

**BIOSYSTEMATIC ANALYSIS OF *ADIANTUM VIVESII* PROCTOR  
(POLYPODIACEAE: ADIANTOIDEAE), A RARE FERN ENDEMIC TO  
NORTHWESTERN PUERTO RICO**

by  
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## Abstract

*Adiantum vivesii* Proctor is a rare fern endemic to the northwestern part of Puerto Rico, known only from its type locality on limestone substrate on privately owned land in the Municipality of Quebradillas. The distribution, abundance, morphology, and cytology of the species were studied to clarify its status as a distinct species or of hybrid or polyploid origin. The population of *A. vivesii* occupies a total area of 21 m x 10 m; eight sympatric species of *Adiantum* are found in the immediate area. Following several deep excavations it was concluded that the entire population of *A. vivesii* is one individual with proliferation by rhizomes. Morphological measurements and light and scanning microscopy were used to describe some parts of *A. vivesii* and *A. tetraphyllum*. Morphometric studies were conducted for 22 characters of the species and the possible parent, *Adiantum tetraphyllum*. Greater variability was obtained within the *A. tetraphyllum* population, and statistically significant differences between the two species were observed for 17 characteristics. Meiotic studies were inconclusive, but no evident difference in chromosome number was observed between the two species. The spores of *A. tetraphyllum* showed normal appearance and were rarely abortive, while those of *A. vivesii* showed greater variability in size and were almost always abortive, suggesting that *A. vivesii* is a sterile hybrid.

## Resumen

*Adiantum vivesii* Proctor es un helecho raro y endémico de la parte noroeste de Puerto Rico, sólo conocido de su localidad tipo en suelos cársicos en terrenos privados del Municipio de Quebradillas. La distribución, abundancia, morfología y citología de la especie fueron estudiados para aclarar el estado de la especie como distinta, un híbrido o de origen poliploide. La población de *A. vivesii* ocupa un área total de 21 m x 10 m; se encuentran ocho especies simpátricas de *Adiantum* en el área inmediata. Después de algunas excavaciones profundas se concluyó que la población de *A. vivesii* consta de un solo individuo con proliferaciones del rizoma. Medidas morfológicas y microscopía de luz y rastreo fueron utilizadas para describir algunas partes de *A. vivesii* y *A. tetraphyllum*. Estudios morfométricos fueron conducidos para 22 caracteres de la especie y del posible progenitor *Adiantum tetraphyllum*. Se obtuvo mayor variabilidad dentro de la población de *A. tetraphyllum*; 17 características fueron encontradas con diferencias estadísticamente significativas entre las especies. Estudios meióticos fueron inconclusos, pero no se observó una diferencia evidente en el número cromosómico entre las dos especies. Las esporas de *A. tetraphyllum* mostraron una apariencia normal y raramente estuvieron abortivos, mientras que las de *Adiantum vivesii* mostraron mayor variabilidad en el tamaño y casi siempre estuvieron abortivas, lo cual sugiere que *A. vivesii* es un híbrido estéril.

## **Dedication**

I dedicate this work first of all to God. To my parents Nefalí and Marina, my sister Marian Talimar and my brother Nefalí for all their endless love and support. Especially in memory of my beloved grandmother María Elena Collazo Rivera (1915-2000).

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## Introduction

Pteridologists have thought that many of the mysteries involving speciation and species concepts in pteridophytes could be addressed only by additional and more intense studies of tropical groups (Haufler, 1996). In fact, evidence indicates that it is in such systems that the most rapid speciation is occurring. Investigations of tropical pteridophytes have suggested that speciation in these plants may differ qualitatively from that in temperate groups (Haufler, 1996).

Probably all named fertile pteridophyte species are morphological species; even when other criteria are employed, morphological discrimination of taxonomic units has been an important component of naming new species. No matter how sophisticated pteridologists become in applying molecular techniques, the practical need to identify plants in the field will demand that they provide morphological criteria for differentiating taxa. Evidence from past research and from new analyses indicates that most pteridophyte species are reproductively isolated units. Fewer data are known on whether populations at opposite ends of the ranges of widespread species retain the capacity to interbreed. Available data have demonstrated that hybrids between congeners are sterile; there are only few cases of fertile F<sub>1</sub>s. Pteridologists know far too little about tropical groups and, through expanded application of biosystematic methods in studying such groups, they may discover that

“biological” species are less numerous than morphological species.

Nevertheless, the evidence to date supports the sterility of hybrids (Haufler, 1996).

Analyses of gametophyte reproductive biology are necessary to develop a complete picture of the biology of fern species as well as to characterize evolutionary tendencies. Analyses of chromosomal behavior at meiosis have been especially important in characterizing suspected hybrid individuals. Blasdell (1963) and Moran (1981) noted the value of spore features for identifying hybrids and determining the ploidy levels of specimens. It may be that tropical species have more specific requirements for successful spore germination and gametophyte maturation than those in temperate zones, and that gametophytes play a more important role in the speciation process in the tropics (Haufler, 1996).

The genus *Adiantum* L. is a large and diverse group of ferns in the Old and New World tropics. Characterized by submarginal sori borne abaxially on recurved lobes of the leaf margin (false indusium), it is one of the most clearly circumscribed genera in the Order Filicales or Polypodiales (Bower, 1928; Tryon, 1964, cited in Paris and Windham, 1988). Proctor puts this genus in the family Polypodiaceae, subfamily Adiantoideae.

*Adiantum* is a large pantropical genus of perhaps nearly 200 species, most numerous in South America, with a few occurring in or extending into temperate regions. In English, many of them are commonly called “maidenhair ferns.” The

generic name *Adiantum* is of Greek origin and means unwetted, referring to the rain-shedding texture of the foliage in many species (Proctor, 1989). The cytology of the genus is fairly consistent, with haploid chromosome numbers being  $n=29, 30$ , or multiples of these. The fossil record of this family is only known definitively from the Eocene.

In Puerto Rico we have a rich and diverse pteridophyte flora. Many of them are well characterized, but in the case of *Adiantum vivesii* Proctor, described by Dr. George R. Proctor in 1985 from specimens collected with Miguel Vives and William Estremera in the San Antonio Ward in the municipality of Quebradillas (Proctor, 1989), we do not know whether it is a distinct species or of hybrid or polyploid origin. This fern is known only from the type locality on limestone substrate in Quebradillas, in northwestern Puerto Rico, where it occurs on privately owned lands. It is endemic to Puerto Rico and has been Federally listed as an endangered species since June 9, 1993 (U.S. Fish and Wildlife Service, 1994).

In his Latin diagnosis of *A. vivesii*, Proctor (1989) distinguishes the species from *A. tetraphyllum* Humb. & Bonpl. ex Willd. based on its fewer pinnae, fewer pinnules on the lateral pinnae, and submedial costa on the pinnules. He believes that *A. tetraphyllum* represents the closest relative of *A. vivesii* (G. R. Proctor, pers. comm.).

Many endangered plant species exist in unique ecological niches, such as those found in bogs, on special soil types, on isolated islands, in mountain ranges, or in valleys. They are rare because they are adapted precisely to their present location and will survive only if their habitat is preserved. Certain types of plants are highly prized by plant fanciers. These particularly include cacti, carnivorous plants, cycads, orchids, ferns, palms, and bromeliads. The professional botanist who is collecting uncommon plants for proper study and understanding needs to exercise caution regarding how many specimens are collected and how the plants are cared for, as well as adhering to laws governing plant collecting (Woodland, 1997).

Destruction or modification of its habitat may be the most significant factor potentially affecting the abundance of this endemic fern. Clearing or development of the area would result in the elimination of the only known population. Also, this species could be an attractive item for collectors. The extreme rarity of this species makes the loss of individual plants even more critical. At the same time, the plant is rhizomatous and it is possible that the entire population consists of only one or a few individuals. The accessibility to the area is difficult; the owner has only allowed the entrance of people who are doing research on the fern. He did not know the exact place to find the population, and it would be very difficult to find it without a guide. Also it is dangerous to go to the area alone because of the cliffs and only people with



experience in the field can get there without problems. Therefore, there is essentially no human impact in the area.

The objectives of this project were the following:

- 1) to determine the distribution and abundance of *Adiantum vivesii*,
- 2) to conduct a morphometric analysis of selected leaves of *Adiantum vivesii* and *Adiantum tetraphyllum* Humb. & Boupl. ex Willd.
- 3) to count the chromosomes of *A. vivesii*,
- 4) to document selected parts of *A. vivesii* and, in some cases, *A. tetraphyllum* using the dissecting microscope and the scanning electron microscope (SEM).
- 5) to determine whether *A. vivesii* makes well formed, viable spores.

The principal goal of this project was to clarify the status of *Adiantum vivesii* as a distinct species or one of hybrid or polyploid origin. In addition, the present study represents the implementation of some tasks specified in the recovery plan for the species (U.S. Fish and Wildlife Service, 1994). Some of those tasks are: to monitor the population, to study the reproductive biology and ecology of the fern, to assess the periodicity of spore production and dispersion, and to assess spore viability and germination requirements.

## Literature Review

### General floristic treatments

In 1960, Scamman revised the maidenhair ferns (*Adiantum*) of Costa Rica; in his article he includes distribution data, a key, and illustrations of the 28 species. A key to all the *Adiantum* species of Central America was done by Seymour (1975). Palacios-Ríos and Riba (1984) made a key to the 20 species of *Adiantum* of Veracruz, including synonymy and brief descriptions. Pteridophytes were included in Britton and Wilson's flora of Puerto Rico and Virgin Islands (Maxon, 1926), and ferns and fern allies of the same region were recently revised by Proctor (1989). He recognizes 408 pteridophyte taxa, among which *Adiantum* includes 15 native species (one with two varieties) and two additional cultivated taxa. He describes each species, including its general and local distribution, habitat, and synonymy. Kepler (1975) published an illustrated guide to the common ferns of the Luquillo Forest. In 1992, Rodríguez published a list of the ferns in the Maricao Forest, and Cedeño-Maldonado (1997) also included pteridophytes in his flora of the Río Maricao drainage. In volume one of Flora Mesoamericana the genus *Adiantum* was treated in three seminatural groups including a total of 36 species. A natural subgeneric classification awaits a worldwide study of the genus (Moran et al., 1995).

Many fern species complexes have been studied. For example, the *Adiantum pedatum* complex has been studied since 1949 by R. Wylie, as well as by Wagner and Boydston (1978), Cody (1983), Paris and Windham (1988), and Rabe and Haufler (1992). Other species complexes that have been studied include the *Cystopteris tennesseensis* complex (Haufler et al., 1990; Paler and Barrington, 1995) and the *Polypodium vulgare* complex (Haufler et al., 1995a, 1995b).

De la Cruz (1998) worked with the fern *Cyathea brooksii* Maxon (Cyatheaceae), apparently restricted in Puerto Rico to serpentine-derived soils within the Maricao Commonwealth Forest. Her study included the distribution, relative abundance, phenology, population ecology, reproductive biology, propagation, and genetic variability of the species.

### **Ecological studies**

As regards studies of *Adiantum*, in addition to those of the *A. pedatum* complex mentioned above, Sharma and Harsh (1992) studied three species of the genus from Rajasthan, in northwestern India. They found that, in addition to ecomorphological variation, the plants also show differences in anatomy and sex expression in the gametophyte.

The distribution patterns of *Adiantum* species at twelve noninundated rain forest sites in Amazonian Peru and Ecuador were studied by Tuomisto-Hanna et al.

(1998). The occurrence and abundance of the species varied among sites with regard to soil texture and the concentration of exchangeable bases and within sites with regard to topography. They concluded that ecological differences among the species clearly explained many of the differences in their distribution patterns at local and regional scales and may be important for the understanding of such patterns at biogeographical scales.

Prado and Palacios-Ríos (1998) discussed the taxonomy and geographical distribution of *Adiantum trapeziforme* L. and *A. pentadactylon* Langsd. & Fisch to establish the distinctness between them. They found that the two species differ morphologically and that confusion between them has resulted from misidentification. They presented a new key to distinguish the species. Also *A. pentadactylon* is an endemic and locally common species in southeastern Brazil while, in contrast, *A. trapeziforme* is a widespread and locally common species from portions of Mexico throughout Mesoamerica, Cuba, Jamaica, the Lesser Antilles to Trinidad, and possibly Venezuela.

### **Morphological studies**

Palmer (1998) used morphological characters to distinguish *Cibotium x heleniae* hyb. nov. from its parents *Cibotium chamissoi* and *Cibotium menziesii*. The characters that he used were: hairs color and distribution on the stipe, and the depth to

costa and angle of the sinuses. He found that plants of *C. x heleniae* are generally intermediate between its known parents. The spores of the hybrid vary in morphology and their fertility also depends on where they were collected. Only in one of the study sites were normal spores collected that germinated and formed gametophytes, but the reason for this variability is not known.

Morphological comparison of parents and potential hybrids and chemosystematic methods are well tested techniques that have been successfully employed to demonstrate hybridity in ferns. While the morphological features of a hybrid usually tend to be intermediate, chemical components often are inherited additively from each parent. Within the ferns, chemical data based on flavonoids have been useful in elucidating the parental origin of hybrids in *Osmunda* and *Asplenium*, and in determining the allopolyploid and autopolyploid origin of two tetraploid chemotypes of *Pityrogramma triangularis* (Conant and Cooper-Driver, 1980).

A morphometric study of hybridization between *Polystichum munitum* and *P. imbricans* was conducted by Mayer (1989) to test for hybridization, because problematic individuals appeared to be intermediate between the two species. Plants were scored for 12 macro- and microscopic characters. Specimens were then grouped according to spore morphology as *munitum*, *imbricans*, or abortive. Plants with abortive spores were morphologically intermediate in most respects, supporting the hybridization hypothesis. Although the two parental species are morphologically

distinct in the mixed populations, they are more similar when sympatric than when allopatric. This convergence could be the result of genetic and/or phenotypic responses to a common environment, or introgression.

Smith (1971) studied the systematics of the neotropical species of *Thelypteris* section *Cyclosorus*, including morphology, chromosome numbers, hybridization, ecology and distribution. He considered morphological characters of the rhizome, stipe and rachis, blade, indumenta, sorus and indusium, spores and sporangia, and gametophyte. Haufler et al. (1990) studied the *Cystopteris tennesseensis* complex, combining morphometric analyses with surveys of chromosomal, isozymic, and gametophytic features to differentiate the allotetraploid *Cystopteris tennesseensis* and its putative diploid progenitors, *C. bulbifera* and *C. protusa*. They found that the isozymic variation in the tetraploid parallels that of the diploids, implying that genetic variability was introduced through recurrent hybridization.

Only in the past quarter of a century have the spores of ferns been used extensively in elucidating systematic relationships. Fern spores display remarkable variation and have been extremely helpful in systematic studies. In many cases particular spore morphologies are distinctive for families or genera, or in some instances individual species. Spore sizes and abortion have been useful in studies of hybridization and polyploidy, and in some cases they have led to the discovery of unknown reproductive variation in ferns. A broad detailed survey of spores is still

needed to increase our understanding of variation patterns and to determine which ones are meaningful taxonomically (Taylor and Mickel, 1974). Blasdell (1963) and Moran (1982, cited in Haufler et al., 1990) noted the value of spore features for identifying hybrids and for determining the ploidy level of specimens of *Cystopteris*. Through analyses of meiotic chromosome behavior, Haufler et al. (1990) were able to provide independent verification for Lovis' (1977) caveat that it may not be appropriate in *Cystopteris* to infer ploidy levels from spore measurements, while also finding that spore size did correlate with several critical genetic features of the species. They agreed with Lovis that spore size comparisons alone do not substitute for direct chromosome analyses, but they found that spores can be extremely valuable in surveying specimens rapidly once strong correlations between spore features and genetic condition are established. In particular, they found that *C. bulbifera* spores were larger than those of *C. protusa*, and spores of tetraploid *C. tennesseensis* showed the further size increase commonly associated with higher ploidy levels.

### **Cytological studies**

Although recent morphological data provide strong evidence for clarifying relationships among members of the *Polypodium virginianum* complex, chromosomal data continue to hold a central position in developing arguments for or against

particular hypotheses about the evolution of *Polypodium* species (Haufler and Wang, 1991).

The significance of chromosomes in the study of pteridophytes starts with Manton's book of 1950. There are still monographs and floras being published in which chromosome numbers may not be listed. If they are listed, the information is sometimes added much as one would characterize a species as either annual or perennial. Stable chromosome numbers such as 29, 37 and 41 in ferns are not quite susceptible to arithmetic manipulations. The most worthwhile attribute of these numbers is to give unity to a genus. The first step in a study of the cytogenetics of a species is the accurate determination of the chromosome number. Unfortunately, this is as far as we have proceeded in many cases, and given the technical difficulties of the material; even this has not been achieved in some cases (Britton, 1974). In leptosporangiate ferns we have a valuable research tool in chromosome counts. Such studies are of particular value in examining hybridization and in supporting genetic and familial relationships (Taylor and Mickel, 1974).

In a series of reviews, Walker (1973, 1984) illustrated the value of chromosomal data in understanding fern phylogeny. Because of the different base numbers in ferns, chromosome counts can be used as evidence for generic and familial circumscription. Within genera, analysis of polyploid series can help to unravel the complexities resulting from reticulate evolutionary events. Finally, the



formation of bivalents during meiosis in sterile hybrids can be used to test hypotheses regarding the genetic similarity of the genomes contained in such hybrids.

### **Hybrid studies**

Many examples of fertile hybrids are known in the ferns, but these usually involve either apogamy or polyploidization (Conant and Cooper-Driver, 1980). Few fertile diploid hybrids with regular reductional meiosis are known. A comprehensive ecological study embracing all aspects of the life cycles of several related species is needed, however, to increase our understanding of the ecological basis of local endemism.

Conant (1983) did a revision of the genus *Alsophila* (Cyatheaceae) in the Americas. His work included the Puerto Rican species, discussing their morphology, cytology, speciation patterns, and hybrids. Conant concluded that it is difficult to determine whether the hybrid plants encountered in the field are F1 or later generation hybrids. Some tend to be rare and intermediate in morphology, suggesting they belong to the first generation.

## Materials and Methods

### Study area

*Adiantum vivesii* Proctor is only found on privately owned land located on the north side of a ridge north of Puerto Rico Hwy. 119 at km.16.1 (18°24'13"N. Lat., 66°54'17"W. Long.) in Barrio San Antonio of the Municipality of Quebradillas. The elevation of the general area ranges from near sea level up to 198 m. *Adiantum vivesii* is found in the limestone or karst region of northwestern Puerto Rico. This region is underlain by limestone rocks of Oligocene or Miocene age. Topography varies throughout the karst region, from extremely rugged to gentle rolling hills. Canyons, sinkholes, and subterranean rivers, as well as these rolling hills, are the most common features of the region. Soils in the limestone hills are generally shallow, well drained, alkaline, and interspersed between limestone outcrops (Soil and Conservation Service, 1975 cited in U. S. Fish and Wildlife, 1994).

The population occurs within semi-evergreen seasonal forest in the subtropical moist forest life zone (Ewel and Whitmore, 1973). This life zone, which covers 58% of the total area in Puerto Rico and the U.S. Virgin Islands, is delineated by a mean annual rainfall ranging from 1000 - 1100 mm to 2000 - 2200 mm and a mean temperature between 18°C and 24°C (Ewel and Whitmore, 1973). *Adiantum vivesii*

occurs in a deeply shaded hollow at the base of a limestone hill in Quebradillas. The ownership of the land is Mr. Ángel Rivera; he knows about the presence of *Adiantum vivesii* in his property and he is willing to cooperate with Puerto Rican government agencies and the U. S. Fish and Wildlife Service to protect this endemic fern.

### **Distribution and abundance**

The population was located precisely on a topographic map (fig. 1). The extent of the population was measured at the beginning of the study using a metric tape measure. The measurement was repeated at the end of the study to identify any changes. Careful excavations were performed around several apparent individuals of *Adiantum vivesii* and *A. tetraphyllum* to determine the extent of connections among them. Two small (ca. 10 cm) pieces of the rhizome were planted in soil from the area to see whether they could form new plants.

Voucher specimens of the *A. vivesii* and *A. tetraphyllum* were deposited at the MAPR herbarium of the University of Puerto Rico, Mayagüez Campus. Other associated species were identified by Mr. Miguel A. Vives, but voucher specimens were not collected. A comparison was made of the characteristics of sympatric *Adiantum* species, based on Proctor (1989).

## **Morphometric analysis**

Specimens were collected of haphazardly selected plants of *Adiantum vivesii* and of *A. tetraphyllum*, which may represent its parent. Dried leaf material was used to conduct a morphometric analysis. Twenty-one parameters were studied for each of twenty blades of each species (table 1; fig.2). Spore measurements were also obtained for *A. vivesii* and *A. tetraphyllum*, as described below.

The data obtained for the 22 characters for the two species studied were analyzed with the MINITAB statistical software package (version 13.20 for Windows) using one-way ANOVA.

## **Chromosome counts**

Fertile material of *Adiantum vivesii* and *A. tetraphyllum* was field-fixed following the standard protocols for chromosome preparations as modified by Haufler and Wang (1991). Pinnae were removed from sporophytes and fixed in Farmer's solution (a 3:1 mixture of 100% ethanol and glacial acetic acid) for at least 24 hours under refrigeration. Groups of fixed sporangia were removed from the sori and placed on a glass slide in a drop of saturated acetocarmine solution (carmine boiled in 45% acetic acid and filtered to remove undissolved crystals); then they were teased apart using steel dissecting needles. The slides were heated with an alcohol burner, then

wrapped in a Kimwipe®, and thumb pressure was applied. The preparation was scanned for cells at late diakinesis or early metaphase of meiosis. To improve the preparation for the purposes of photographic documentation, the selected cells were marked and the slide was supported by glass rods while inverted in a 45% acetic acid solution. After the coverslip floated off, extraneous material was carefully removed, an additional drop of stain was applied, the coverslip was replaced, and the preparation was again squashed. The second squash usually puts all chromosomes in a single plane and separates them maximally from each other (Haufler and Wang, 1991). Photographs were taken with color film (ASA 400) using an Olympus BHS microscope and an Olympus PM-C35B photomicrography system. The photographs were scanned in a Microtek E-3 Plus scanner, and processed using Adobe Photoshop 5.0 and Adobe Page Maker 6.5 programs and printed on high quality glossy paper on an Epson Stylus Photo printer.

### **Light microscopy**

Fresh or dried pinnules, sori, and sporangia of *Adiantum vivesii* and *A. tetraphyllum* were observed under the the SZ-PT Olympus dissecting microscope at magnifications of 100x to 630x. Photographs were taken, scanned, processed, and printed as described in the previous section.

Under the dissecting microscope, one frond of each species was examined for areas where there were mature or well developed sporangia. A drop of Permuto<sup>®</sup> was placed in the center of a slide and the point of a dissecting needle was dipped into the solution to wet the tip in order to provide a “glue” for picking up the spores. The spores were spread on the slide; this procedure was repeated several times to accumulate a hundred or more spores in the drop. The spores were covered by slowly lowering a cover glass over them. Spore morphology was observed and spore size was measured at 400x using a calibrated ocular micrometer and an Olympus BHS microscope. The total length (including the perispore) of fifty spores of each species was measured.

### **Scanning electron microscopy (SEM)**

Rhizomes and fertile pinnules of *A. vivesii* were cleaned to remove surface contaminants, cut into small pieces and fixed in FAA or Farmer’s solution for at least 4 hours. The fixative was discarded and two rinses with 70% ethanol were done. The samples were dehydrated in an ascending ethanol series (75%, 85%, 95% for 10 minutes each, and 100% , 3 changes for 10 minutes each). The dehydrated specimens were desiccated by transferring them to the prechilled critical point dryer chamber (EMS 850 System) after quickly blotting off most of the alcohol. The specimens were mounted onto aluminum stubs with double sticky tape. The samples were

quickly transferred to a clean desiccator, which was tightly closed and stored at least for two hours.

For spores of *A. vivesii* and *A. tetraphyllum*, a different procedure was required in preparation for SEM studies. Spores were collected by allowing sporangia on fresh leaves to dehisce in clean newspaper overnight. The spores were then submitted to an acetolysis procedure, which were conducted under a fume hood. Mature spores of *Adiantum vivesii* and *A. tetraphyllum* were placed in 1.5 ml microtest tubes, which were placed inside a 100 ml beaker with ice water to reduce the rate of acetolysis. (The acetolysis mixture is violently reactive to water, and the reaction is highly exothermic.) Three drops of concentrated sulfuric acid and 27 drops of acetic anhydride were added to the microtest tubes containing the spores. Only acetolysis-resistant spore walls should persist, and it is inferred that these are chemically composed of the substance called sporopollenin (Zetsche, 1932, cited in Pérez-Muñoz, 1991). Glacial acetic acid (0.75 ml) was added to the tube, which was centrifuged for ten minutes, the time required for pellet formation. The pellet was washed twice with glacial acetic acid and then with distilled water. Spores were transferred to an aluminum stub with double sticky tape and air-dried.

All samples, including rhizomes, fertile pinnules, and spores were coated with gold/palladium in a sputter-coater (Bio-Rad, model E-5000), and examined under the JEOL 5410 LV Scanning Microscope to see whether all studied parts were well

formed. Photographs were taken with a Polaroid 4" x 5" camera with Polaroid 55 film. The photographs were scanned, processed and printed as described previously.



## Results

### Distribution and abundance

*Adiantum vivesii* is restricted to a single location in Quebradillas. The extent of the population at the beginning of the study ( March, 1999) was 17 m x 9 m; its extent at the end of the study (March, 2000) was 21 m x 10 m. Seven or eight deep excavations were made at different points throughout the entire population, and in most of them rhizome connections between the apparent individuals were found (fig. 3A). One rhizome was photographed; it was about half a meter in length (fig. 3B). The excavations made to the population of *A. tetraphyllum* showed that each apparent plant was a single plant. Two 10 cm rhizome segments were collected, one in March 1999 and in March 2000. They were planted in soil from the area, and grew into healthy plants within about three months that have survived to the present (fig. 3C-D). Small numbers of fiddleheads and young blades were observed on each visit to the study site. The production of sporangia was seen throughout the year. On all visits, attempts were made to find fern gametophytes, but none were seen.

On one visit, in March of 1999, Miguel A. Vives prepared a list of pteridophytes and flowering plants that were growing in the immediate area of *A. vivesii* (table 2). In total, 25 species of pteridophytes and 74 species of spermatophytes were found near the population. Eight species of the genus *Adiantum*

were found in the vicinity. They were *A. cristatum*, *A. fragile*, *A. latifolium*, *A. melanoleucum*, *A. pulverulentum*, *A. tenerum*, *A. tetraphyllum* and *A. wilsonii*. Of these, only *A. tetraphyllum* was growing within the area occupied by *A. vivesii*. The characteristics of *A. vivesii* and the eight sympatric *Adiantum* species were compared (table 3). The most similar species to *A. vivesii* appear to be *A. tetraphyllum* and *A. latifolium*. The wide-creeping rhizome and the 2-pinnate blade are features that *A. vivesii* shared with both species. *Adiantum latifolium* have 6 to 16 pairs of pinnules per pinnae, *A. tetraphyllum* have 18 to 27 and *A. vivesii* have 10 to 13 (table 3).

### **Morphometric analysis**

The results of a morphometric analysis of *Adiantum vivesii* and *A. tetraphyllum* based on 21 vegetative characters and one spore character (table 1) are presented in figures 4 - 25. According to a series of one-way ANOVAs, significant differences were observed between the two species for 16 of the vegetative characters as well as spore size (table 4).

### **Chromosome counts**

After two years of work and numerous attempts at acetocarmine squashes, no preparations of *A. vivesii* sporogenous tissue were obtained from which its

chromosome number could be determined. Different stages of sporogenesis in the spore mother cells were found and photographically documented for the species (figs. 26A-F). Although similar preparations of *A. tetraphyllum* were not photographed, it appeared that the two species had similar chromosome numbers, and that in any case *A. vivesii* does not have twice the chromosome number of *A. tetraphyllum*.

### **Light microscopy**

The presence of sori on the margins of the pinnules with false indusia covering the sporangia was observed throughout the year (fig. 27A). The sori of *A. vivesii* contain both immature and mature sporangia at the same time (fig. 27B). A globose appearance of the group of mature sporangia was observed in fresh pinnules of *A. vivesii* (fig. 27C). Empty, open, mature sporangia were observed on many dried fronds of *A. tetraphyllum* (fig. 27D).

Contraction of the annulus and rupture of lip cells occurred in mature sporangia exposed to light under the dissecting microscope; variability in the size and shape of the spores of *A. vivesii* was then evident (fig. 28A). The spores of *A. vivesii* range from 10 to 19  $\mu\text{m}$  in size and those of *A. tetraphyllum* from 19 to 23  $\mu\text{m}$ . Under the microscope many of the spores of *A. vivesii* seemed blank, with a prune-like surface due to the presence of depressions; many spores were black or with black areas (figs. 28B-C). In *Adiantum tetraphyllum*, the spores were regularly shaped (figs.

28D-E), and showed less variation in size than those of *A. vivesii* (Table 4; figs. 25, 28D).

### **Scanning electron microscopy (SEM)**

Rhizomes of *A. vivesii* appeared healthy and with many rhizoids important for vegetative reproduction (fig. 29A). The sori with columns of immature sporangia on the false indusium have a normal appearance (fig. 29B). The groups of unopened mature sporangia had a grape-like appearance; also, the stalk and annulus seemed normal (fig. 29C-D). The spores of *A. vivesii* have a spherical shape when observed under the scanning electron microscope (fig. 30A). The spores of *A. tetraphyllum* have a tetrahedral shape and are all about the same size (fig. 30B-C).

## Discussion

*Adiantum vivesii* may be a single plant. Hybrid plants, including ferns, may have processes to reproduce and colonize new habitats; in this case *A. vivesii* used rhizome proliferation to increase its area of occupation. Apparent new individuals were seen, but they were all rhizome-connected (fig. 3A). Probably over time and due to climatic factors such as rain, the decomposition of the rhizomes caused that the connections were lost at a few points. The spreading of the species in the area is by vegetative reproduction due to rhizome proliferation; long rhizomes were found in many of the excavations (fig. 3B). It is concluded that the entire population is only one individual that has proliferated by rhizomes. In contrast, the excavations made to *A. tetraphyllum* demonstrated that each single plant do not have any rhizome connection with another single plant of the same species in the area. The ability of the rhizome to produce new plants was demonstrated (figs. 3C-D). The population is healthy and, over one year of monitoring from March 1999 to March 2000, it increased in size by about 37%. Throughout the year sporangia and spores were produced, but signs of sexual reproduction as gametophytes or small plants were not observed.

In the immediate area of the population of *A. vivesii* eight species of the genus *Adiantum* were found, but only *A. tetraphyllum* grows within the *A. vivesii* population (table 2). If *A. vivesii* is a hybrid, one of the seven species of *Adiantum* could be another parental species.

*Adiantum vivesii* appears to be a distinct morphological taxon. The best morphological features that can be used in the field to distinguish *A. vivesii* from *A. tetraphyllum* are the number of lateral pinnae and the number of pinnules on each lateral pinna, which are fewer in *A. vivesii* (figs. 8, 13). The leaf of *A. vivesii* is very similar to that of *A. tetraphyllum*; for this reason Proctor thought that *A. tetraphyllum* is the closest relative of this new species. The morphometric analysis showed great variability in many features, but significant differences were not found at the level of the entire frond. Petiole length, blade length and width, the blade length/width ratio and the mean length of the lateral pinnae showed slight differences; many of the values overlap between the two species (figs. 4-7, 9). *Adiantum tetraphyllum* showed more within-species variability, apparently because the material used in the analysis represented many individuals. It is believe that *Adiantum vivesii* showed less variation because all the fronds belonged to the same individual (table 3, figs. 4-25). *Adiantum vivesii* does not appear to be a good biological species, capable of sexual reproduction. The presence of sori on the pinnules does not mean that the species spores are capable of germinating and forming a new plant. The plant uses the

rhizome for its vegetative reproduction and the entire population seems to be connected by them (fig. 3A).

Examination of the hybrid and parental plants of *Woodsia* revealed morphological intermediacy occurring in several characters on the hybrid plants (Brooks, 1982). Palmer (1998) described morphological intermediacy in a hybrid of the genus *Cibotium*, in comparison with its known parents.

The gross morphology helps in the identification of intermediacy between parental species, but the spores must also be studied to know if the new plant is a hybrid. Sterile intertaxa with defective spores are much more common than any other types of hybrids (Wagner and Chen, 1965). Considering the high incidence of sterile nothospecies among pteridophytes, the use of spore abortion as a means of identification can be extremely important, especially where the parental species are subtly differentiated on the basis of gross morphology.

Sterile hybrids tend to have a greater variation in spore size than normal sexual species (Wagner and Chen, 1965). The greater variation in spore size in *A. vivesii* is mainly produced by spore abortion (figs. 28A-C). Palmer (1998), in his work with the *Cibotium* hybrid, found variability in spore morphology. Mayer (1989) states that plants of the genus *Polystichum* that have abortive spores were morphologically intermediate, supporting the hybridization hypothesis.

The variability in spore morphology and spore size are supported by the studies of Whittier and Wagner (1971) and Wagner et al. (1986). Normal species such as *A. tetraphyllum* have at least some abortive spores (fig. 28E), but many fewer than in sterile hybrids (Wagner and Chen, 1965). The forms of the spores of *A. vivesii* are quite different because of the collapse of the exospore associated with the absence of the protoplast. The empty spores were commonly filled with air producing optically black areas within the exospore (fig. 28B-C).

The mature sori of *A. vivesii* (figs. 27C, 29B-D) seemed to be more compactly constructed than those of *A. tetraphyllum*, with the sporangia appearing as more or less globular objects tightly grouped together; this is consistent with the sorus of a hybrid. Wagner and Chen (1965) found the abortion of sporangia and spores to be an important tool in the detection of *Dryopteris* hybrids. Abortion can be characterized as significant variation from the normal products of sporogenesis. Spore samples showing exaggerated variability in spore size will often one or both show of the following symptoms: Spore collapse may go hand in hand with loss of the protoplast. Usually massive perisporial development occurs, together with a reduction in the size of the protoplasts as compared to the parents (Wagner and Chen, 1965).

Wagner and Chen (1965) demonstrated that greater variation in spore size, which is mainly produced by spore abortion, is characteristic of a sterile hybrid. The phenomenon of spore abortion may be traced back to the process that produces the



spores. The important point is that in hybrids the chromosomes tend to be distributed irregularly to the young spores, and the spores differ genetically among themselves.

Wagner and Chen (1965) proposed the following steps that are involved in the detection of a hybrid found in nature: 1) Recognition that the majority or all the characteristics of the plant in question are intermediate between those of two distinct and well understood species. 2) Observation that the sori contain abortive sporangia and spores. 3) Determination that the chromosomes, when studied in cytological preparations under the compound microscope, do not behave normally in the process of spore production. In this investigation only the last point was left unresolved. The chromosome counts in both the immature sporangia and root tips do not present clearly the chromosome number of the species.

In the case of *Tectaria estremarana* Proctor & Evans, which is believed to be of autopolyploid origin, its chromosome number is twice that of its parent, *Tectaria cicutaria* (L.) Copeland, Philipp. This is not the case for *A. vivesii*, where the acetocarmine squashes did not differ substantially in appearance from those of *A. tetraphyllum* and approximately the same number of chromosomes is suggested for both species. It was demonstrated that *A. tetraphyllum* in Trinidad is diploid and tetraploid, without morphological differences (Jermy and Walker, 1985, cited in Moran et al., 1995).

*Adiantum vivesii* is certainly a distinct morphological taxon that has maintained itself and in fact is spreading vegetatively. Long-term monitoring of the species is recommended, since not much difference in population size was observed over a single year. Annual visits to the site would be reasonable to detect changes in the size and status of the population. The habitat is safe for *A. vivesii* because there is little or no human impact. Only a hurricane or major constructions (for example, a road or housing development) could endanger the species' stability.

As a species incapable of sexual reproduction, *A. vivesii* is not expected to colonize other localities on its own. If it were deemed useful to pursue its conservation *ex situ*, this could readily be done by rhizomes (fig. 3C-D). Other suitable habitats should have the similar environmental conditions, and should preferably share associated species (table 2) with the present site.

## Conclusions

1. *Adiantum vivesii* is restricted to its type locality in Quebradillas.
2. The population probably consists only of one individual with rhizome proliferations.
3. *Adiantum vivesii* appears to be a distinct morphological taxon.
4. *Adiantum vivesii* does not appear to be a good biological species, capable of sexual reproduction.
5. *Adiantum vivesii* does not appear to be a polyploid, at least of *Adiantum tetraphyllum*.
6. *Adiantum vivesii* appears to be sterile, and may be hybrid. One of its parents may be *A. tetraphyllum*; the other is unknown.

## Recommendations

1. Further cytological studies of *Adiantum vivesii*.
2. Additional studies of spores obtained from various fronds.
3. Spore germination experiments.
4. Molecular systematics of *Adiantum vivesii* and all sympatric species of *Adiantum*.
5. Vegetative propagation *ex situ* in similar habitats.
6. Long-term monitoring of the population.

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## **Tables**

**Table 1.** List of features included in morphometric analyses. Asterisks indicate those illustrated in Figure 2.

1-9 FEATURES OF ENTIRE LEAF

- \* 1. Length of petiole (cm)
- \* 2. Length of blade (cm)
- \* 3. Width of blade (cm)
- 4. Length / width of blade
- 5. Number of lateral pinnae
- \* 6. Mean length of lateral pinnae (cm)
- \* 7. Mean width of lateral pinnae (cm)
- 8. Mean length / width of lateral pinnae
- \* 9. Distance between first and second lateral pinnae (cm)

10-13 FEATURES OF LATERAL PINNAE

- 10. Mean number of pinnules on lateral pinnae
- \* 11. Mean length of longest pinnule (cm)
- \* 12. Mean width of longest pinnule (cm)
- 13. Mean length / width of longest pinnule

14-21 FEATURES OF TERMINAL PINNA

- \* 14. Length of terminal pinna (cm)
- \* 15. Width of terminal pinna (cm)
- 16. Length / width of terminal pinna
- \* 17. Length of petiolule (cm)
- \* 18. Number of pinnules on terminal pinna
- \* 19. Length of largest pinnule on terminal pinna (cm)
- \* 20. Width of largest pinnule on terminal pinna (cm)
- 21. Length / width of largest pinnule on terminal pinna (cm)

22. SPORE FEATURE

- 22. Spore size (mm)

**Table 2:** Species associated with *Adiantum vivesii*, according to Miguel A. Vives.

<b>SPECIES</b>	<b>FAMILY</b>	<b>SUBFAMILY</b>
<b>Pteridophyta</b>		
<i>Cyathea arborea</i> (L.) Sm.	Cyatheaceae	
<i>Adiantum cristatum</i> L.	Polypodiaceae	Adiantoideae
<i>Adiantum fragile</i> Sw.	Polypodiaceae	Adiantoideae
<i>Adiantum latifolium</i> Lam.	Polypodiaceae	Adiantoideae
<i>Adiantum melanoleucum</i> Willd.	Polypodiaceae	Adiantoideae
<i>Adiantum pulverulentum</i> L.	Polypodiaceae	Adiantoideae
<i>Adiantum tenerum</i> Sw.	Polypodiaceae	Adiantoideae
<i>Adiantum tetraphyllum</i> Humb. & Bonpl. ex Willd.	Polypodiaceae	Adiantoideae
<i>Adiantum vivesii</i> Proctor	Polypodiaceae	Adiantoideae
<i>Adiantum wilsonii</i> Hook.	Polypodiaceae	Adiantoideae
<i>Arachniodes chaerophylloides</i> (Poir.) Proctor	Polypodiaceae	Dryopteridoideae
<i>Bolbitis pergamentaceae</i> (Maxon) Ching	Polypodiaceae	Dryopteridoideae
<i>Diplazium</i> sp.	Polypodiaceae	Athyrioideae
<i>Polypodium latifolium</i> Vahl	Polypodiaceae	Polypodioideae
<i>Polypodium phyllitidis</i> L.	Polypodiaceae	Polypodioideae
<i>Fadyenia hookeri</i> (Sweet) Maxon	Polypodiaceae	Tectarioideae
<i>Hypoderris brownii</i> J. Smith	Polypodiaceae	Tectarioideae
<i>Tectaria cicutaria</i> (L.) Copel.	Polypodiaceae	Tectarioideae

**Table 2** (cont.).

<b>SPECIES</b>	<b>FAMILY</b>	<b>SUBFAMILY</b>
<i>Tectaria heracleifolia</i> (Willd.) Underw.	Polypodiaceae	Tectarioideae
<i>Tectaria trifoliata</i> (L.) Cav.	Polypodiaceae	Tectarioideae
<i>Thelypteris guadalupensis</i> (Wikström) Proctor	Polypodiaceae	Thelypteridoideae
<i>Thelypteris hildae</i> Proctor	Polypodiaceae	Thelypteridoideae
<i>Thelypteris pennata</i> (Poir.) Morton	Polypodiaceae	Thelypteridoideae
<i>Psilotum nudum</i> (L.) P. Beauv.	Psilotaceae	
<i>Anemia adiantifolia</i> (L.) Sw.	Schizaeaceae	
<i>Selaginella laxifolia</i> Baker	Selaginellaceae	
<i>Selaginella subcaulescens</i> Baker	Selaginellaceae	

**Table 2** (cont.).

<b>SPECIES</b>	<b>FAMILY</b>
<b>Spermatophyta</b>	
<i>Justicia</i> sp.	Acanthaceae
<i>Ruellia coccinea</i> (L.) Vahl	Acanthaceae
<i>Comocladia glabra</i> (Schultes) Spreng.	Anacardiaceae
<i>Anthurium crenatum</i> (L.) Kunth	Araceae
<i>Didymopanax morototoni</i> (Aubl.) Decne. & Planch	Araliaceae
<i>Prestoea borinquena</i> (R. Grah.) Nichols.	Asteraceae
<i>Spathodea campanulata</i> Beauv.	Bignoniaceae
<i>Tabebuia haemantha</i> (Bert.) DC.	Bignoniaceae
<i>Tabebuia heterophylla</i> (DC.) Britton	Bignoniaceae
<i>Ceiba pentandra</i> (L.) Gaertn.	Bombaceae
<i>Bourreria domingensis</i> (DC.) Griseb.	Boraginaceae
<i>Cordia laevigata</i> (Lam.)	Boraginaceae
<i>Cordia sulcata</i> DC.	Boraginaceae
<i>Guzmania</i> sp.	Bromeliaceae
<i>Tetragastris balsamifera</i> (Sw.) Oken	Burseraceae
<i>Cecropia schreberiana</i> Mig.	Cecropiaceae
<i>Gyminda latifolia</i> (Sw.) Urb.	Celastraceae

**Table 2** (cont.).

<b>SPECIES</b>	<b>FAMILY</b>
<i>Calophyllum calaba</i> L.	Clusiaceae
<i>Clusia rosea</i> Jacq.	Clusiaceae
<i>Diospyros sintenisii</i> (Krug & Urb.) Standl.	Ebenaceae
<i>Sloanea amygdalina</i> Griseb.	Eleocarpaceae
<i>Drypetes ilicifolia</i> Krug & Urb.	Euforbiaceae
<i>Gymnanthes lucida</i> Sw.	Euphorbiaceae
<i>Andira inermis</i> (W.Wright) DC.	Fabaceae
<i>Casearia guianensis</i> (Aubl.) Urb.	Flacourtiaceae
<i>Homalium racemosum</i> Jacq.	Flacourtiaceae
<i>Gesneria pedunculosa</i> (DC.) Fritsch	Gesneriaceae
<i>Ottoschulzia rhodoxylon</i> (Urb.) Urb.	Icacinaceae
<i>Ocotea leucoxylon</i> (Sw.) Mez	Lauraceae
<i>Mouriri helleri</i> Britton	Melastomataceae
<i>Tetrazygia angustifolia</i> (Sw.) DC.	Melastomataceae
<i>Guarea guidonea</i> (L.) Sleumer	Meliaceae
<i>Inga vera</i> Willd.	Mimosaceae
<i>Artocarpus altilis</i> (Parkinson) Fosberg	Moraceae
<i>Pseudolmedia spuria</i> (Sw.) Griseb.	Moraceae
<i>Trophis racemosa</i> (L.) Urb.	Moraceae

**Table 2** (cont.).

<b>SPECIES</b>	<b>FAMILY</b>
<i>Ardisia obovata</i> Desv.	Myrsinaceae
<i>Eugenia confusa</i> DC.	Myrtaceae
<i>Eugenia ligustrina</i> (Sw.) Willd.	Myrtaceae
<i>Myrcia paganii</i> Krug & Urb.	Myrtaceae
<i>Pimenta racemosa</i> (Mill.) J.W. Moore	Myrtaceae
<i>Psidium amplexicaule</i> Pers.	Myrtaceae
<i>Syzygium jambos</i> (L.) Alston	Myrtaceae
<i>Guapira fragans</i> (Dum.-Cours.) Little	Nyctaginaceae
<i>Neea buxifolia</i> (Hook. f.) Heimerl	Nyctaginaceae
<i>Oeceoclades maculata</i> (Lindl.) Lindl.	Orchidaceae
<i>Roystonea regia</i> (H.B.K.) O.F. Cook	Palmae
<i>Sabal causiarum</i> (O.F. Cook) Becc.	Palmae
<i>Piper aduncum</i> L.	Piperaceae
<i>Securidace virgata</i> Sw.	Polygalaceae
<i>Coccoloba costata</i> C. Wright	Polygonaceae
<i>Coccoloba diversifolia</i> Jacq.	Polygonaceae
<i>Coccoloba pubescens</i> L.	Polygonaceae
<i>Coccoloba pyrifolia</i> Desf.	Polygonaceae
<i>Clematis dioica</i> L.	Ranunculaceae



**Table 2** (cont.).

<b>SPECIES</b>	<b>FAMILY</b>
<i>Krugiodendron ferreum</i> (Vahl) Urb.	Rhamnaceae
<i>Reynosia krugii</i> Urb.	Rhamnaceae
<i>Cassipourea guianensis</i> Aubl.	Rhizophoraceae
<i>Prunus myrtifolia</i> (L.) Urb.	Rosaceae
<i>Coffea arabica</i> L.	Rubiaceae
<i>Gonzalagunia</i> sp.	Rubiaceae
<i>Neolaugeria resinosa</i> (Vahl) Nicolson	Rubiaceae
<i>Randia aculeata</i> L.	Rubiaceae
<i>Rondeletia inermis</i> (Spreng.) Krug & Urb.	Rubiaceae
<i>Amyris elemifera</i> L.	Rutaceae
<i>Pilocarpus racemosus</i> L.	Rutaceae
<i>Zanthoxylum martinicense</i> (Lam.) P. Wilson	Rutaceae
<i>Cupania americana</i> L.	Sapindaceae
<i>Thouinia striata</i> Radlk.	Sapindaceae
<i>Manilkara bidentata</i> (A.DC.) Chev.	Sapotaceae
<i>Brunfelsia portoricensis</i> Krug & Urb.	Solanaceae
<i>Symplocos martinicensis</i> Jacq.	Symplocaceae

**Table 3.** Comparison of some characteristics of sympatric *Adiantum* species, based on Proctor (1989). “No information” means that Proctor did not include it in his book.

<b>Species</b>	<b>Rhizome</b>	<b>Blade</b>	<b>Pinnae</b>	<b>Pinnules</b>	<b>Sori</b>
<i>Adiantum vivesii</i>	creeping	2-pinnate	2 or 3 pinnae	10-13 pairs	up to 5 per pinnule
<i>Adiantum fragile</i>	short	3-4 pinnate at base	no information	no information	singly or in pairs
<i>Adiantum latifolium</i>	wide-creeping	2-pinnate	1-4 pairs	6-16 pairs	up to 12 per pinnule
<i>Adiantum melanoleucum</i>	creeping	2-pinnate at base	2-8 pairs	no information	2-6 per pinnule
<i>Adiantum puberulentum</i>	short-creeping	2-pinnate	3-9 pairs	no information	usually solitary
<i>Adiantum pyramidale</i>	creeping	2-pinnate	2-11 pairs	no information	up to 9 per pinnule
<i>Adiantum tenerum</i>	short-creeping	3-5-pinnate at base	no information	no information	in pairs
<i>Adiantum tetraphyllum</i>	creeping	2-pinnate	2-6 pairs	18-27 pairs	no information
<i>Adiantum wilsonii</i>	short- to long-creeping	1-pinnate	1-3 pairs	no information	no information

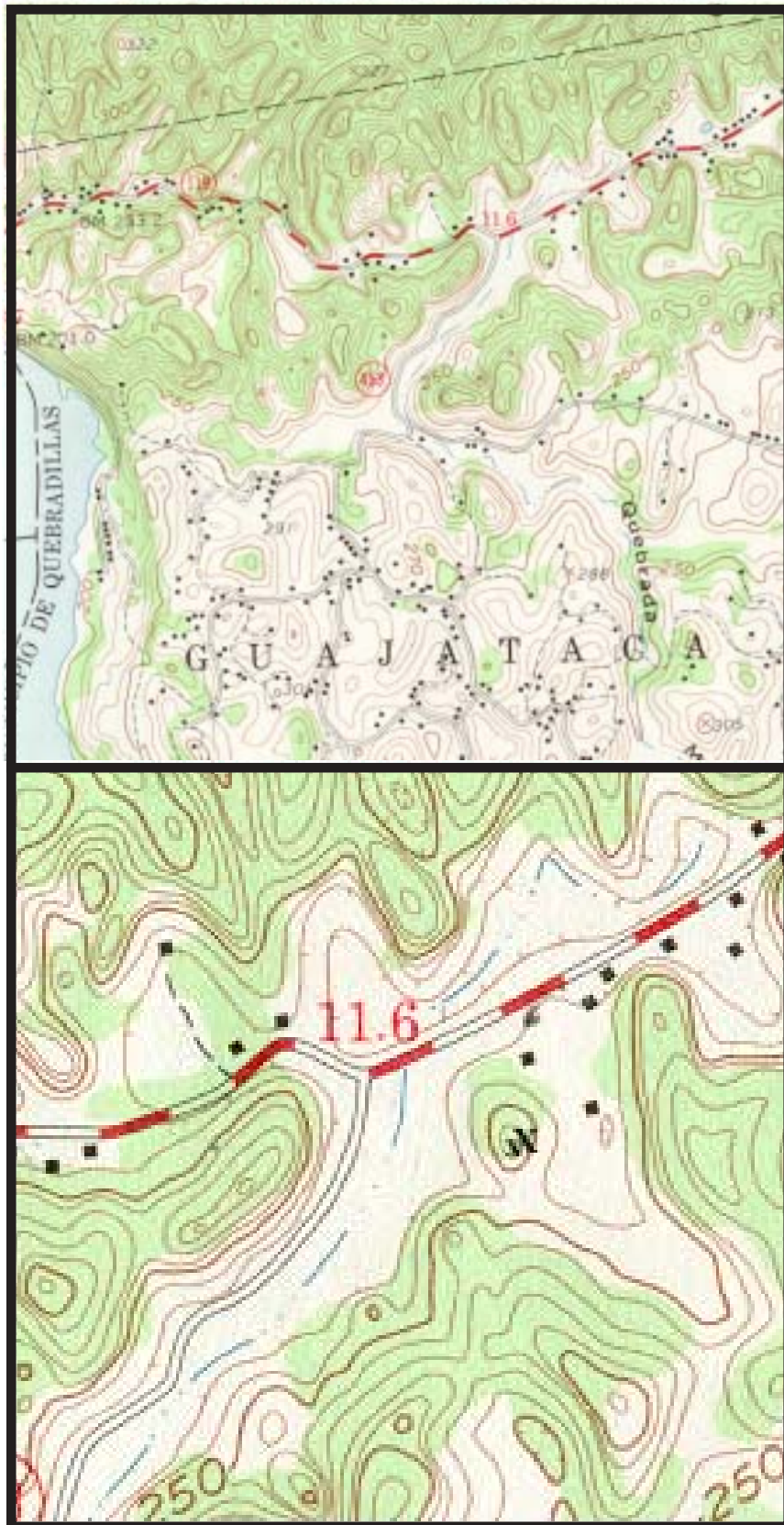
**Table 4.** Results of a morphometric analysis of *Adiantum vivessii* and *A. tetraphyllum* based on 21 vegetative characters and one spore character. For each species, the mean and standard deviation (in parentheses) are given, together with the probability value (P), obtained by a one-way ANOVA, and its level of significance: n.s. = not significant ( $P > 0.05$ ), \* = significant ( $0.01 < P \leq 0.05$ ), \*\* = highly significant ( $0.001 < P \leq 0.01$ ), \*\*\* = very highly significant ( $P \leq 0.001$ ). For the vegetative characters, n = 20 for each species; for the spore character, n = 50.

<b>Character</b>	<i>Adiantum vivessii</i>	<i>Adiantum tetraphyllum</i>	<b>Probability</b>
Petiole length (cm)	31.8 (6.56)	30.5 (10.31)	0.637 n.s.
Blade length (cm)	23.8 (2.52)	24.5 (2.87)	0.374 n.s.
Blade width (cm)	28.3 (3.72)	28.3 (3.44)	0.997 n.s.
Blade length/width	0.86 (0.06)	0.87 (0.08)	0.588 n.s.
Number of lateral pinnae	2.60 (0.50)	5.10 (1.12)	0.000 ***
Lateral pinnae mean length (cm)	14.4 (2.00)	14.9 (1.57)	0.408 n.s.
Lateral pinnae mean width (cm)	5.62 (0.79)	3.91 (0.67)	0.000 ***
Lateral pinnae mean length/width	2.62 (0.47)	3.89 (0.65)	0.000 ***
Distance between first and second lateral pinnae	2.63 (0.62)	2.05 (0.60)	0.004 **
Mean number of pinnules on lateral pinnae	9.94 (1.30)	20.0 (1.69)	0.000 ***

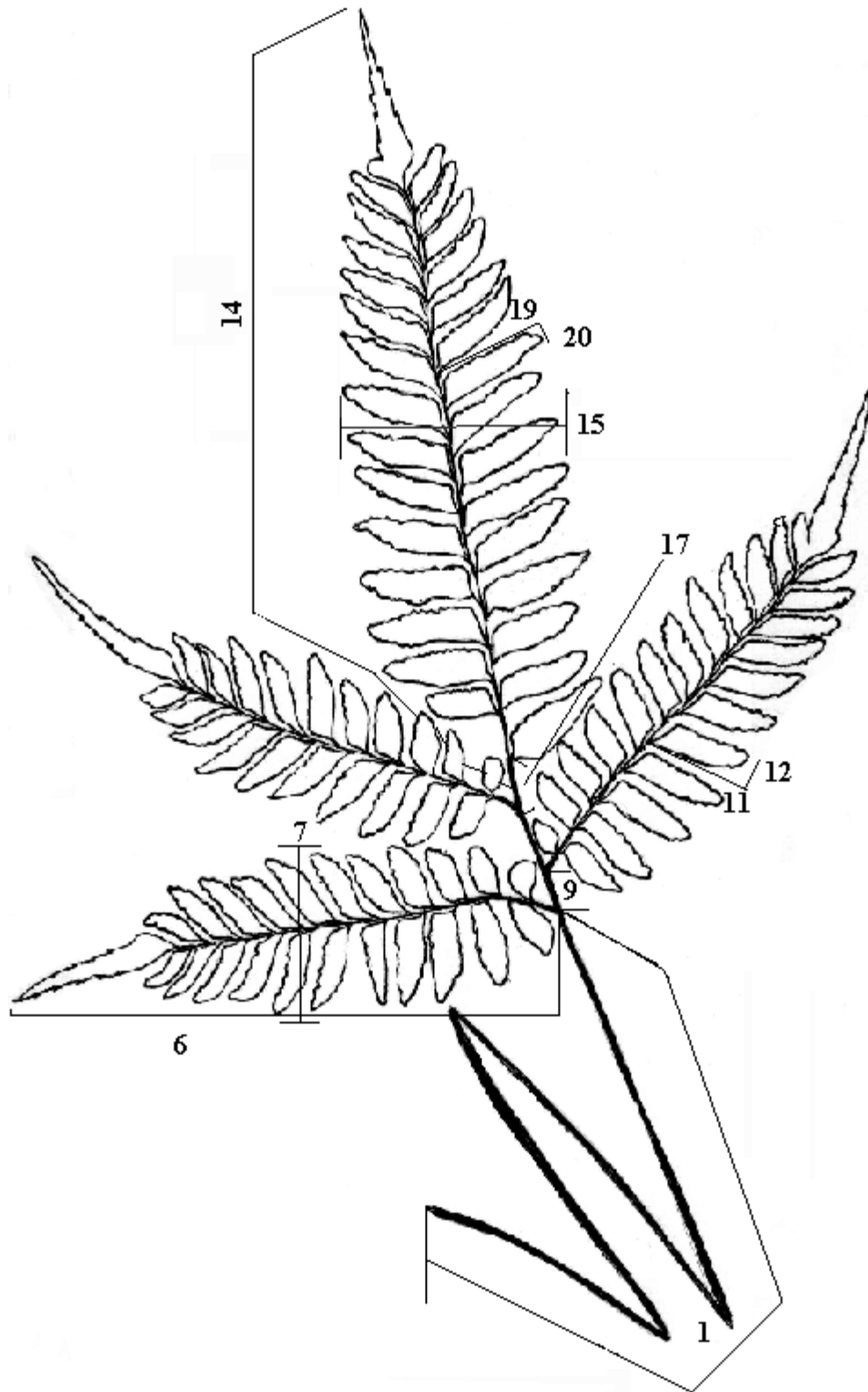
**Table 4** (cont.).

<b>Character</b>	<i>Adiantum vivesii</i>	<i>Adiantum tetraphyllum</i>	<b>Probability</b>
Mean length of longest pinnule on lateral pinna (cm)	3.26 (0.49)	2.20 (0.38)	0.000 ***
Mean width of longest pinnule on lateral pinna (cm)	0.83 (0.13)	0.69 (0.10)	0.000 ***
Mean length/width of longest pinnule on lateral pinna	3.98 (0.34)	3.20 (0.37)	0.000 ***
Terminal pinna length (cm)	21.1 (2.28)	19.4 (1.98)	0.016 *
Terminal pinna width (cm)	7.38 (0.93)	4.96 (0.89)	0.000 ***
Terminal pinna length/width	2.92 (0.45)	4.02 (0.67)	0.000 ***
Petiolule length (cm)	1.24 (0.38)	0.76 (0.22)	0.000 ***
Number of pinnules on terminal pinna	13.8 (1.20)	24.3 (1.81)	0.000 ***
Largest pinnule length on terminal pinna (cm)	4.04 (0.47)	2.72 (0.45)	0.000 ***
Largest pinnule width on terminal pinna (cm)	1.00 (0.13)	0.82 (0.12)	0.000 ***
Largest pinnule length/width on terminal pinna	4.11 (0.39)	3.32 (0.37)	0.000 ***
Spore size ( $\mu\text{m}$ )	13.7 (1.44)	20.8 (0.95)	0.000 ***

## **Figures**



**Figure 1.** Topographic map of barrio San Antonio, Quebradillas.



**Figure 2.** Leaf features included in morphometric analysis.



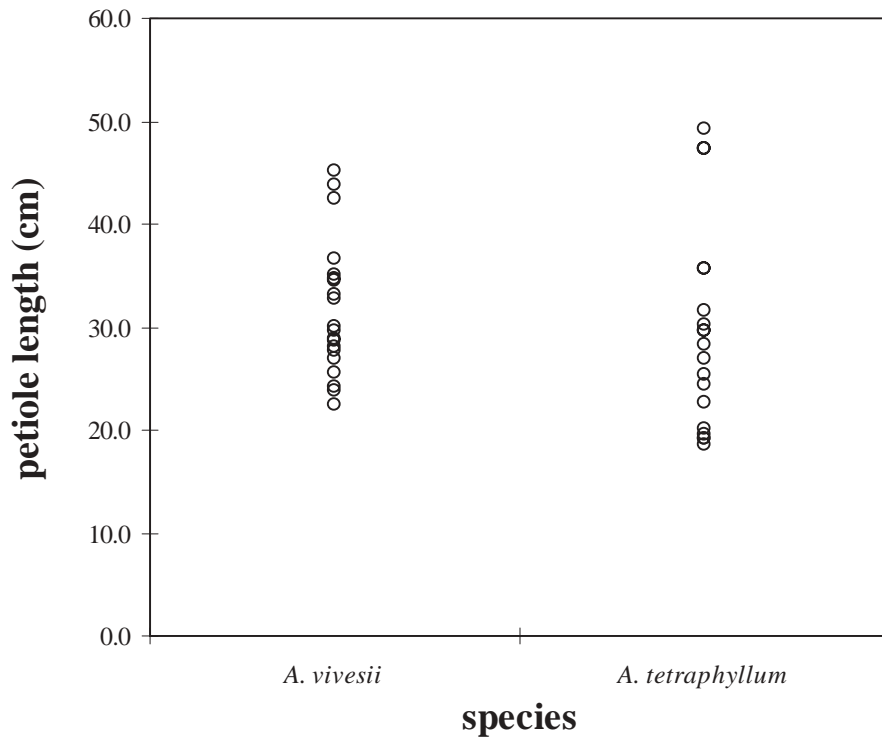
**Figure 3A.** Rhizome connections between the apparent individuals of *Adiantum vivesii*.

**3B.** Rhizome of *Adiantum vivesii* about half a meter long.

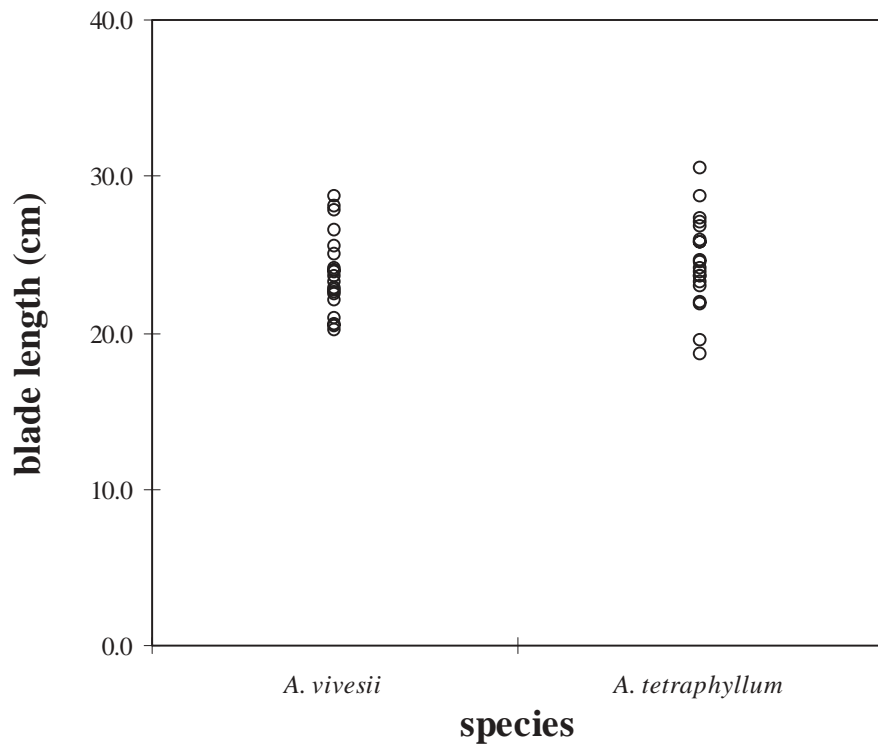
**3C.** New plant of *Adiantum vivesii*, grown from rhizome collected in March 1999.

**3D.** New plant of *Adiantum. vivesii*, grown from rhizome collected in March 2000.

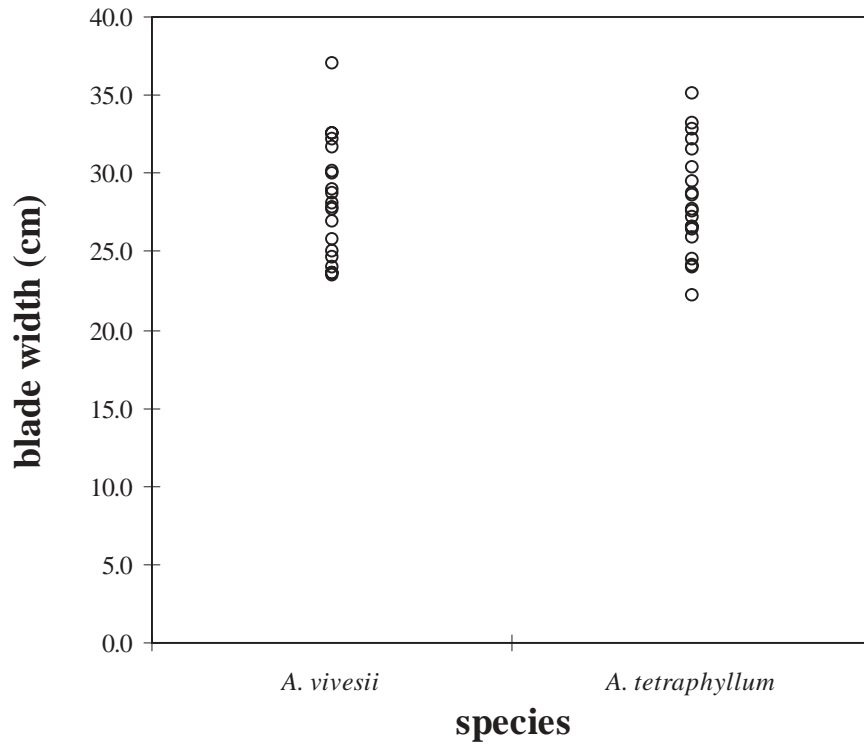




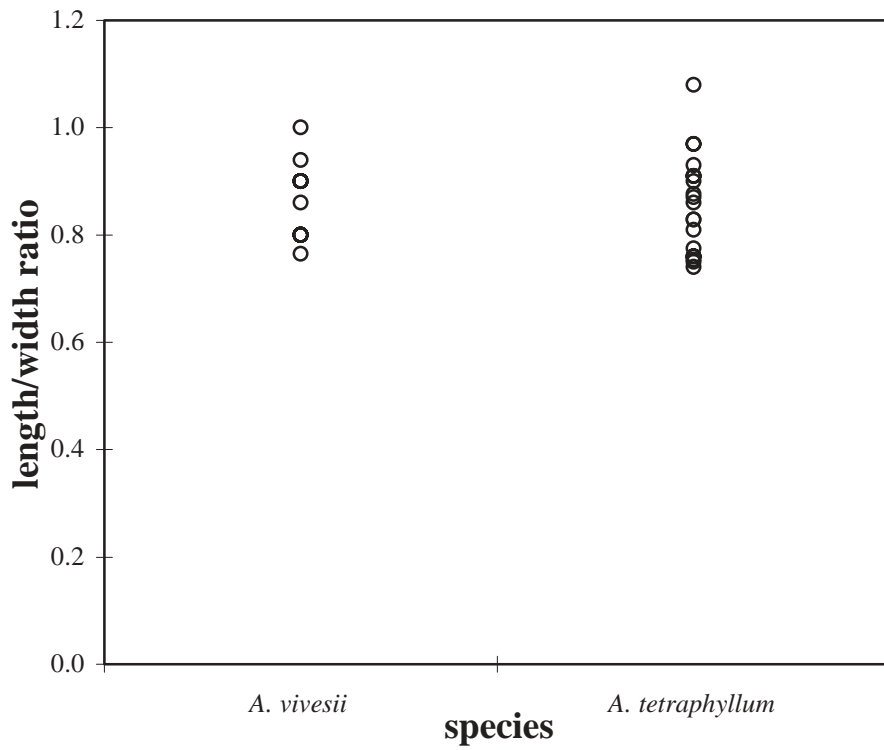
**Figure 4.** Dotplot of petiole length in *Adiantum vivesii* and *Adiantum tetraphyllum*. Mean length of the petiole was 31.7 cm for *A. vivesii* and 30.4 cm for *A. tetraphyllum*.



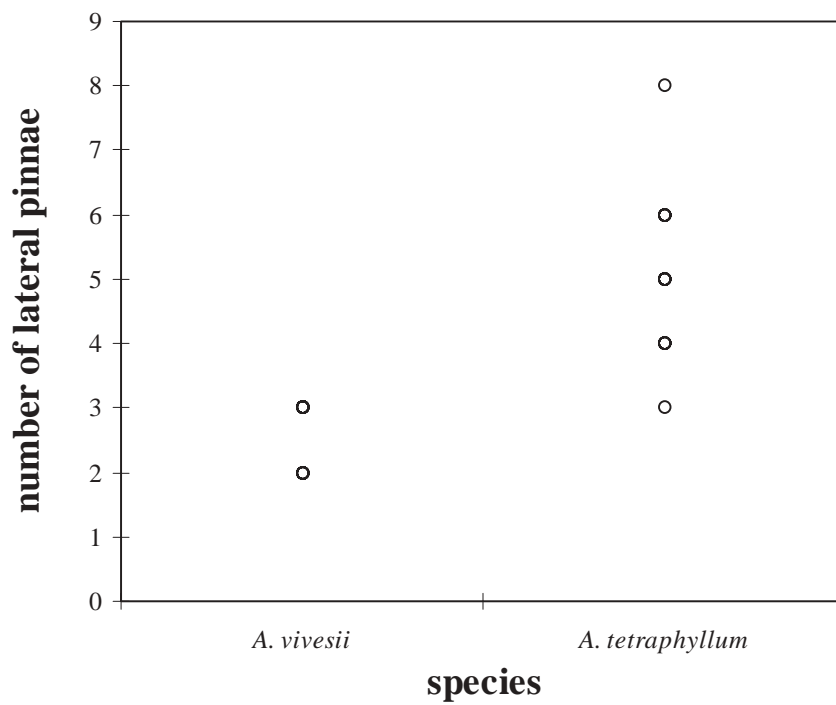
**Figure 5.** Dotplot of blade length in *Adiantum vivesii* and *Adiantum tetraphyllum*. Mean blade length was 23.8 cm for *A. vivesii* and 24.5 cm for *A. tetraphyllum*.



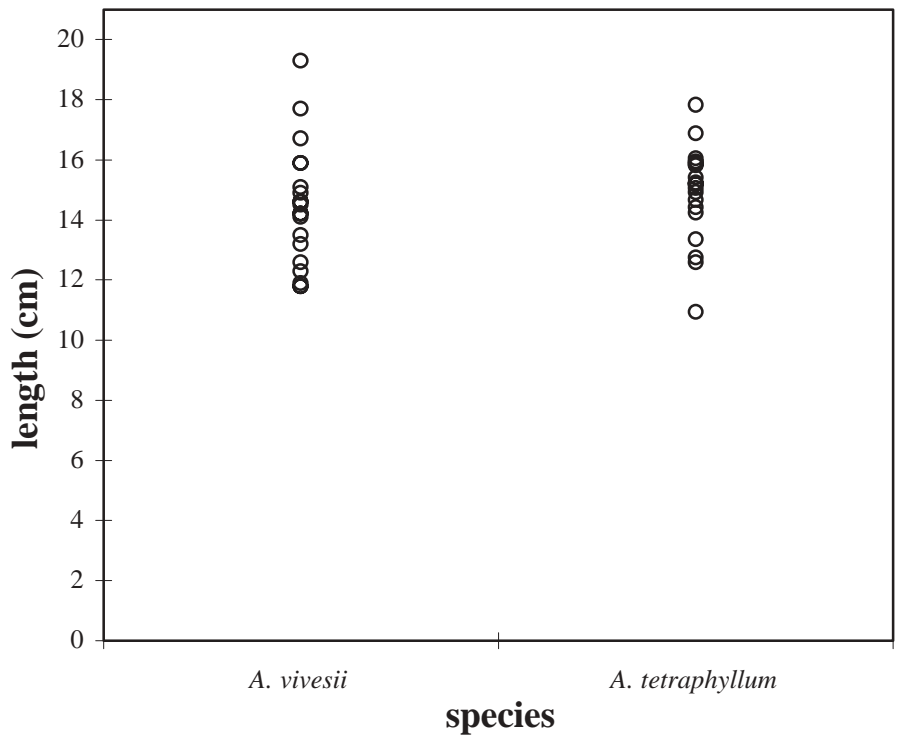
**Figure 6.** Dotplot of blade width in *Adiantum vivesii* and *Adiantum tetraphyllum*. Mean blade width was 28.2 cm for *A. vivesii* and 28.2 cm for *A. tetraphyllum*.



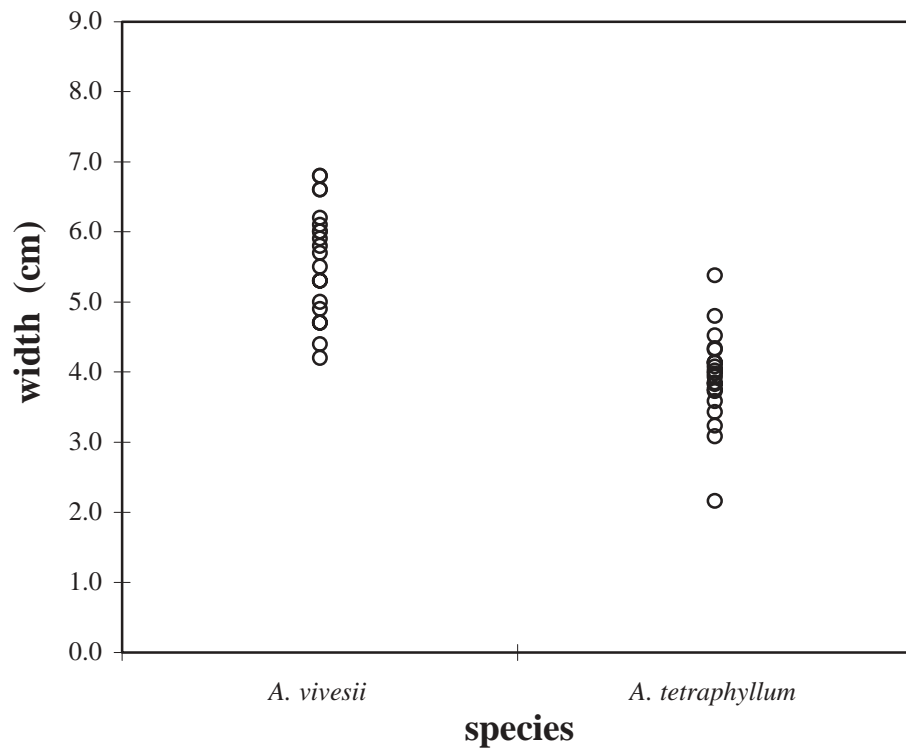
**Figure 7.** Dotplot of blade length/width in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean blade length/width was 0.86 for *A. vivesii* and 0.87 for *A. tetraphyllum*.



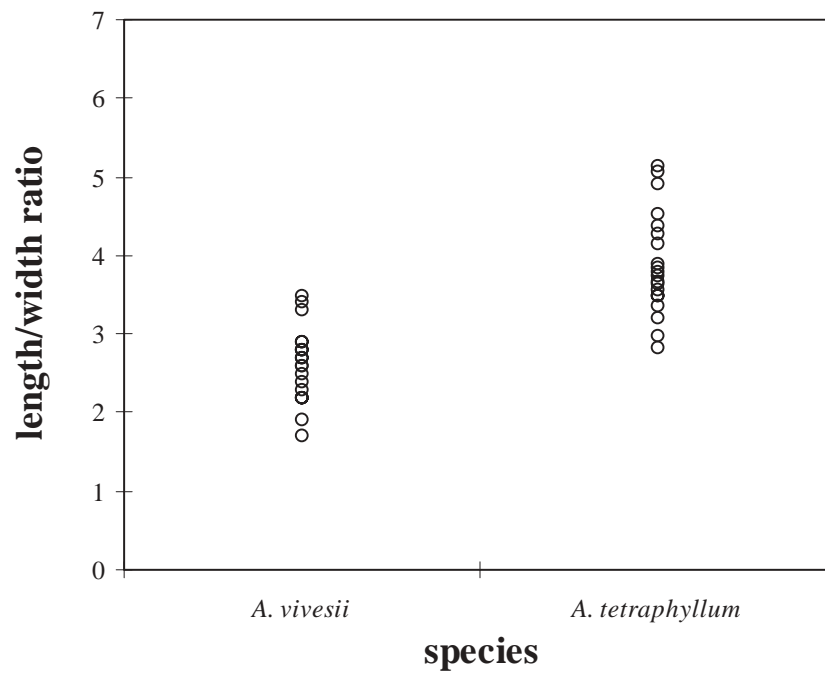
**Figure 8.** Dotplot of number of lateral pinnae in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean number was 2.6 for *A. vivesii* and was 5.1 for *A. tetraphyllum*.



**Figure 9.** Dotplot of mean length of lateral pinnae in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean length was 14.4 cm for *A. vivesii* and 14.9 cm for *A. tetraphyllum*.

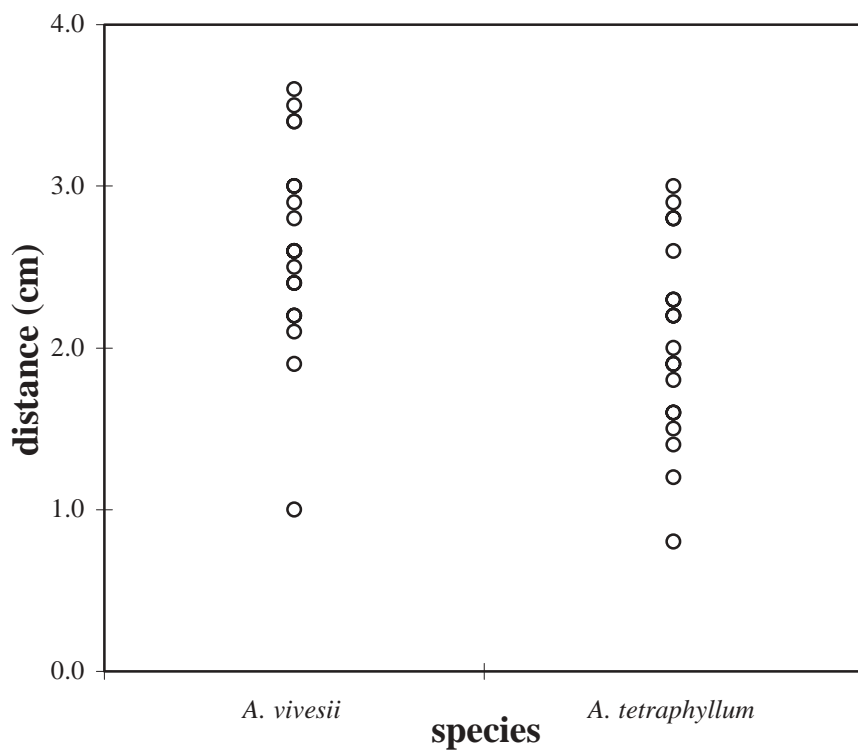


**Figure 10.** Dotplot of mean width of lateral pinnae in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean width was 5.6 cm for *A. vivesii* and 5.7 cm for *A. tetraphyllum*.

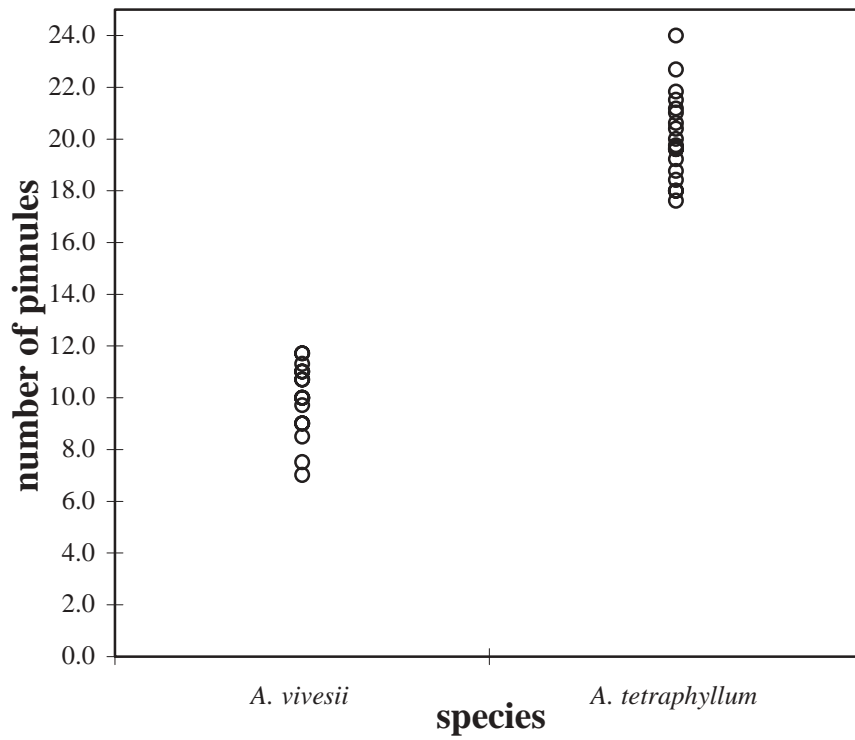


**Figure 11.** Dotplot of mean length/width of lateral pinnae in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean length/width was 2.6 for *A. vivesii* and 3.9 for *A. tetraphyllum*.

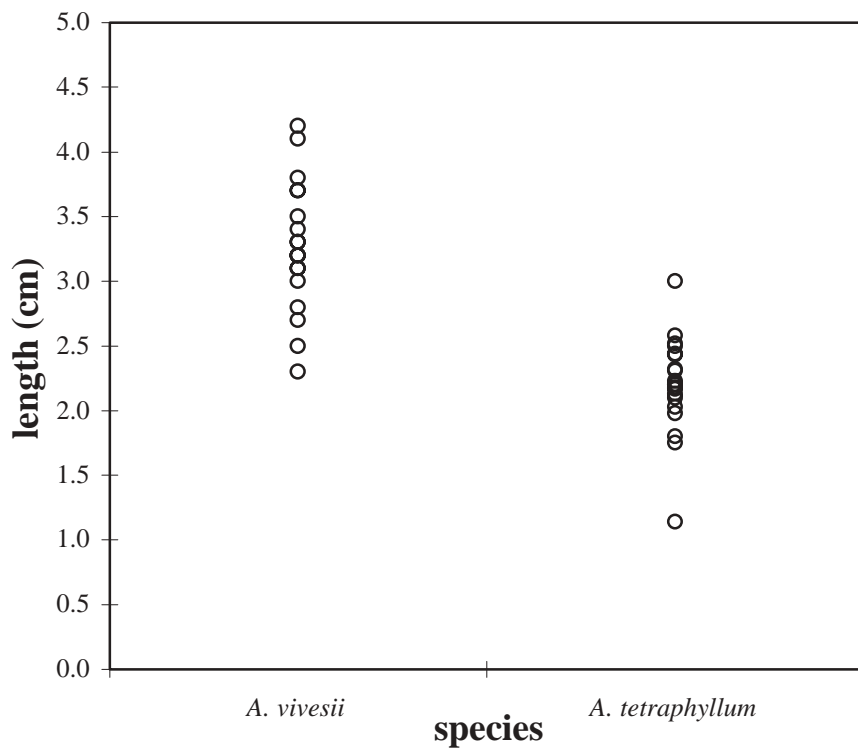




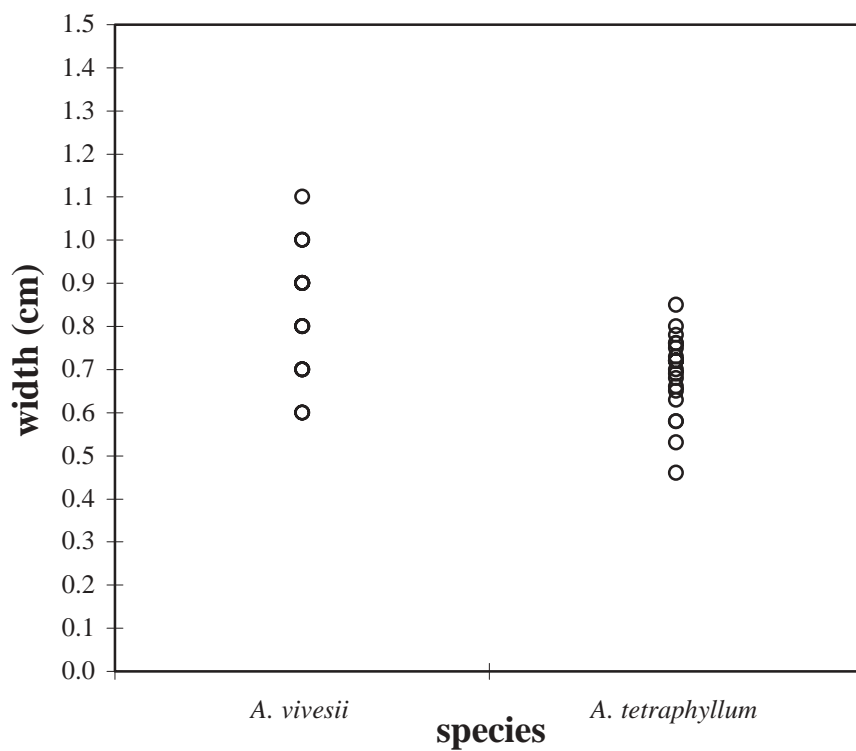
**Figure 12.** Dotplot of distance between first and second lateral pinnae in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean distance was 2.6 for *A. vivesii* cm and 2.0 cm for *A. tetraphyllum*.



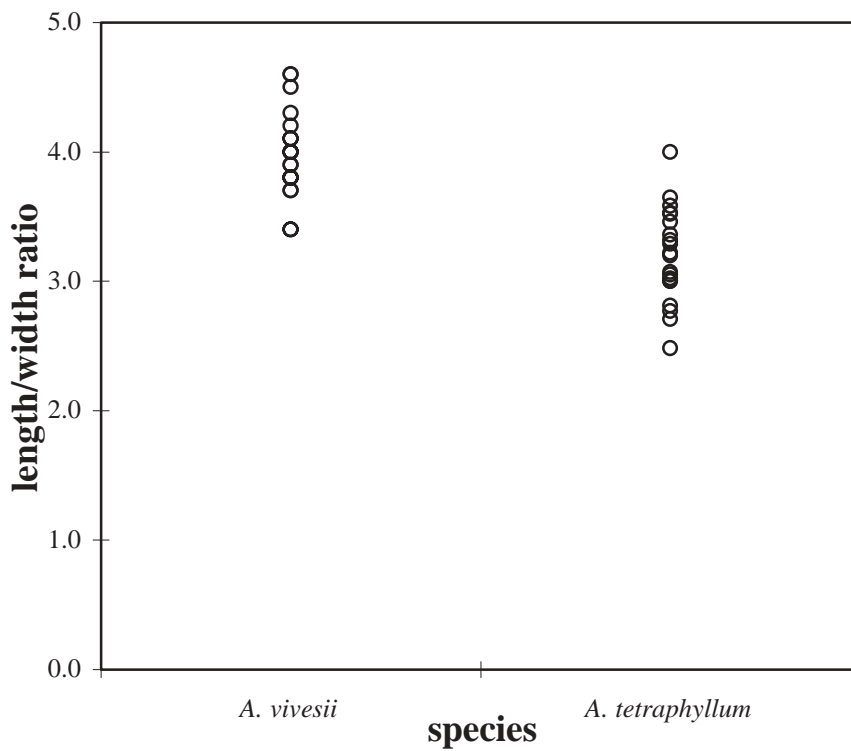
**Figure 13.** Dotplot of mean number of pinnules on lateral pinnae in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean number was 9.9 for *A. vivesii* and 19.9 for *A. tetraphyllum*.



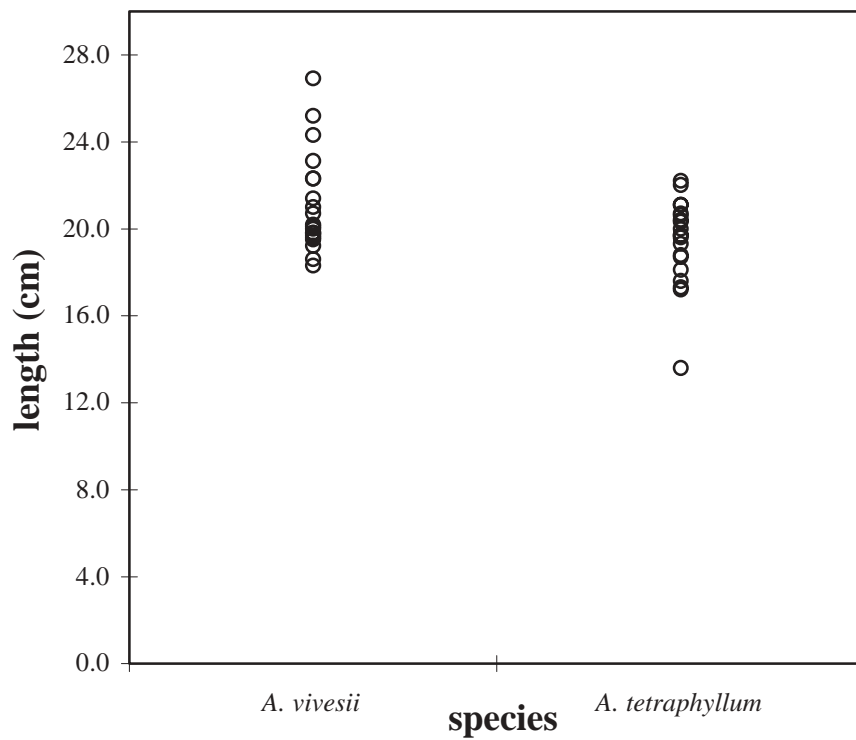
**Figure 14.** Dotplot of mean length of longest pinnule on lateral pinnae in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean length was 3.2 cm for *A. vivesii* and 2.2 cm for *A. tetraphyllum*.



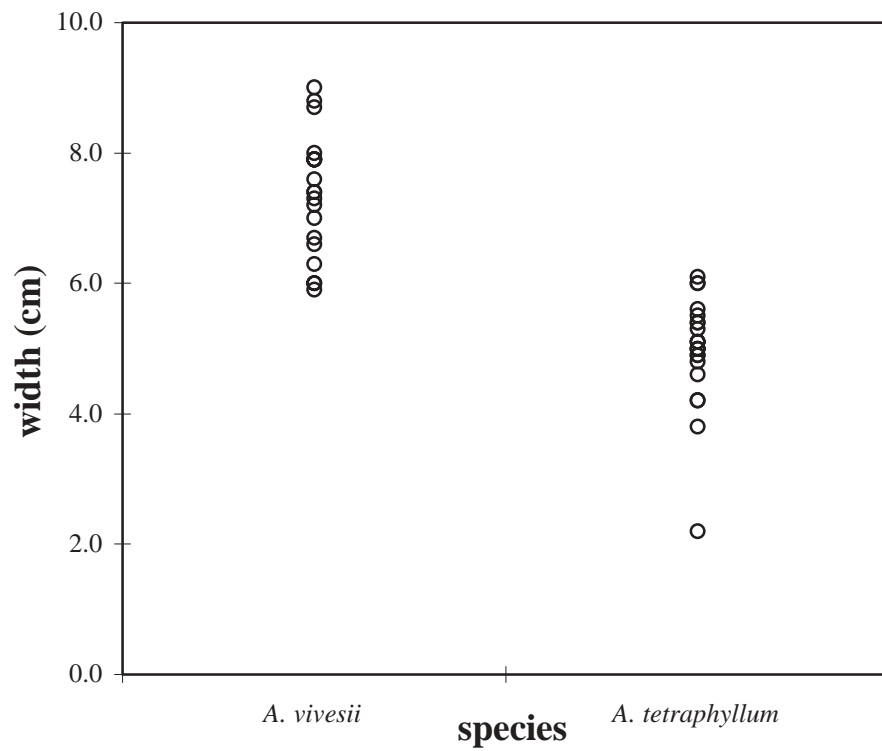
**Figure 15.** Dotplot of mean width of longest pinnule on lateral pinnae in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean width was 0.83 cm for *A. vivesii* and 0.68 cm for *A. tetraphyllum*.



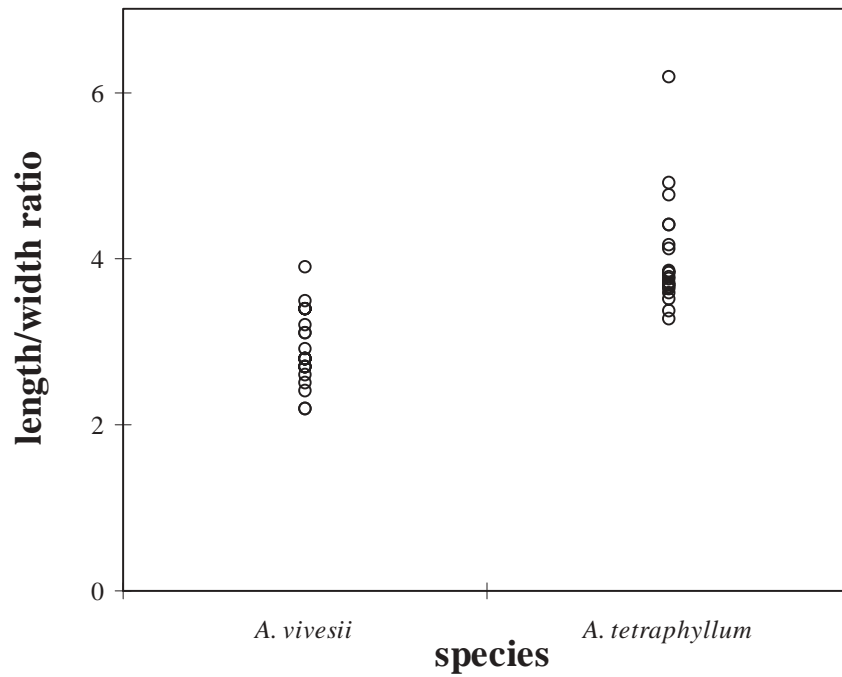
**Figure 16.** Dotplot of mean length/width of longest pinnule on lateral pinnae in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean ratio was 3.9 for *A. vivesii* and 3.2 for *A. tetraphyllum*.



**Figure 17.** Dotplot of length of terminal pinnae in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean length was 21.1 cm for *A. vivesii* and 19.4 cm for *A. tetraphyllum*.

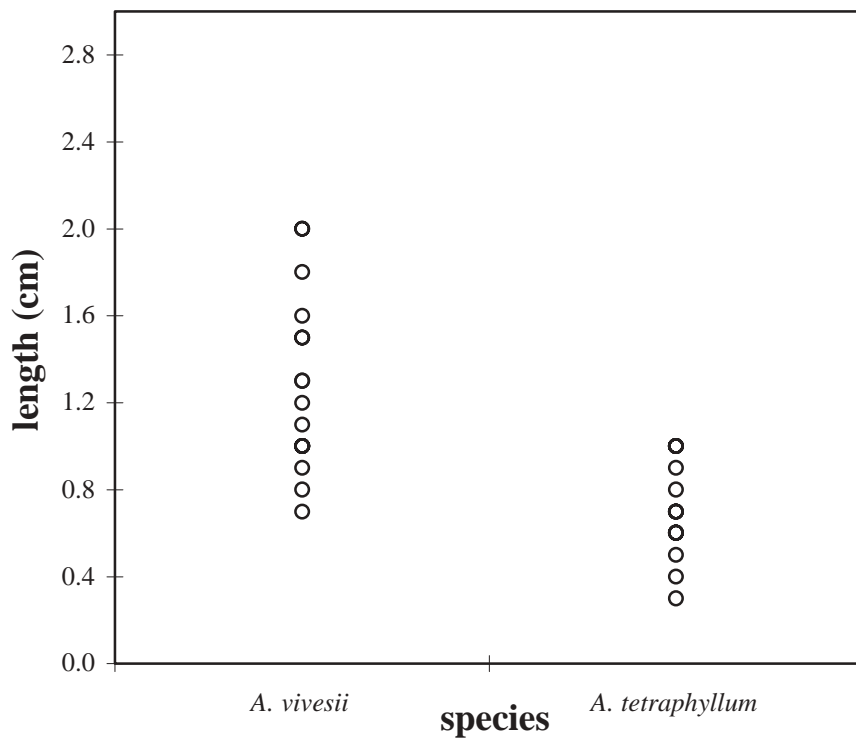


**Figure 18.** Dotplot of width of terminal pinnae in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean width was 7.4 cm for *A. vivesii* and 4.9 cm for *A. tetraphyllum*.

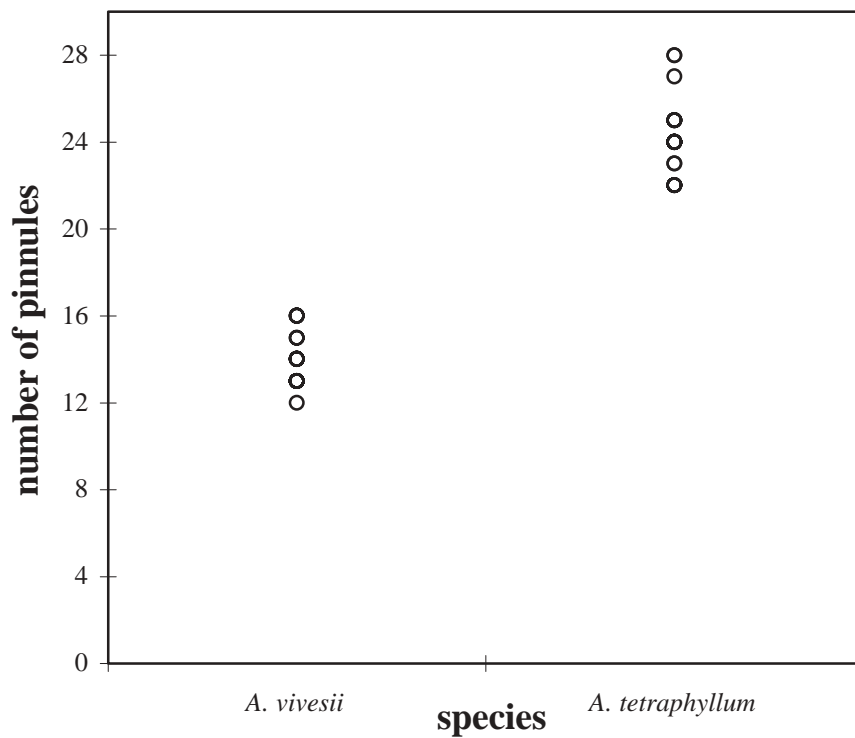


**Figure 19.** Dotplot of length/width of terminal pinnae in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean length/width was 2.9 for *A. vivesii* and 4.0 for *A. tetraphyllum*.

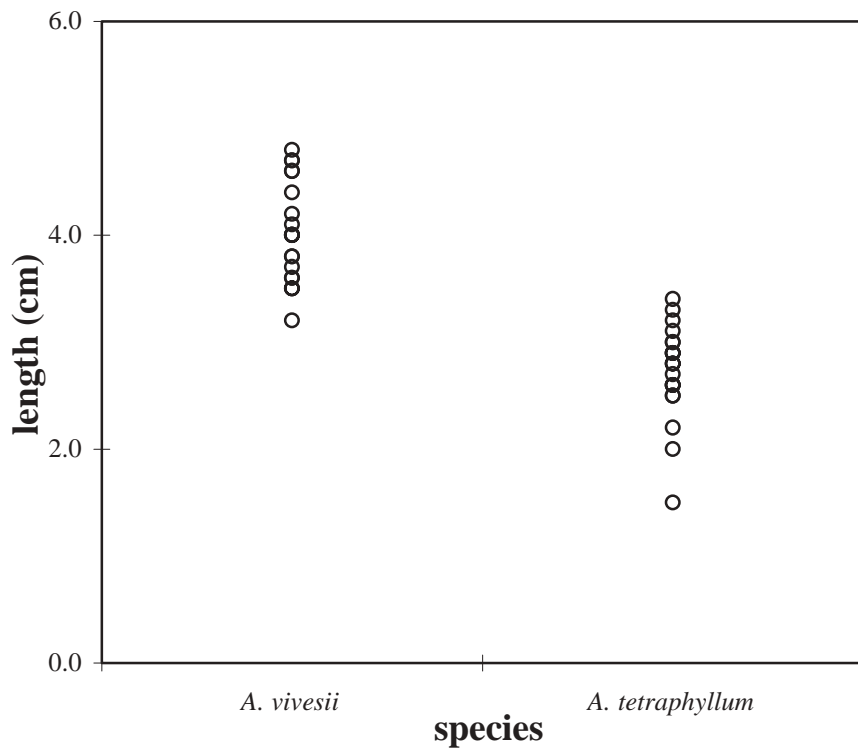




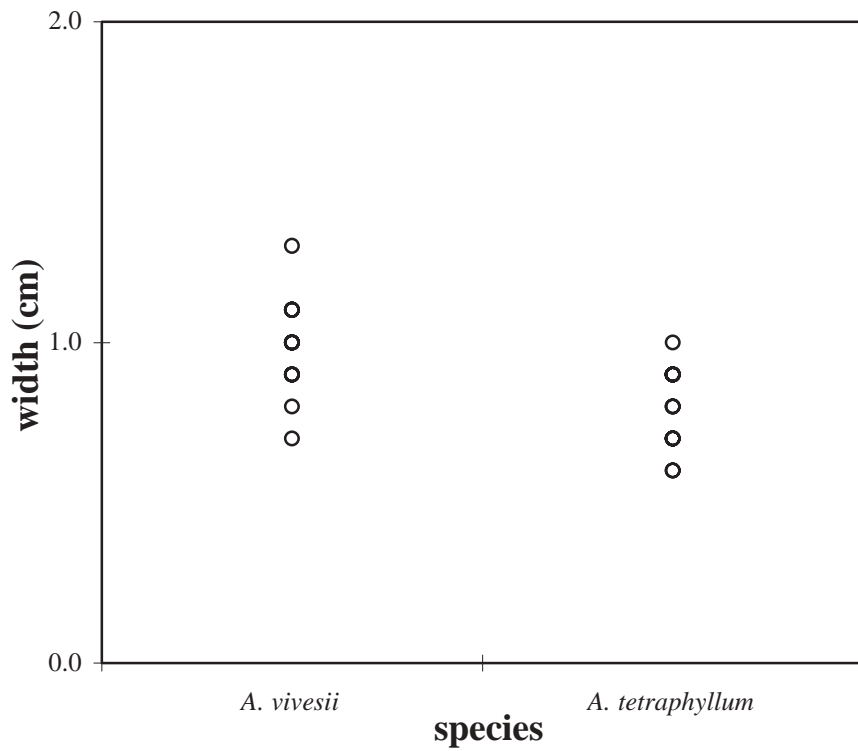
**Figure 20.** Dotplot of petiolule length in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean length was 1.23 cm for *A. vivesii* and 0.75 cm for *A. tetraphyllum*



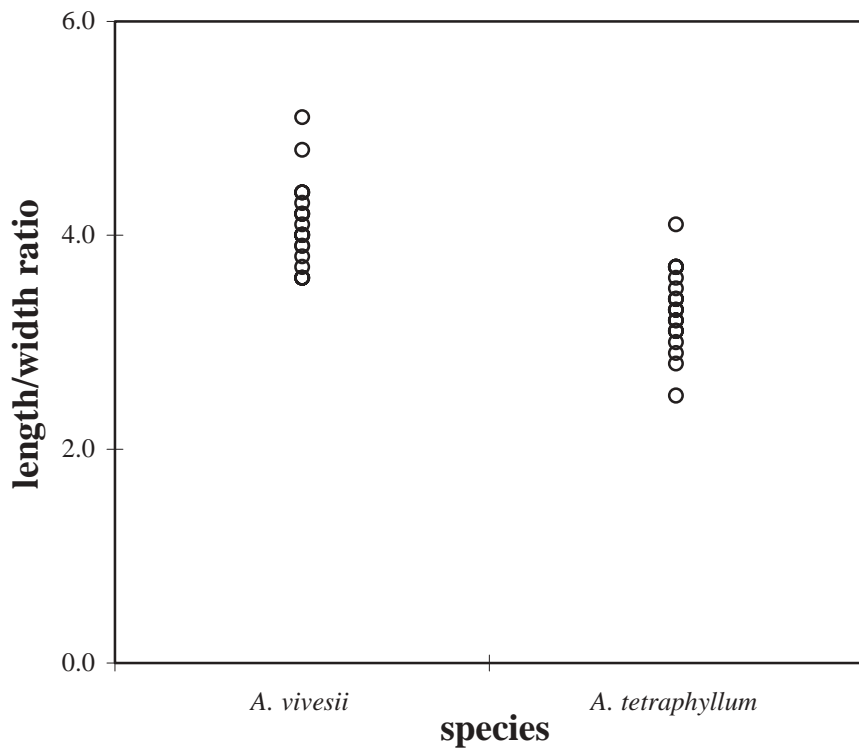
**Figure 21.** Dotplot of number of pinnules on terminal pinnae in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean number was 13.8 for *A. vivesii* and 24.3 cm for *A. tetraphyllum*



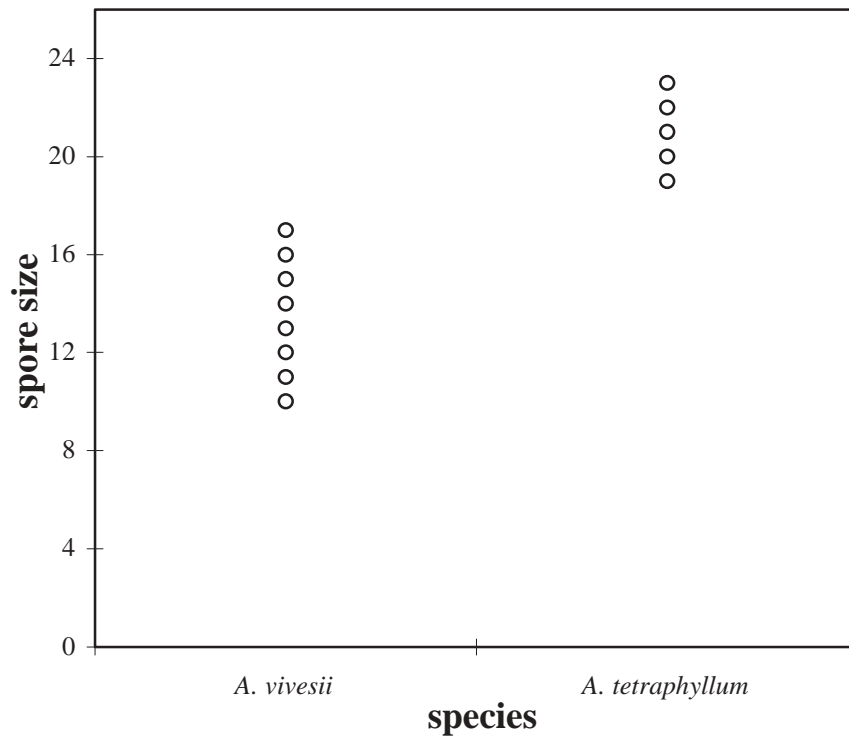
**Figure 22.** Dotplot of length of largest pinnule on terminal pinnae in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean length was 4.0 cm for *A. vivesii* and 2.7 cm in *A. tetraphyllum*.



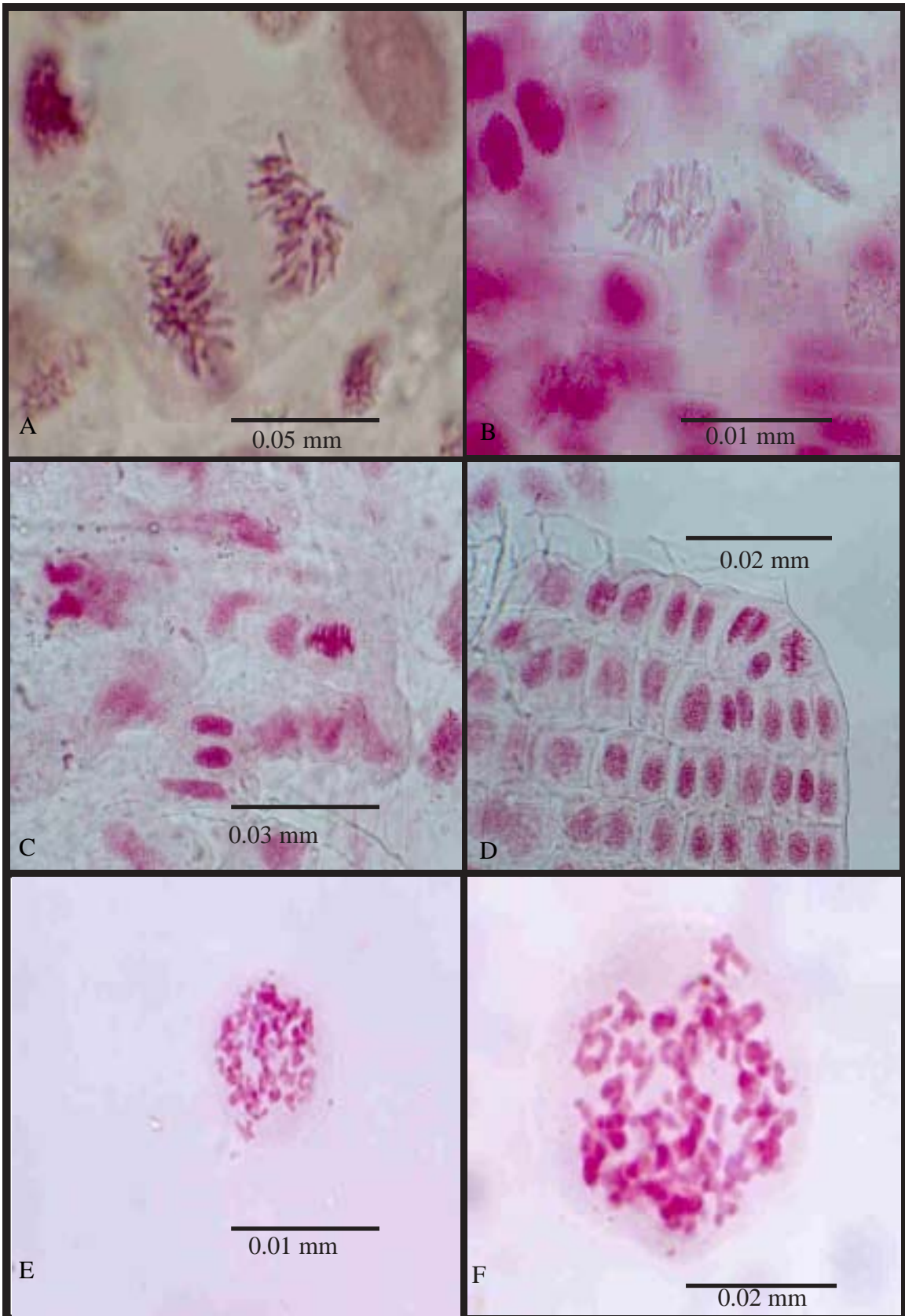
**Figure 23.** Dotplot of width of largest pinnule on terminal pinnae in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean width was 0.99 cm for *A. vivesii* and 0.82 cm for *A. tetraphyllum*



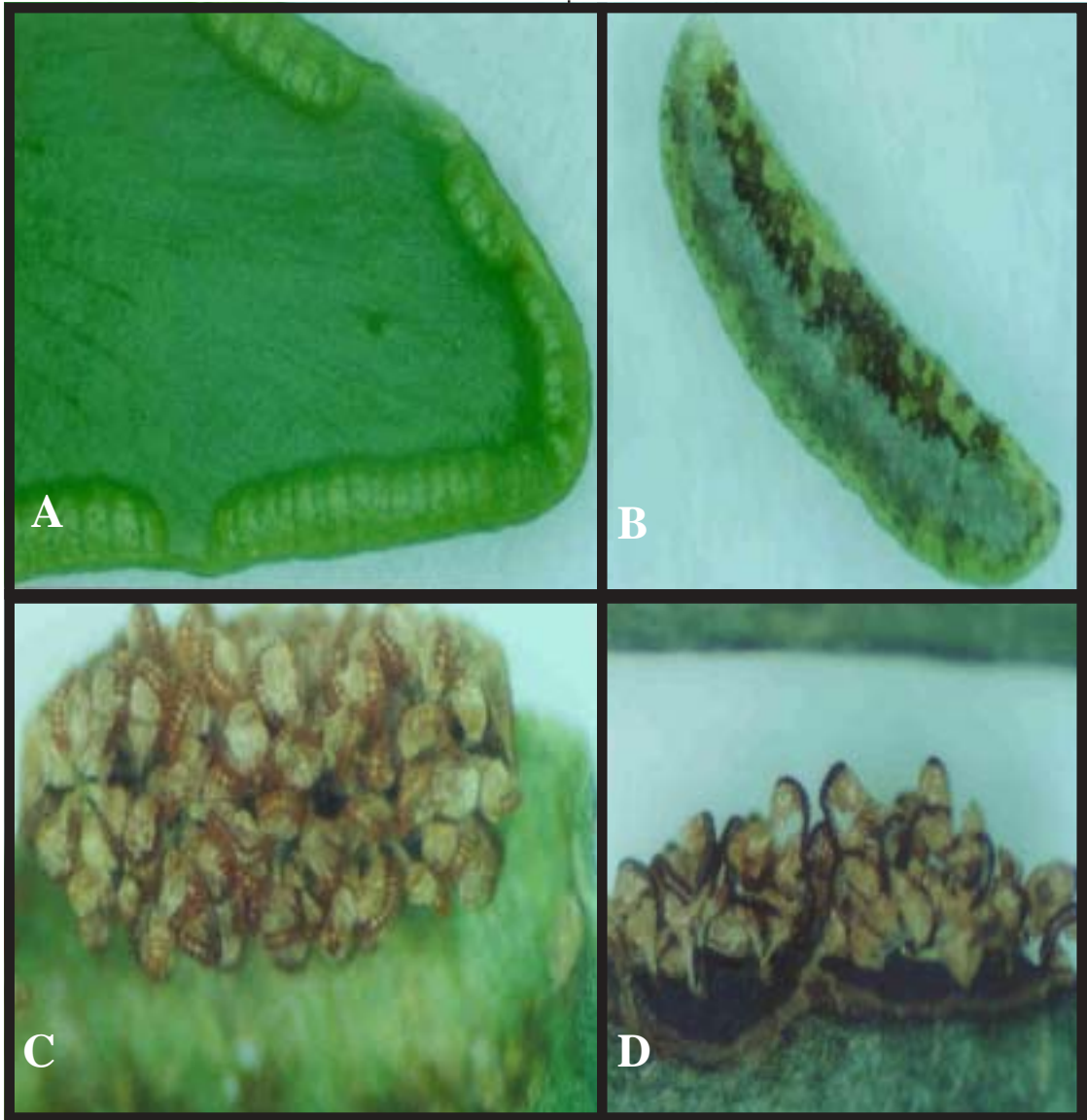
**Figure 24.** Dotplot of length/width of largest pinnule on terminal pinnae in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean length/width was 4.1 for *A. vivesii* and 3.3 for *A. tetraphyllum*



**Figure 25.** Dotplot of spore size (mm) in *Adiantum vivesii* and *Adiantum tetraphyllum*. The mean spore size was 13.6 mm for *A. vivesii* and 20.8 mm for *A. tetraphyllum*.



**Figure 26.** Stages of meiosis in sporogenous tissue in *Adiantum vivesii*.



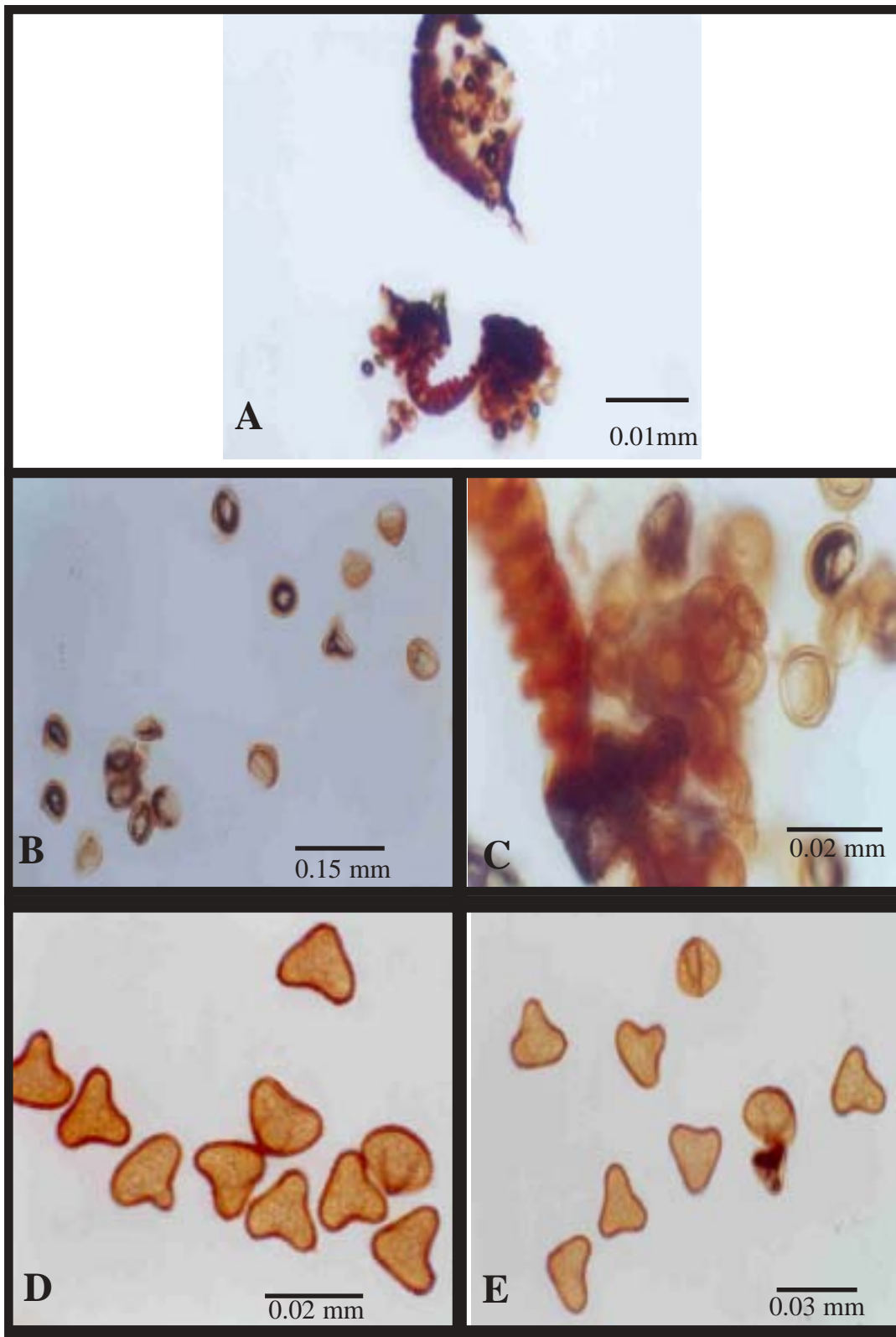
**Figure 27A.** *Adiantum vivesii* pinnule with sori.

**27B.** *Adiantum vivesii* mature sporangia.

**27C.** Mature sporangia of *Adiantum vivesii*.

**27D.** Mature opened sporangia of *Adiantum tetraphyllum*





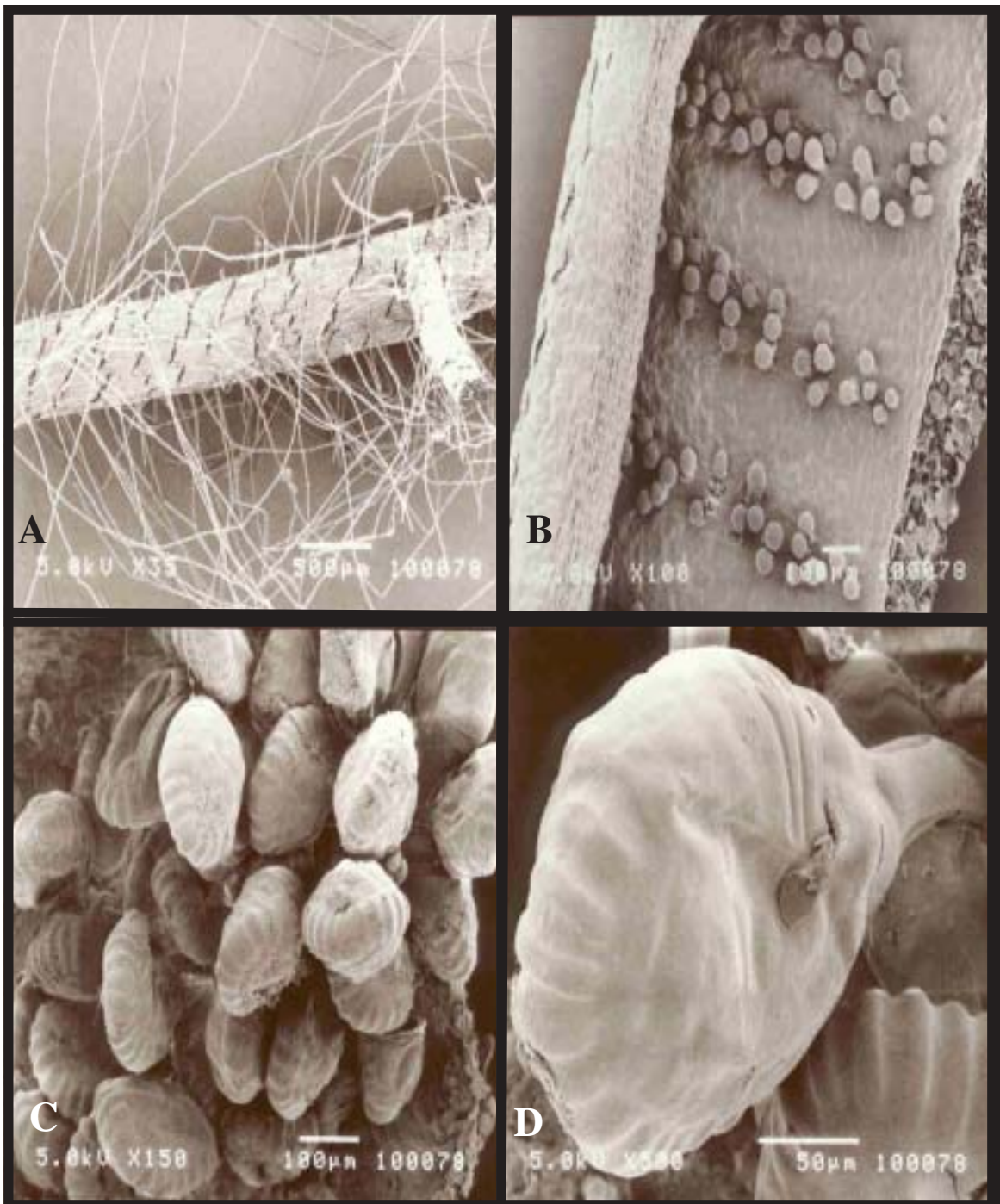
**Figure 28A.** Contraction of annulus and lip cells of *Adiantum vivesii*.

**28B.** Abortive spores of *Adiantum vivesii* (20x).

**28C.** Close-up of abortive spores of *Adiantum vivesii* (40x).

**28D.** *Adiantum tetraphyllum* normal spores (20x)

**28E.** One abortive spore in *Adiantum tetraphyllum*.

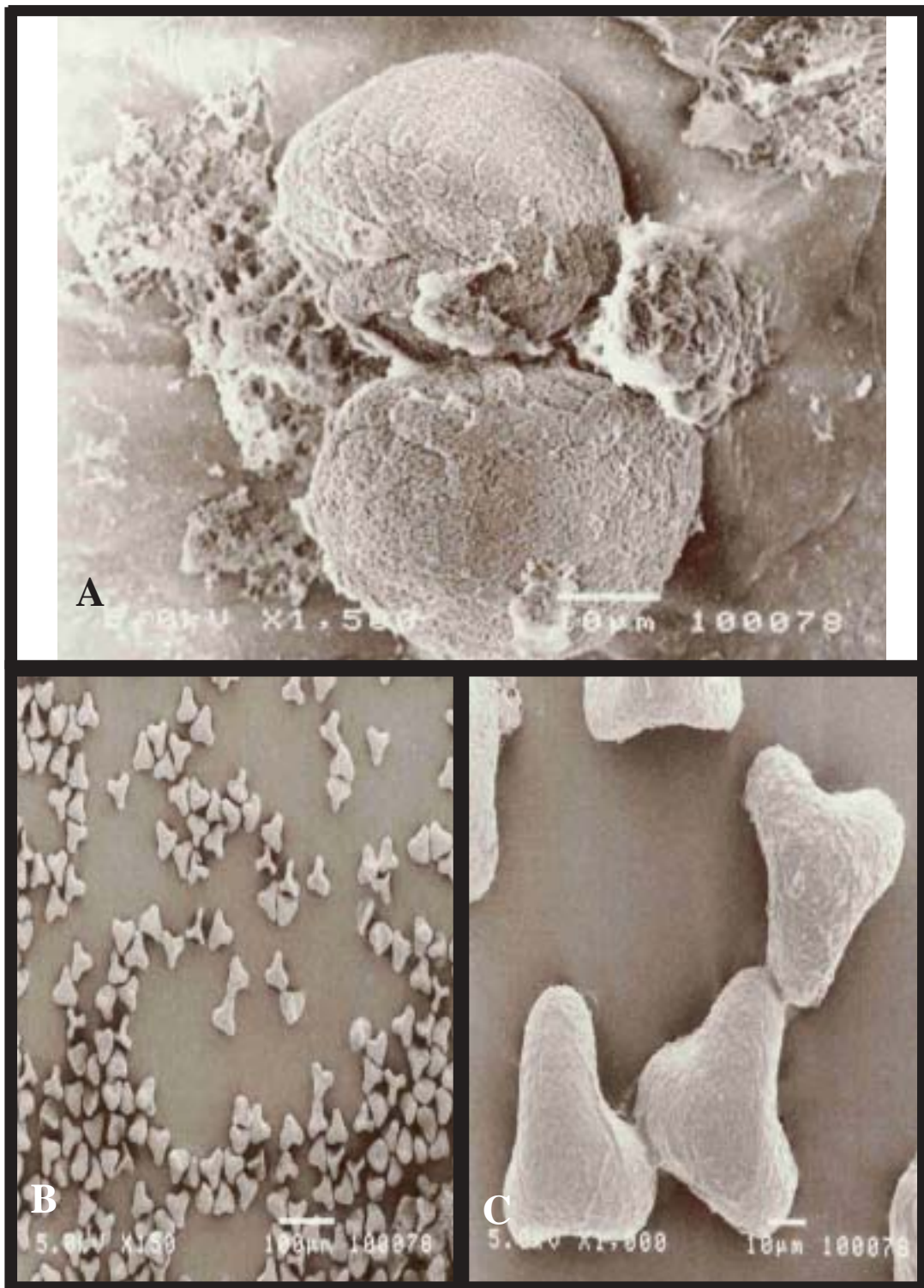


**Figure 29A.** Scanning photograph of rhizome in *Adiantum vivesii*.

**29B.** Scanning photograph of immature sporangia in *Adiantum. vivesii*.

**29C.** Scanning photograph of mature sporangia in *Adiantum vivesii*

**29D.** Close-up of a scanning photograph of mature sporangia in *Adiantum vivesii*.



**Figure 30A.** Scanning photograph of the spores of *Adiantum vivesii*.

**30B.** Scanning photograph of *Adiantum tetraphyllum* spores.

**30C.** Close-up of a scanning photograph of *Adiantum tetraphyllum* spores.