

# Vascular Plant Morphology

## Laboratory 6

### Class Pteropsida

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The class Pteropsida is characterized by pteridophytic plants that have large, dissected leaves, and sporangia that are borne on the leaves. Most species are homosporous, but some are heterosporous. Plants typically have unipolar growth, primary tissues only, and mesarch xylem maturation. Stelar architecture is of the simonostelic or dictyostelic types in most species.

Next to flowering plants, ferns are the most diverse group of living land plants. Groups of ferns, or fern-like plants are derived from the trimerophyte complex, and can be traced back to the Middle Devonian. Several groups (Cladoxylales, Stauropteridales, Zygopteridales) are known only as fossils, but other groups (Ophioglossales, Marattiales, Filicales and Hydropteridales) have living species.

In the past few years our understanding of fern relationships has changed rapidly as the results of systematic studies that use molecular and paleontological data have augmented traditional morphological systematics. Since the last time this course was taught the changes have been dramatic enough to cause us to rethink many of our ideas. The two phylogenetic trees that follow are the latest revisions of our ideas. We will use both to present a better understanding of fern systematics, and to emphasize how scientists must be prepared to constantly revise and update our ideas.

Compare the cladograms follow. You will note both similarities and significant differences because the studies that produced these results were conducted to address quite different questions. Note that the living eusporangiate and leptosporangiate ferns are in the same general arrangement to the leptosporangiates in both cladograms, but there are differences in the taxa included in each. Because the second study uses a much larger number of leptosporangiate taxa and many more characters that are phylogenetically informative for the leptosporangiates, it is probably more accurate for this part of the tree.

This first cladogram represents the results of a study that asks the question "what is a fern". Therefore it includes all ferns and fern-like plants, the psilotophytes (Psilotum and Tmesipteris) and all other groups that may be related to ferns. What are the relationships among ferns? Do they form a monophyletic group? Note also the relationships of the equisetophytes and of the lignophytes (these are the seed plants and their relatives, the progymnosperms). Do you recognize any of the other taxa used in this analysis? Do they occur in the positions you would suspect them to occupy by what we have already covered in this course?

Strict consensus tree of 12 most parsimonious trees of 429 steps from the analysis of 101 morphological characters of 51 living and extinct taxa (Rothwell, 1999). The arrow indicates the node at which the Hydropteridales is attached.

This second cladogram represents the results of a study that addresses the relationships

among leptosporangiate ferns. It also includes eusporangiate ferns with living species (i.e., Ophioglossales and Marattiales), but these are used primarily as outgroups. (Do you know what an "outgroup" is?)

Figure 11 of Pryer, Smith and Skog (1995), showing the results of a combined analysis of morphological and molecular (*rbcL* gene sequencing) data for living species of fern taxa.

## Order Ophioglossales

The Ophioglossales are broadly included within the "ferns" but differ from the remaining ferns in several respects. Their phylogenetic relationship to the remaining ferns is obscure, and it has been suggested that they may be more closely allied with the progymnosperms. The best fossil record for the order consists of Botrychium specimens from basal Paleocene deposits. These tell us little about the origin and progenitors of the order, but they do demonstrate that modern-appearing species had evolved by the early Tertiary. Therefore, the group is quite ancient.

Three genera are usually included in the Ophioglossales. Botrychium has about 35 species in tropical and temperate regions, and is sometimes called the grape fern or moonwort. Ophioglossum, with about 45 species, is also widespread, and some forms are called adders-tongue ferns. Helminthostachys is monotypic and occurs only in Malaysia and Polynesia.

The Ophioglossales have a number of features that are rarely if ever found in other fernlike plants and some of which are characteristic of progymnosperms and seed plants. (1) The stele of the stem is a eustele, and its component bundles are collateral. No other fernlike plants are known to have a eustele. Eusteles with collateral bundles are characteristic of seed plants and some progymnosperms. (2) In the stem of Botrychium, there is what appears to be secondary xylem produced from a vascular cambium. No other fernlike plants, including the other members of the Ophioglossales, have secondary growth. Progymnosperms and gymnosperms have secondary growth from a vascular cambium, but it is bifacial whereas the cambium in Botrychium is unifacial (it produces secondary xylem but not secondary phloem. Another possibility is that the radial alignment of the trachieds in Botrychium results from the activity of a primary thickening meristem. If true, there may be no vascular cambium in this genus. (3) Primary xylem maturation is endarch, like extant gymnosperms but unlike true ferns, progymnosperms, or primitive gymnosperms. (4) The tracheids in Botrychium have circular or elliptical bordered pits, like those of progymnosperms and seed plants, while those of Ophioglossum have the scalariform pitting that is characteristic of true ferns.

Although the similarities between Ophioglossales and progymnosperms are striking, the characters do not strongly support a phylogenetic relationship between the two groups. The four characters listed above all appear to be derived, and hence a valid basis for inferring phylogenetic relationship. However, since they all vary within either the Ophioglossales or the progymnosperms, it is quite possible that they were independently derived in the two groups. On the other hand, the only similarity between Ophioglossales and true ferns are the homosporous life cycle and the fernlike foliage. The homosporous life cycle is considered to be ancestral among vascular plants, and therefore not a good character for inferring close relationships between groups or vascular plants. Fern-like leaves are known to have evolved at least twice and probably many more times in the Paleozoic, and therefore, this similarity is very likely due to convergent evolution. The Ophioglossales may well represent an independent lineage not closely related to any other known plants, living or extinct.

## Botrychium

Examine a portion of a frond having vegetative segment (**trophophore**) and a fertile segment (**sporophore**). Note that the fertile part branches several times and that the sporangia are massive and slightly stalked. A line of dehiscence may be seen on the relatively mature sporangia. The sporophore of this genus is thought by many morphologists to represent a pair of fused, erect pinnae, so that the sporangia are foliar (i.e., they are borne on the leaf).

Herbarium specimens of complete plants of Botrychium are in the laboratory. Examine these and note the form of the stem and the fleshy roots. Sketch a whole plant of one of the specimens.

Examine a prepared slide of a transverse section of the rhizome of Botrychium. Note the central pith and the surrounding area of primary xylem (of which there is very little) and the radial files of tracheids and vascular rays that have been interpreted by some authors as secondary xylem. Gaps that may be visible in the vascular cylinder are associated with leaf divergence but are not equivalent to the leaf gaps of a siphonostele. In the former there are parenchymatous breaks in the cylinder of secondary xylem, while in the latter there are gaps in an otherwise uninterrupted cylinder of primary xylem.

The stele in Botrychium is a eustele that lacks leaf gaps in the primary xylem. Note the phloem region is rather indistinct. The cortex is made up of soft tissue with thin-walled cells. Root traces may be present in the section.

Examine a prepared slide cut through a portion of the sporophore spike of Botrychium. Note the thickness of the sporangial walls and the layer of several cells inside of the wall proper. The latter tissue is nutritive in function and is called tapetum. Within the sporangium you will see numerous sporocytes (spore mother cells) or spores, depending upon the developmental stage. Very numerous spores are produced, in excess of 2000 per sporangium.

## Ophioglossum

Examine the herbarium specimen of Ophioglossum to gain an idea of the appearance of the genus. Note the short, thick stem, and the leaf that is divided into a trophophore and a sporophore. How does the sporophore differ from that of Botrychium? Sketch the complete plant.

Examine a cross-section of the sporophore. Note that the sporangia are arranged in two series. These sporangia are embedded and fused into a compact structure. Can you tell how the sporangia open? Examine an herbarium specimen to check this. Note the thickness of the sporangial walls, the tapetum, and the large number of sporocytes or spores depending upon the developmental stage.

Examine transverse sections of the root, rhizome, and stipe. Note the arrangement of the tissues in each.

### Order Marattiales

The Order Marattiales includes about 200 species of living ferns that are generally grouped into either six or seven genera. These genera include: Marattia, a pantropical genus; Danaea, a genus confined to the tropical new world; and Angiopters, an old world genus. In addition, there are three relatively rare genera which will not be dealt with here. In this course we are going to regard all the living genera as members of a single family, the Marattiaceae. The marattiales are eusporangiate ferns.

In addition to the living Marattiales, extinct arborescent forms are known from conspicuous Carboniferous and Permian fossils. These are generally assigned to the family Psaroniaceae, based on the genus Psaronius, a common form of petrified stem.

The Marattiales is a distinctive group of ferns that have some features in common with the Filicales (sometimes called the true ferns). The overall habit of a typical marattialean is much like that of the Filicales, as is the vascular pitting, that is scalariform. The marattialeans have their sporangia arranged into more-or-less fused groups (synangia) on the lower (abaxial) surface of the frond. This grouping is a **sorus** of fused sporangia. The sorus is characteristic of most members of the Filicales. Although some marattialeans (particularly the genus Angiopters) have sporangia that are barely fused (only at their bases), for the purposes of this course we will consider all marattialeans to be synangiate.

As stressed above, a distinctive feature of the Marattiales is the presence of synangia. In this group, one of the major features of evolution of the sori has involved the fusion of the sporangia. In some cases, the resulting structure (synangium) is embedded in the lower portion of the leaf while in others it is fully exposed on the surface. In laboratory we will concentrate primarily on these characteristic spore-producing structures of the most common genera.

Examine an herbarium specimen of the frond Angiopters or Danaea. Note the location of sporangia. To study the details of this sporangial arrangement, obtain a portion of a fertile pinna. Can you identify individual sporangia? How would you describe the arrangement of the sporangia in each synangium? Where did the sporangia dehisce? Draw a typical synangium as it appears from above.

Examine a prepared slide of fertile Angiopters frond, cut so as to run through several sporangia of a synangium. Note the thick walls, the tapetal layer, and numerous spores of sporocytes depending upon the developmental stage present. Can you tell where the sporangia will dehisce? Draw a typical sporangium. Examine a transverse section of the rhizome of Marattia.

Now examine an isolated pinnule of Marattia. Compare the synangia to those of Angiopteris. Draw a single synangium.

Examine a prepared slide of synangia of Marattia. Note the nature of the sporangial walls, the large number of spores, and the area of dehiscence.

### Fossil Marattiales

The ancestors of the earliest marattialean are completely unknown, but by the beginning of the Pennsylvanian period, large tree ferns with the essential features of this group had evolved. These forms became extinct for the most part by the end of the paleozoic, and were much more complex in their vegetative anatomy than are any living marattialeans. Their fertile parts were generally less modified than those of most of the living forms, and the sporangia tended to be less fused.

Examine a specimen of Psaronius and note the position of the fronds on the stem (leaf scars) and the vascular tissue in the stem. The stele is a complex polycyclic dictyostele with many leaf gaps.

Examine a transverse section through a permineralized Psaronius stem. This specimen shows the cellular details of the tissues. Locate the numerous cycles of xylem and phloem. Note how leaf gaps occur in all of the cycles. You should be able to see leaf traces. Some adventitious roots that formed a thick mantle about the stem are present surrounding the stem proper. These roots are actinostelic. Diagram this transverse section.

The Psaroniaceae produced large fronds that had foliage of a type called Pecopteris. Examine a specimen of a part of a typical frond. Note that the frond had an appearance much like that of numerous living ferns. Specimens of isolated pinnae are also present. This is the most common way in which foliage of these plants occurs.

Examine fertile frond material of Psaroniaceae. Several genera of fertile parts are known and are differentiated upon the features of the sporangia, such as numbers, degree of fusion, etc.

Perhaps the most common type of Psaronius fertile structure found in a permineralized condition (that is, with the cellular anatomy preserved) is Scolecopteris. Examine a specimen and note the features of this fertile part. Which of the genera of living marattialeans is it most like? Draw a typical cluster of sporangia of Scolecopteris.

A reconstruction of a Psaroniaceae fern is on demonstration. Note its general appearance. How does this compare with living members of the order?

## Order Filicales

This is the single largest order of non-seed-producing vascular plants and contains approximately 10,000 species grouped into about 300 genera. The Filicales are often called the true ferns or leptosporangiate ferns. The exact number of families is subject to a great deal of debate. Some authors delimit as many as 35 or 40 families, while others recognize as few as seven. To consider this order in detail is beyond the scope of this course. Much is known concerning the morphology and anatomy of the ferns, which are extremely diverse in form and structure. We will deal with certain basic features of the order and its more easily recognizable families.

In this course we will recognize the same seven families included in Gifford and Foster (1989). Because of time constraints, however, we will examine members of only four. The seven families are listed below. Note also the molecular phylogeny that recently was published by Hasebe, et al. (1994; on the next page). This provides a first-approximation, testable hypothesis for phylogenetic relationships among filicalean and heterosporous leptosporangiate ferns. How does it compare to the Pryer, Smith and Skog (1995) tree?

Osmundaceae - 3 living genera forming a natural intermediate in numerous respects between the eusporangiate ferns and the families below.

Schizaeaceae - 4 living genera forming a relatively primitive and possibly unnatural group.

Adiantaceae - Ferns with sporangia in lines along the veins, vertical annulus, and often with a false indusium.

Gleicheniaceae - 2 living genera forming a relatively primitive natural group.

Mataoniaceae - Indusiate ferns with simultaneous maturation of sori and an oblique annulus on the sporangium.

Cyatheaceae - Largest family of ferns in the classification used in Gifford and Foster (1989). The eight subfamilies recognized in this scheme (pages 303-305) include tree ferns (i.e., Cyatheiroideae) and filmy ferns (i.e., Hymenophylloideae), as well as a large number of highly advanced forms. The latter belong to several lineages and represent some of the most highly derived ferns.

Polypodiaceae - As used by Bower, this family is an unnatural grouping of the most advanced filicalean ferns. In this course, however, we will view the family in the much more restricted sense of Wagner. This is consistent with the treatment found in Gifford and Foster (1989). Representatives of the family typically have sporangia grouped into sori with mixed development and have no indusium. Sporangia are small with a vertical annulus and a small number of spores. The rhizome usually is dictyostelic, and the pinnules of the fronds may have anastomosing veins (i.e., net venation).

Species of the filicales generally have sporangia present in definite groupings. The

individual grouping or cluster is the **sorus** (pl. **sori**), whose members not only are related in position, but are also related developmentally. The central area of the sorus is sometimes modified into a mound-like or rod-like projection and is called the **receptacle**.

If all of the sporangia have synchronous development the sorus is called **simple**. In other instances, the development is in a rather specific direction within the sorus. This is called a **graduate sorus**. The most common type of graduate sorus involves the maturation of successive sporangia toward the margin of the sorus from the sural center. In members of the order that are interpreted as being the most highly advanced, the sporangia of a sorus develop over a relatively long period of time, and the sequence of development is not in any definite order. The term **mixed sorus** is applied to this mode of sporangial development, and the ferns having this feature are sometimes referred to as the Maoist. The evolutionary trend in sporangial development appear to be simple to graduate to mixed.

Some members of the Filicales lack sori. The family Osmundaceae apparently represents a relatively primitive group that is non-sural. The sural condition found in the vast majority of the Filicales appears to have evolved from the non-sural condition. However, a second group of non-sural filicaleans appears, on the basis of numerous features, to be highly advanced within the order. Some of these forms have the so-called acrostichoid condition which has been derived from ancestral forms having sori.

#### Family Osmundaceae

Examine the herbarium specimen of Osmunda. Note the structure of the fertile frond. Examine fertile frond material of Osmunda. Note the form of the sporangium, its relatively massive size, its stout stalk, and its mode of dehiscence. A lateral patch of cells with thick walls makes up the annulus. This sporangium is the most primitive type found among the Filicales. Draw a typical sporangium of Osmunda. Examine transverse sections of the rhizome of Osmunda. What type of stellar configuration does each display?

#### Family Schizaeaceae

Examine an herbarium specimen of Lygodium. What is the plant habit? Examine fertile frond material of Lygodium or some other member of the family. Note that the sporangia are not in sori. Each sporangium has a sizable stalk and an annulus that forms a cap at the distal end or tip of the sporangium. This apical annulus is characteristic of the Schizaeaceae. How does the sporangium dehisce? Draw a typical sporangium of a member of the Schizaeaceae.

#### Family Polypodiaceae

This small family contains ferns with naked sori and, sporangia that typically have a small capsule with a vertical annulus and a narrow stalk. Examine a plant of Polypodium. Note the disposition of the sori. Now place some sporangia on a slide and draw a typical sporangium. Polypodium has sporangia that are considered to be of the most highly derived type for filicalean ferns. Is this sporangium in the pre- or postdehiscence configuration? How does it function in spore dispersal?

## Family Cyatheaceae

Most of the filicales are assignable to the Cyatheaceae as described by Gifford and Foster (1989), and many of the members are assignable to highly derived lineages. Two of the more ancient and distinct subfamilies that we will cover in this lab are the filmy ferns (Hymenophylloideae) and the tree ferns (Cyatheoideae).

### Subfamily Hymenophylloideae

Examine a specimen of Trichomanes. Note that the sori have elongate receptacles and that these sori are marginal. The annulus is horizontally oblique on the individual sporangia and they dehisce obliquely laterally. Unlike the previous families, the development of the sporangia is gradate in Hymenophyllodeae. The Gleicheniaceae, Schizaeaceae, and Osmundaceae have simple sori. Draw a typical sporangium of Hymenophyllaceae.

### Subfamily Cyatheoideae

The Cyatheoideae are characteristically tree ferns with distinct vegetative anatomy, habit, and fertile parts. Examine a section of a Cyathea stem. Identify the leaf and bundle scars. Are secondary tissues present? Examine a portion of the fertile frond of Alsophila and note the sori which are superficial and have cup-shaped indusia. The sporangia are relatively small compared to those of the families above and have an oblique annulus about the sporangium. Dehiscence is like that of the Hymenophyllaceae. Draw a typical sporangium of Alsophila.

## Gametophytes

The members of the order Filicales are homosporous and usually produce gametophytes that are potentially bisexual. The fern gametophyte is often called a prothallium. In the typical case the mature gametophyte is bright green, surface-living, and somewhat heart-shaped in outline. This prothallium is relatively thin and generally bears rhizoids on the lower surface. The lower surface is the place where the sex organs also develop.

Examine living or prepared slides of fern gametophyte noting the general features. Note the apical notch, the area where new cells are produced. You should be able to locate sex organs with relatively low magnifications. The antheridia are globose and have a wall consisting of a

single layer of cells. Developing sperm appear as coiled or curved structures within the antheridium. Where on the lower surface are these male sex organs produced? How are the sperm released? Draw the prothallium indicating several antheridia. Draw a single antheridium showing its cellular detail.

Examine living gametophytes or prepared slides and locate archegonia. You will be able to identify these by the prominent necks which project above the level of the thallus. Note that relatively few tiers of cells are present in the neck. If an early developmental stage is present you will see an elongate cell, the neck canal cell, in the central part of the neck. In older stages a canal is present in the neck. This canal leads to the egg. Where on the gametophyte do the archegonia develop?

### **Heterosporous Leptosporangiate Ferns**

There are five genera of living leptosporangiate heterosporous ferns, Marsilea, Regnellidium, Pilularia, Salvinia and Azolla. The first three traditionally have been thought to be derived from among the Schizaeaceae and to have evolved heterospory independently of the last two, which are thought to be derived from among the Hymenophyllaceae. However, recent paleobotanical and molecular studies have independently suggested that the heterosporous ferns are monophyletic (Rothwell and Stockey, 1994; Hesebe, et al., 1994). Note the relationship of Marsilea and Salvinia in the Hasebe et al. tree (presented above), and compare this to the tree from Rothwell and Stockey (presented on the next page).

### **Order Hydropteridales**

#### Family Marsileaceae

The Marsileae is the first family of heterosporous aquatic ferns. The sporophyte consists of a horizontal rhizome that bears roots and upright leaves. Special sporangia-bearing structures called **sporocarps** are borne on the stipe. The three genera show distinctive variation with regard to leaf form. Marsilea has four leaflets, Regnellidium has two, and Pilularia fronds are simple grass-like structures. All three genera exhibit circinate vernation.

Relationships Among Heterosporous Ferns from Rothwell and Stockey (1994).

### Marsilea

Examine the living plant and the herbarium specimen of Marsilea. Identify the rhizome, leaves, roots, and sporocarps. Sketch the whole plant.

A Marsilea sporocarp was scarified and placed in water three hours before class. Examine the sporophore and saclike sori that have emerged from the sporocarp. You will be able to see megasporangia and microsporangia. How many megaspores can you see in each megasporangium? Draw a megasporangium and microsporangium.

During the following laboratory period you should be able to see swimming sperm. Examine the finger bowl for these. Also examine the young Marsilea sporophytes from a sporocarp that was germinated about three weeks ago. Can you identify the parts? Sketch a young sporophyte.

### Regnellidium

Examine a plant of Regnellidium (if available). Compare the morphology of this genus to that of Marsilea (above) and Pilularia (below). Concentrate particularly on the morphology of the leaves. Do you find crosiers? Draw a frond of each of the three genera.

### Pilularia

Examine a plant of Pilularia (if available). Be careful not to damage the specimen, as it cannot be replaced. Identify the various parts. Is this specimen fertile? Locate some developing leaves. Sketch the whole plant of Pilularia. Be sure to include a developing leaf.

### Families Salviniaceae and Azollaceae

The Salviniaceae and Azollaceae are represented by two extant genera. Fossils of Salvinia occur in mid Cretaceous and more recent rocks. Azolla is usually the smaller of the two and has a fossil record extending back to the Upper Cretaceous. As in the Marsileales the sporangia of Salvinia and Azolla are enclosed in sporocarps.

### Salvinia

Examine the living or preserved specimen of Salvinia. Identify the leaves and stem. The root-like structures are actually modified leaves. Salvinia has no true roots. Draw the whole plant.

### Azolla

Examine the living specimens of Azolla. Identify the leaves, stem and roots. Now examine the prepared slides of Azolla sporocarps. Become familiar with all the parts; this may require looking at more than one slide. How do the sporocarps of the Salviniaceae compare to those of Marsileales? Be sure you understand the structure and function of the megasporangiate and microsporangiate parts. Draw a megaspore as you have reconstructed it in your mind. Examine the demonstration slide of microsporangiate massulae. Identify the microspores. Sketch this structure.

## Literature

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