximal pinnules or segments on basal pinnae usually not stalked; basal pinnae pinnate for less than 1/3 their length; frond apex occupying approximately 1/6 - 2/5 of blade length.
 - g. Pinnules distinctly falcate, oblique, acute, always broadly attached to costules, never stalked or free; pinna margins parallel to costae for most of their length. 6. D. drepanolobium
 - g. Pinnules not falcate, usually straight, obtuse, often stalked or free (at least on basal pinnae).
 - h. Stipe scales present: petiolules mostly less than 0.7 cm. long. 7. D. lonchophyllum
 - h. Stipes scales absent; petiolules mostly more than 0.5 cm. long. 8. D. subsilvaticum
- 1. D. cristatum (Desr.) Alston, J. Bot. Brit. & For. 74:173.

1936.

Rhizome erect; fronds 42-62 cm. long, with stipe 2/3 to nearly as long as blade, abundantly dark-brown scaly at base; pinna bases subequilateral, more strongly produced acroscopically, (3)5-8 pairs; basal petiolules 0.2-0.4 cm. long; basal pinnae 2.0-3.0 cm. wide and 7.0-12.0 cm. long, mostly 3-4 times longer than broad, shallowly lobed to pinnatifid, lobes mostly obtuse

0.4-0.7 cm. long and up to 1.5 times longer than broad, no free pinnules; 16-23 costules; median pinnae base angle 90-97°; sori on median pinnae curved or not, in one or several series, 0.5-0.9 cm. long; frond apex spreading 12-15 cm. long, approximately 1-1.5 times longer than broad, occupying 1/2 of the frond lamina, basal segments of apex diverging from rachis at 60-68°.

DISTRIBUTION: West Indies. In wet forests and shaded ravines, 20-700 m.

COMMENTS: The circumscription of D. cristatum and D. arboreum has been tenuous historically. Modern authors synonymize D. arboreum (Willd.) Presl. (based on a Venezuelan type) under D. cristatum (Desr.) Alston (based on a type from Martinique). No one has proposed a distinct West Indies taxon. However, this study has shown that the West Indies material is a well-defined taxon, so the separation of the two taxa and the resurrection of D. arboreum is necessary. Diplazium cristatum in the current sense is restricted to the West Indies. It differs from D. arboreum primarily in frond length, number of pinnae, petiolule length, median pinna base angle, degree of dissection, number of costules, and apex proportions. The species as a whole remains clearly distinct from other taxa in the complex. Diplazium cristatum may represent an isolated group undergoing speciation. It would be interesting to study D. cristatum and D. arboreum in Cuba and Trinidad where they both occur and to conduct further cytological studies to see if D. cristatum and D. arboreum are

consistently 4X and 2X, respectively, as reported by Mickel et al. (1966), Walker (1966), and Smith and Mickel (1977).

2. D. tablazianum Christ, Bull. Herb. Boiss. II, 7:270. 1907.

Rhizome erect to sub-erect, slender; fronds 53-66 cm. long, with stipe $1/2$ - $3/4$ as long as blade, stipe base essentially glabrous; pinna bases unequilateral, more strongly produced acroscopically, 11-16 pairs; basal petiolules 0.3-0.7 cm. long; basal pinnae 2.0-3.0 cm. wide, 6.0-9.0 cm. long, 2.5-5 times longer than broad, shallowly lobed, lobes obtuse 0.2-0.7 cm. long and 0.5-1.5 times as long as broad, without free pinnules, 12-18 costules; median pinnae base angle 85-125°; sori on median pinnae in one to several series along costae or costules, 0.3-0.8 cm. long; frond apex 7.0-10.0 cm. long, 1.5-3 times longer than broad, occupying about $1/4$ of the frond lamina, basal segments diverging from rachis at 41-64°.

DISTRIBUTION: Costa Rica and Panama, Honduras. In wet shaded ravines and forests, (50)500-2000 m.

COMMENTS: Diplazium tablazianum remains distinct based on this study despite its similarities to D. subsilvaticum. Differing from the latter in being smaller and less dissected, with further ecological study D. tablazianum may prove to be an immature or less vigorous form of D. subsilvaticum. Contrary to other reports of a wide distribution throughout Mexico and Central America, in this investigation the two taxa were recorded only from Costa Rica and Panama and at the same elevation, and one atypical specimen of D. tablazianum was seen from Honduras.

3. D. werckleanum Christ, Bull. Herb. Boiss. II, 4:969. 1904.

Rhizome erect; fronds 58-88(100) cm. long, with stipe mostly as long as blade, abundantly dark-brownish scaly at base; blade simply pinnate to pinnate and barely lobed; pinnae 7-15 pairs, strongly excavate basiscopically; basal petiolules 0.4-0.9 cm. long; basal pinnae 1.7-2.8 cm. wide, 7.0-12.0 cm. long, mostly 5 times longer than broad, 18-27 costules; leaf tissue thick, subcoriaceous, light green below and dark green above; median pinnae base angle 75-100° wide; sori on median pinnae 0.6-1.2 cm. long, curved along veins in one to several series; frond apex rather abruptly pinnatifid with broadly triangular segments, 7.0-15.0 cm. long, 1.5-2.5 times longer than broad occupying about 1/4 of the frond lamina, basal segments of apex diverging from rachis at 46-73°.

DISTRIBUTION: Southern Mexico through Panama. In Pre-montane and Lower montane shady, wet forests and ravines, (600) 1050-2000 (2700) m.

COMMENTS: Diplazium werckleanum is the most easily recognized and distinct taxon in the D. lonchophyllum complex because it is the only one with simply pinnate fronds and long petiolules. The frond apex which rather abruptly becomes pinnatifid, is composed of short broadly falcate to triangular segments giving the frond apex a distinctive shape. Diplazium werckleanum may show some lobing of the basal pinnae. Whether this is inherent in its genome or is a result of hybridization is not known.

While its distribution coincides with D. lonchophyllum and D. arboreum, the latter generally occurs at considerably lower elevations so hybridization more likely may occur with D. lonchophyllum.

4. D. arboreum (Willd.) Presl, Tent. Pterid. 114. 1836.

Rhizome erect; fronds 68-80 cm. long, with stipe about 4/5 as long as blade, abundantly dark-brown scaly at base; blade mostly pinnate-pinnatifid; pinna bases subequilateral, more strongly produced acroscopically, 7-10 pairs; basal petiolules 0.4-0.7 cm. long; basal pinnae 3.0-5.0 cm. wide and 14-18 cm. long, 3-4 times longer than broad, pinnatifid, lobes mostly obtuse 1.1-1.5 cm. long and 1-1.5 times longer than broad, rarely with free pinnules, 23-26 costules; median pinna base angle 115-170°; sori on median pinnae curved or not, in one or several series, 0.5-0.6 cm. long; frond apex spreading, 10-16 cm. long, 1-1.5 times longer than broad, occupying 1/4 of the frond lamina, basal segments of apex diverging from rachis at 60-75°.

DISTRIBUTION: Rare in southern Mexico. Common in Central America through South America except Guayana, Uruguay, and Chile. In wet forests and shaded ravines, 20-700 m.

COMMENTS: Modern taxonomists synonymize D. arboreum under D. cristatum. As discussed under D. cristatum, they consider D. cristatum sensu lato to be one species ranging from Central America and the West Indies through South America. This study

has shown though, that the West Indies and Central American material are distinct. Based on type localities, D. cristatum should be applied to the West Indies taxon, and D. arboreum to the remainder.

Distinguishing between D. arboreum and D. lonchophyllum has traditionally been problematic. However, the shape and size of its apex, number of pinna pairs, and degree of dissection adequately differentiate D. arboreum from D. lonchophyllum. Since this study has shown that D. arboreum, rarely if ever, occurs in Mexico, shallowly lobed Mexican specimens are probably D. lonchophyllum.

5. D. franconis Liebm., Kongel. Danske Vidensk. Selsk. Skr., Naturvidensk. Afd. (5)1:256. 1849.

Rhizome robust, erect; fronds (61)89-123 cm. long, with stipe 0.5-1.5 times as long as lamina, stipe base abundantly covered with light brown chartaceous scales; blade bipinnate to tripinnate; (10)13-21 pinna pairs; basal petiolules 0.3-1.5 cm. long; basal pinnae 5.0-12.5(15.0) cm. wide, 12-27(32) cm. long, 2-3 times longer than broad, pinnate to bipinnate, ultimate segments obtuse or acute, pinnules 2.2-6.3 cm. long, 2.5-5 times longer than broad, pinnules mostly stalked, 19-27(34) costules; median pinna base angle (78)90-128(137)°; sori on median pinnae straight, along costules, 0.2-0.5 cm. long; frond apex 2.0-8.0 cm. long, 1.5-3 times longer than broad, occupying less than 1/6 of the lamina, basal segments diverging from rachis at 36-70°.

DISTRIBUTION: Southern Mexico through Costa Rica. In wet forests and deeply shaded ravines, sometimes over limestone, (50)1000-1500 m.

COMMENTS: Diplazium franconis is the most dissected and has the longest, widest fronds of any members of this complex. In this treatment, D. franconis includes D. donnell-smithii Christ, a strictly tri-pinnate form. Further study may show the two to be distinct entities. While several specimens were observed to be intermediate between D. franconis and D. drepanolobium or D. lonchophyllum, typical D. franconis is distinguishable from D. drepanolobium by having stalked pinnules, generally straight, obtuse or acute, not falcate, pinnules, and a shorter frond apex, and from D. lonchophyllum in overall size, dissection, a tendency for lobing of pinnules, and in having a shorter frond apex.

6. D. drepanolobium A. R. Smith, Amer. Fern J. 70:21. 1980.

Rhizome erect, fronds 90-133 cm. long, with stipe 1/2 to as long as blade, stipe base with dark brown scales; blade becoming bipinnate; pinna 14-18 pairs, bases inequilateral, more strongly produced acroscopically; basal petiolules 0.3-0.7 cm. long; basal pinnae 2.8-5.5 cm. wide, 10.0-22.0 cm. long, 3-5 times longer than broad, pinnatifid to pinnate, lobes strongly oblique, falcate, and acute, 1.3-3.0 cm. long, 2-4 times longer than broad, pinnules always adnate to midrib, 22-27(35) costules; median pinna base angle 90-108°; sori on median pinnae pinnate, straight, along

costules, 0.3-0.6 cm. long; frond apex long attenuate, 8.0-13.0 (17.0) cm. long, 1.5-3 times longer than broad and occupying less than 1/4 of lamina; basal segments diverging from rachis at 30-73°.

DISTRIBUTION: Southern Mexico. Lower montane and Montane rain forests, seasonal Evergreen forest, sometimes over limestone, 200-2500 m.

COMMENTS: Diplazium drepanolobium appears to be a well-defined taxon of restricted occurrence in five states of Mexico. Similar to D. lonchophyllum in dissection, its larger size, acutely falcate, oblique pinnules, and their adnate, broad attachment to the pinna midrib serve to separate the two taxa. In addition, the acroscopic and basispic pinna margins run parallel to the pinna midrib for most of their length in D. drepanolobium. This feature is not so obvious in any other taxon.

7. D. lonchophyllum Kunze, Linnaea 13:141. 1839.

Rhizome erect, fronds 41-99 cm. long, with stipe 1/4 to as long as blade, stipe base with light brown scales; blade pinnate-pinnatifid to bipinnate; pinna (9)11-15(21) pairs, bases inequilateral, more strongly produced acroscopically; basal petiolules 0.3-0.7(0.9) cm. long; basal pinnae 2.0-6.0(8.5) cm. wide, 7.0-20.0 cm. long, 2-5 times longer than broad, pinnatifid to pinnate, lobes obtuse or acute, 0.6-3.0(3.6) cm. long, 1-4 times as long as wide, pinnules on basal pinnae often free, (13)15-26 costules; median pinna base angle (70)90-115°; sori

on median pinnae pinnate in several series along costules, $\pm$ straight 0.2-0.6(0.8) cm. long; frond apex 6.0-13.0(17.0) cm. long, 1.5-3 times longer than broad, occupying 1/10-1/4 of the lamina, basal segments diverging from rachis 40-68°.

DISTRIBUTION: Disjunct on Weeks Island, Iberia Parish, Louisiana. Common in Mexico through Nicaragua, rare in Costa Rica and Panama. Common in wet, shaded slopes and ravines, (0)600-1660 m.

COMMENTS: Although there is some overlap geographically and altitudinally with D. franconis, D. werckleanum, and D. drepanolobium, there are good characters by which to differentiate D. lonchophyllum from them. Diplazium lonchophyllum is more dissected, larger, its stipe is proportionately shorter, petiolules shorter, proximal basiscopic basal pinna vein angle wider, and apex segments less spreading than D. werckleanum. It differs from D. drepanolobium in lacking the obviously sharp falcate pinnules and by usually having stalked or at least narrowly attached proximal pinnules on the basal pinnae. Diplazium franconis is more dissected, larger, has wider pinnae, longer petiolules, and a shorter apex. Robust D. lonchophyllum and immature D. franconis may be difficult to identify. The apex length, shape, and size in relation to the lamina length may help but this group could use more work. Specimens from Louisiana fall well within the range of variation for D. lonchophyllum and deserve no special merit. The established populations of D. lonchophyllum in Louisiana are most likely the

result of long distance spore dispersal by hurricanes from the south (Reese and Thieret, 1966; R. M. Tryon, 1970).

8. D. subsilvaticum Christ in Pitt. Prim. Fl. Costar. 3(1):28. 1901.

Rhizome erect to sub-erect, slender; fronds 63-104 cm. long, with stipe 1/4 to as long as blade, stipe base essentially glabrous; blade pinnate-pinnatifid to bipinnate; pinna 11-15(21) pairs, bases inequilateral, more strongly produced acroscopically; basal petiolules 0.3-0.7(0.9) cm. long, basal pinnae 2.4-4.0 cm. wide, 7.0-13.0 cm. long, 2.5-4.5 times longer than broad, lobed to partially pinnate, lobes obtuse 0.8-1.5(2.0) cm. long and up to twice as long as wide, proximal acroscopic pinnules sessile or stalked, (14)17-20 costules; median pinna base angle about 95°; sori on median pinnae in several series, ± straight, 0.2-0.9 cm. long; frond apex long attenuate, (6)8-11(13) cm. long, about 1.5-3 times longer than broad, occupying less than 1/4 of the lamina, basal segments diverging from rachis at 37-57°.

DISTRIBUTION: Costa Rica and Panama (Guatemala). In wet, shaded ravines and forests, 500-2000 m.

COMMENTS: This taxon is similar to D. lonchophyllum in a number of features but its lack of stipe base scales, longer petiolules, and proportionately wider pinnules serve to separate the two. In addition, D. subsilvaticum is restricted to Costa Rica and Panama where D. lonchophyllum rarely occurs. Maxon (1909) described a scaleless species, D. prominulum, from Guatemala.

While the type description and type do not fit exactly anything in this study, it is probable that D. prominulum is referable to D. subsilvaticum. However, in this study, no specimens of D. subsilvaticum were seen north of Costa Rica other than the type of D. prominulum.

CHAPTER VI

CONCLUSIONS

The objectives of the present study were to delimit reliably D. lonchophyllum from other members of the D. lonchophyllum complex, to assess the taxonomic status of disjunct D. lonchophyllum plants in Louisiana, and to provide a foundation for further biosystematic studies in this group. Morphological data analyzed by numerical methods and data from gametophytes, spores, stomates, cytology, distribution, and greenhouse culture of living plants were used in assessing taxonomic relationships. The results indicate that eight species are present in the D. lonchophyllum complex and that D. lonchophyllum is a distinct species.

Analysis of morphology using numerical methods suggests that eight morphological groups are present. The results of cluster analysis and PCA using the first three principal axes show fairly good separation between the eight taxa. Factor analysis revealed that the characters important in separating most of the groups are stable morphological characters. The eight groups are considered species groups because of this in conjunction with consistent group composition in cluster analysis and PCA. However, further study may suggest revision of the taxonomic status of D. tablazianum and D. subsilvaticum (considered distinct species here). This study has resulted in the splitting of D. cristatum

sensu lato into two species, D. cristatum sensu stricto and D. arboreum.

The taxa in the D. lonchophyllum complex can be distinguished by a combination of morphological characters: overall size and degree of dissection, number of pinna pairs, characters of the frond apex, presence or absence of stipe base scales, and petiolule length. The latter four characters have not been used previously to distinguish these taxa.

Diplazium lonchophyllum exhibits features which are intermediate in the group yet in combination, are distinct. The species' center of distribution seems to be southern Mexico through Guatemala, and it is rare in Costa Rica and Panama.

Data from gametophyte studies showed that a female to hermaphroditic pattern is common to all samples except one which was initially male. One sample exhibited a positive response to antheridiogen. Three collections of D. lonchophyllum had distinctive gametophyte shapes. All samples displayed considerable intra- and inter-populational variability. Caution is emphasized in placing any significance on these results because of the great amount of variation observed from single spore sources and limited species available as spore sources.

The remaining observations made on spore lengths, spore morphology using SEM, stomate patterns, frond flavonoid chemistry, cytology, and greenhouse culture of plants, yielded few good intraspecific characteristics or conclusive results. The spores

of D. werckleanum were largest and had sharp, abrupt perine ridges as opposed to loosely folded and anastomosing rugulae in the other taxa. All taxa had similar anomocytic to polocytic stomate patterns except D. werckleanum which had mostly polocytic stomates. Flavonoids were not detected. Cytology revealed only that the Louisiana plants are probably tetraploid.

While the preliminary nature of this study cannot be over-emphasized, the study is useful in providing a basis for further biosystematic inquiry in this group. The results have shown specifically that: separation of taxa can be facilitated using several features in addition to frond dissection and size; plants from Louisiana are good D. lonchophyllum; Diplazium lonchophyllum can easily be distinguished from D. arboreum and D. subsilvaticum, two problem areas previously, and; species distributions are more limited than earlier thought. Groups that especially need further work are the species pairs D. cristatum and D. arboreum in their areas of distributional overlap, D. tablazianum and D. subsilvaticum, and D. lonchophyllum and D. franconis. In order to understand the biological relationships between members of the complex and to understand the nature of phenotypic variation, morphological work must be supplemented with field observations, controlled environment growth studies, hybridization and cytology studies, and more chemical investigation. These plants as they occur in nature will likely provide us with more answers than they ever will as herbarium specimens.

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APPENDIX

APPENDIX A

SPECIMENS EXAMINED IN NUMERICAL ANALYSES*

D. arboreum

BELIZE: Toledo; 28/8. Gentle 6397(US).

COSTA RICA: Cartago; 22/2. Maxon 183(US), 30/10. Weber 6166(US).

HONDURAS: Atlántida; 29/9. Standley 54206(US); Santa Barbara; 23/3. Thieme 5682(US).

PANAMA: Canal Zone; 24/4. Killip 2805(US), 26/6. Nee 9458(MO); Chiriqui; 25/5 Croat 21986(US).

WEST INDIES: Cuba; 27/7. Palmer, Riley 228(US), 31/11. Maxon 4121(US).

D. cristatum

WEST INDIES: Dominican Republic; 32/12. Miller 1203(US), 35/15. Ekman 10570(US), 37/17. Türckheim 2723(US), 38/18. Abbott 2634(US), 39/19. Lellinger 449(US); Guadeloupe; 34/14. Proctor 20116(US); Jamaica; 36/16. Maxon 9331(US), 40/20. Maxon 9056(US); Trinidad; 33/13. Fendler 13(US).

D. drepanolobium

MEXICO: Chiapas; 71. Breedlove, Thorne 20760(CAS), 72.

*Number before slash corresponds to OTU number in analysis of 150 OTU set, number after slash corresponds to OTUs in 100 OTU set. If no slash, then specimen was used only in 150 OTU set.

Breedlove 38676(CAS), 78. Breedlove, Smith 22451(F), 79. Breedlove 32852(CAS), 80. Breedlove 24163(CAS); Mexico; 122. Hinton 4225(US); Morelos; 120. Lyonnet 3140(US); Oaxaca; 73. Wendt et al. 2761(US); Tabasco; 74. Cowan 1725(CAS), 75. Cowan 3131(CAS), 76. Cowan 3162(CAS), 77. Cowan 2904(CAS).

D. franconis

COSTA RICA: Heredia; 150. Stolze 1576(US); San José; 111. Tonduz 17570(F).

EL SALVADOR: Chalatenango; 144. Seiler 380(F); San Salvador; 143. Seiler 336(F); 145. Seiler 825(F).

GUATEMALA: Alta Verapaz; 139. Standley 90314(F); Chimaltenango; 137. Standley 62040(F); Izabal; 141. Steyermark 39147(F); Quezaltenango; 138. Standley 85673(F); San Marcos; 140. Steyermark 37675(F); Solola; 142. Williams et al. 23179(F).

HONDURAS: Intibuca; 146. Molina 6289(F).

MEXICO: Chiapas; 131. Breedlove, Thorne 21415(CAS), 132. Breedlove, Smith 22598(NY); Mexico; 134. Hinton 2768(US); Oaxaca; 116. Morton, Makrinius 2388(F), 133. Hallberg 1560(NY); San Luis Potosi; 98. Pennell 17969(US), 115. Pringle 3958(US); Vera Cruz; 135. Ventura 266(F), 136. Ventura 3271(NY).

NICARAGUA: Madriz; 147. Williams, Molina 20255(F); Matagalpa; 148. Williams et al. 23841(F), 149. Williams et al. 23847(F).

D. lonchophyllum

COSTA RICA: Cartago; 130/100. Williams 16139(US); San José; 109/79. Maxon 133(US), 110/80. Brenes 21411(F).

EL SALVADOR: Ahuachapan; 103/73. Standley 20016(US); San Salvador; 101/71. Seiler 452(F), 102/72. Seiler 1086(F), 128/98. Seiler 218(NY).

GUATEMALA: Amatitlan; 125/95. Smith 2724(US); Escuintla; 100/70. Standley 89582a(US); Guatemala; 126/96. Smith 2445(US); Sacatepéquez; 99/69. Standley 81022(F); Santa Rosa; 127/97. Standley 79142(US).

HONDURAS: Comayagua; 104/74. Williams, Molina 12526(F), 105/75. Williams 13682(F).

MEXICO: Chiapas; 89/59. Breedlove, Smith 22138(F), 118/88. Breedlove 25231(CAS), 123/93. Breedlove 25106(CAS), 124/94. Breedlove, Smith 21624(CAS); Guerrero; 90/60. Hinton 14048(MO); Hidalgo; 91/61. Sanchez 624(US); Jalisco; 92/62. Pringle 2025(CAS); Michoan; 121/91. Hinton et al. 16258(US); Morelos; 93/63. Lyonnet 1345(US); Nayarit; 94/64. Jones 23478(MO), 119/89. Palmer 1943(US); Nuevo Leon; 95/65. Lacás 432(F), 117/87. Leavenworth 92(F); Oaxaca; 96/66. Hallberg 1396(NY); Veracruz; 97/67. Smith 2189(MO), 114/84. Mickel 742(NY).

NICARAGUA: Bluefields; 21/1. Proctor et al. 27255(NY); Granada; 129/99. Atwood A304(MSC); Monagua; 106/76. Standley 8341(F); Masaya; 107/77. Standley 8716(F), 108/78. Standley 8717(US).

UNITED STATES: Louisiana, Iberia Parish; Avery Island; 113/83. Correll 9491(US); Weeks Island; 81/51. Sessions 5(TENN), 82/52. Sessions 10(TENN), 83/53. Sessions 17(TENN), 84/54. Sessions 43(TENN), 85/55. Sessions 44(TENN), 86/56. Sessions 45(TENN), 87/57. Sessions 46(TENN), 88/58 Sessions 48(TENN), 112/82. Sessions 47(TENN).

D. subsilvaticum

COSTA RICA: Alajuela; 50/30. Stolze 1465(US), 69/49. Burger, Stolze 4974(F), 70/50. Lellinger, White 1642(US); Alajuela, Puntarenas, and Guanacaste; 43/23. Dryer 236(F), 44/24. Dryer 365(F); Cartago; 56/36. Williams 19744(F); Puntarenas; 68/48. Mickel 3063(NY); San Jose; 53/33. Stolze 1427(F), 66/46. Skutch 4735(MO), 67/47. Burger, Stolze 4880(F).

PANAMA: Chiriqui; 58/38. Duke et al. 13639(MO), 59/39. Croat 13588(F), 60/40. Croat 10562(MO), 62/42. Cornman 920(MO), 63/43. McDaniel 10160(TENN), 64/44. Seibert 249(MO); Darien; 61/41. Duke, Elias E13695A(MO).

D. tablazianum

COSTA RICA: Alajuela; 41/21. Standley, Torres 47821(US), 54/34. Lellinger, White 1638(US); Cartago; 45/25. Armond 159(CAS), 46/26. Standley, Torres 51188(US), 49/29. Lent 660(F), Heredia; 42/22. Standley, Valerio 49242(US), 51/31. Levin 16(F); Limon; 57/37. Burger, Antonio 10910(F); Puntarenas; 47/27. Burger, Gentry 8681(F); San Jose; 48/28. Scamman, Holdridge 7982(US), 52/32. Scamann 7671(US).

PANAMA: Chiriqui; 55/35. Croat 15803(MO), 65/45. Killip 5038(US).

D. werckleanum

COSTA RICA: Heredia; 16. Levin 32(F), 18. Lellinger, White 1691(US), 17. Smith 22(F).

EL SALVADOR: Santa Ana; 7. Seiler 275(F), 8. Seiler 623(F), 9. Seiler 952(F).

GUATEMALA: Chiquimula; 5. Steyermark 31695(F); El Quiche; 4. Molina 30301(F); Jalapa; 6. Steyermark 32331(F).

HONDURAS: Morazan; 10. Williams, Molina 14830(US), 11. Molina et al. 17018(F), 12. Hawkes et al. 2019(F).

MEXICO: Chiapas; 1. Purpus 352(US), 2. Breedlove 34693(MO), 3. Breedlove 35217(MO).

NICARAGUA: Esteli; 13. Atwood A135(MSC); Jinotega; 14. Standley 10693(F); Matagalpa; 15. Williams et al. 24973(F).

PANAMA: Chiriqui; 19. Dwyer et al. 557(MO), 20. Cornman 1061(US).

VITA

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