

**ANATOMICAL AND PALYNOLOGICAL STUDIES ON  
SOME FILICALES FROM NEELUM VALLEY,  
MUZAFFARABAD, AZAD KASHMIR.**

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**Abstract**

Four ferns viz., *Polystichum nigroplacium* (Christ) Diels (Aspidiaceae), *Woodwardia unigemmata* (Makino) Nakai (Blechnaceae), *Asplenium adiantum nigrum* L (Aspleniaceae) and *Adiantum capillus veneris* L. (Adiantaceae) were collected from Neelum Valley, Muzaffarabad, Azad Kashmir and identified. The anatomy of the rachis of *Polystichum nigroplacium*, *Woodwardia unigemmata* and *Asplenium adiantum nigrum* showed di-tri-tetrarch stele in the rachis. The rachis anatomy of *Adiantum capillus veneris* revealed a thick walled heavily cutinized epidermis and hypodermis while the ground tissue was made up of parenchymatous cells with air spaces. The ornamentation pattern of the spores of *Adiantum capillus veneris* is unique and can be referred as means of identification of the species.

**Introduction**

Ferns appear to have fewer taxonomically informative morphological characters than seed plants because they lack flowers, which provide valuable characters for analyzing evolutionary relationships (Yatabe *et al.*, 2001). In systematic research on the ferns, cytotaxonomical and enzyme electrophoretic data have revealed cryptic species in many lineages such as *Adiantum*, *Botrychium*, *Pityrogramma* etc., (Paris *et al.*, 1989).

Filicophyta represent the most dominant successful group of lower vascular plants. The group has been classified accordingly by different workers into Eusporangiate, Osmundidae and Leptosporangiate or Filicales (Hirmer, 1927; Holttum, 1947, 1949). Filicalean ferns are well known as components of Cretaceous and Tertiary vegetation, where they usually have been included in floristic treatments of compressed angiosperm dominated assemblages (Tidwell & Ash, 1994; Collinson, 1996).

A significant component of recent phylogenetic research regarding the filicalean ferns has been the demonstration that several extant families with relatively early fossil records, including the Gleicheniaceae, Matoniaceae, Osmundaceae and Schizaeaceae occupy basal or near basal phylogenetic positions (Hasebe *et al.*, 1995; Pryer *et al.*, 1995; Pryer *et al.*, 2004; Stevenson & Loconte, 1996; Rothwell, 1999).

According to Pryer *et al.*, (1995), there is good support for Osmundaceae being the most basal leptosporangiate fern family. This is concordant with the fossil record for Osmundaceae which extends into the late Permian, one of the oldest known extant leptosporangiate fern families (Stewart & Rothwell, 1993). The strong bootstrap support for the leptosporangiate clade (including Osmundaceae) indicates that despite its few eusporangiate-like features, Osmundaceae is more closely related to leptosporangiate ferns than it is to any other group.

According to Christensen (1938) there are about 235 genera and 8700 species showing the importance of this group that majority of true ferns are assignable to Leptosporangiatae. Next to the flowering plants, the Leptosporangiate ferns are the most diverse group of living land plants. Recent estimates place their diversity at about 12,000 species in 300 genera (Camus *et al.*, 1992). Most of the fern flora of Pakistan has been categorized as belonging to Leptosporangiatae except the genera *Ophioglossum*, *Botrychium*, *Osmunda*, *Salvinia*, *Azolla* and *Marsilea*. The plains of Pakistan are quite dry for ferns and only a few fern species like *Adiantum*, *Goniopteris*, *Pteris* and *Ophioglossum* are found in these areas, but the number of ferns increases with the increase of height where temperature decreases and moist conditions are favourable. Filicales have been studied extensively (Pigg & Rothwell, 2001; Duckett & Ligrone, 2003; Yatabe *et al.*, 2001; Kentner & Mesler, 2000; Carlquist & Schneider, 2001; Hererro *et al.*, 2001).

The fern flora of Pakistan is not completely known, especially the morphology, anatomy, and palynology has not been investigated although some work has been done on certain ferns (Murtaza *et al.*, 2004; Zamurad, 1982). The present studies were undertaken to describe the anatomy and palynology of selected ferns of the order Filicales found in the Neelum Valley, Muzaffarabad, Azad Kashmir.

### Materials and Methods

The specimens of four ferns viz., *Polystichum nigroplacium*, *Woodwardia unigemmata*, *Asplenium adiantum nigrum* and *Adiantum capillus veneris* were collected fresh along with rhizome. The material was preserved in acetic alcohol (1:3) till further use. For anatomical studies the transverse sections of the rachis were made. The sections were stained with 2% saffranin. The sporangia were detached from the fertile fronds with the help of needle under binocular microscope and then mounted in glycerin jelly on a glass slide. For spore investigations, the sori were crushed with glass rod and passed through 1.5 inch diam., funnel placed in a centrifuge tube and plucked with loosely placed glass wool. The sieved spores treated with 5% KOH solution to remove the oils and humic acid. The spores were chlorinated as suggested by Bhutta & Sadiq (1987). After chlorination the spores were mounted in 2 % glycerin jelly already stained with 2% saffranin. The mounted slides were sealed using synthetic enamel on a ringing disc. After staining with 2% saffranin solution the sections were mounted in Euperal for microscopic studies (Murtaza *et al.*, 2004). Microphotographs were taken on Photomicroscope Kyowa Medilux-12. All the specimens and other material used during this study were deposited in The Department of Botany, Azad Jammu and Kashmir University, Muzaffarabad.

### Results and Discussion

**Anatomy of rachis:** In the rachis of *Polystichum nigroplacium* the leaf trace is intermediate where the petiole receives 2-3 strands, two stronger which are adaxil and unite together upward dividing into 4-6 strands (Fig. 1), whereas in *Dryopteris* there are two strands in the rachis (Zamrud, 1982). Similarly the *Athyrium* also have two strands but they fuse upward. Hypodermis is composed of rounded and thick walled cells. The ground tissue consists of loosely packed parenchymatous cells.

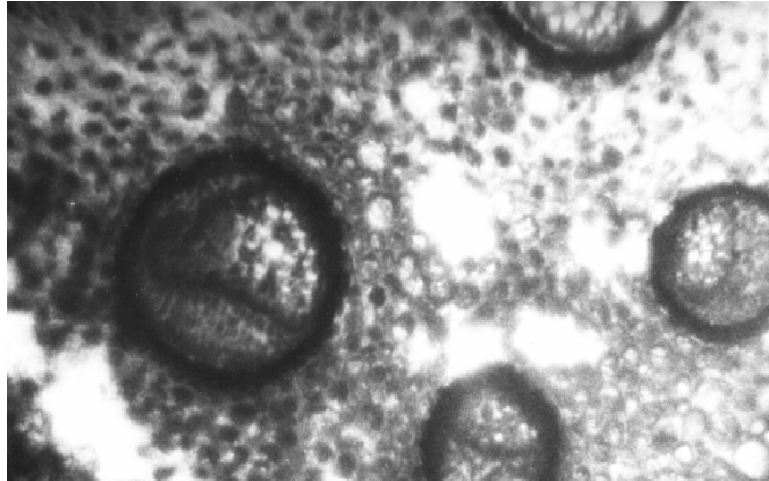


Fig. 1. T. S of rachis of *Polysticum nigroplacium* showing two large and two small meristemes.

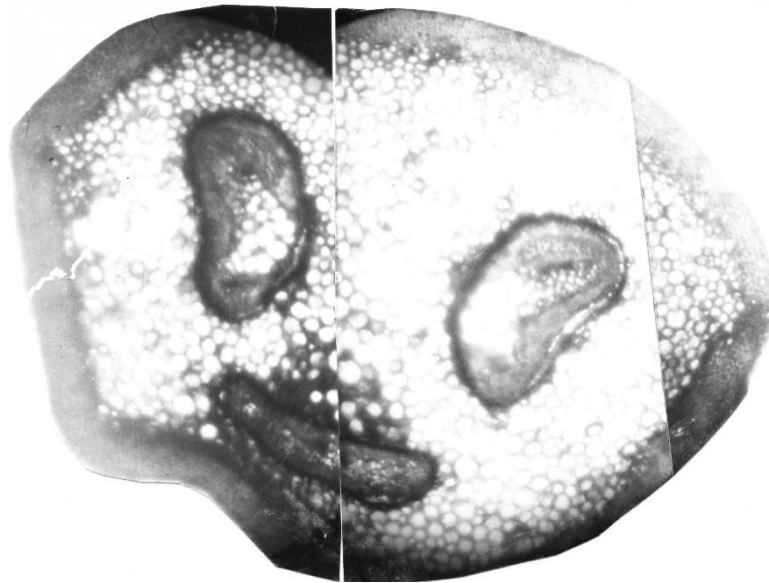


Fig. 2. Transverse section of rachis *Woodwardia unigemmata* showing 3 meristemes.

The cross section of rachis of *Woodwardia unigemmata* shows 3 meristemes supplying their connections to the laterally attached pinnules. The outer most region is mainly formed of sclerenchymatous cells functioning probably as mechanical tissue while the ground tissue is composed of parenchyma without air spaces. The 3 meristemes has exarch protoxylem plate (Fig. 2). Pigg & Rothwell (2001) found 2 large adaxial vascular bundles that occur laterally and adaxially, flanking an arc of 4–6 smaller bundles in *Woodwardia virginica* showing that there is a great variability in the stelar system of the genus.

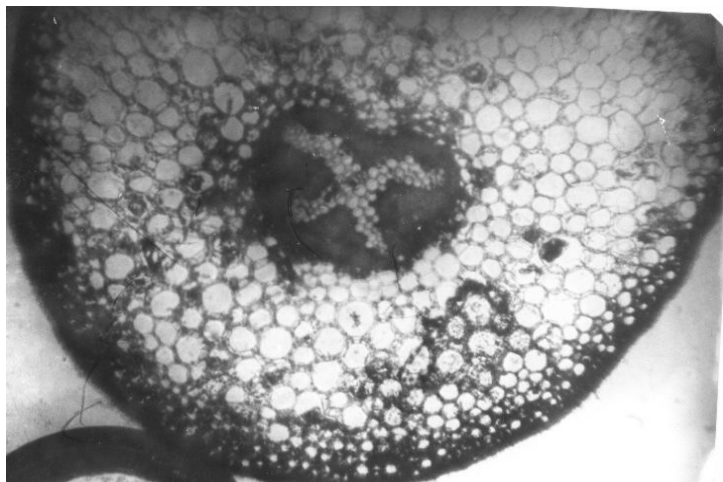


Fig. 3. A transverse section of rachis *Asplenium adiantum nigrum* showing actinostele.

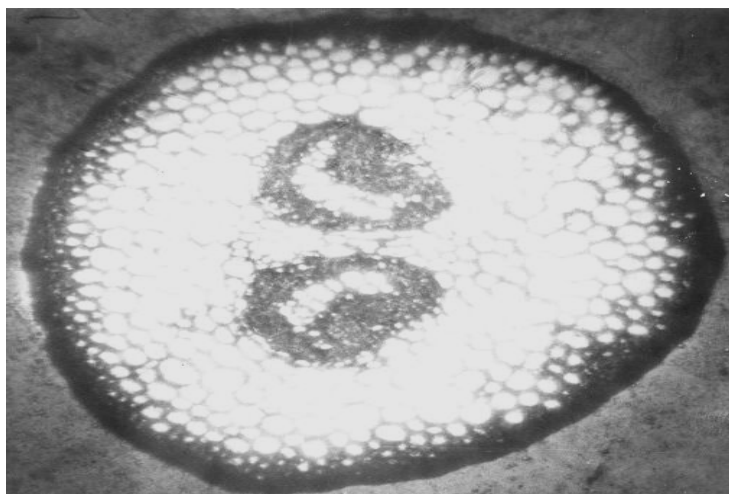


Fig. 4. *Adiantum capillus veneris*. T.S of rachis with two C-shaped Meristele.

Anatomy of rachis of *Asplenium adiantum nigrum* shows that the hypodermis is composed of thick walled cells while the ground tissue is of uniform parenchymatous cells. Within the ground tissue a four armed actinostele is located. The arms of the actinostele are of unequal size (Fig. 3).

The rachis anatomy of *Adiantum capillus veneris* revealed that there is a thick walled heavily cutinized epidermis and hypodermis while the ground tissue is made up of parenchymatous cells with air spaces. In the ground tissue there are two meristele supplied by rhizome (Fig. 4). Further upward each meristele is divided into two sub meristele to enter each pinnule thus the dichotomy of the meristele in the rachis up to the tip of pinnule remain constant.

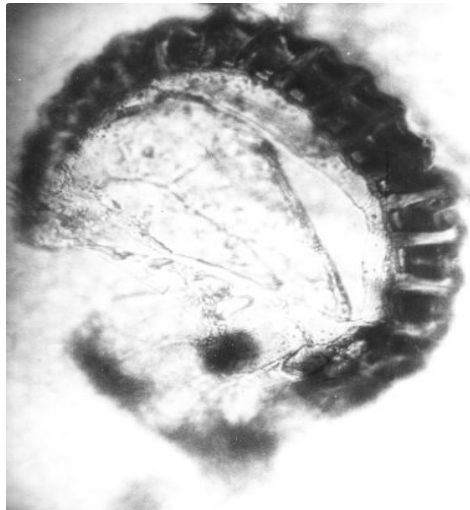


Fig. 5. *Polysticum nigroplacium*. Lateral view of sporangium with incomplete annulus.

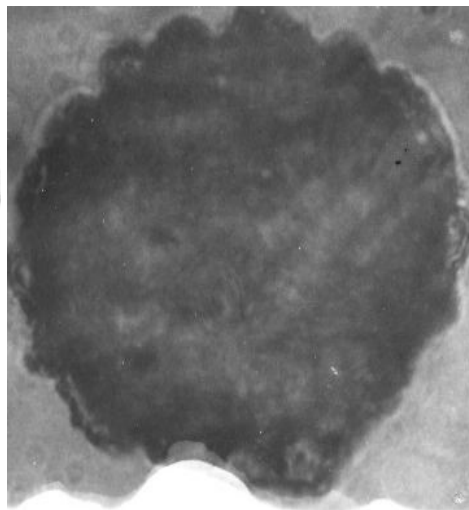


Fig. 6. Bilateral spore of *Polysticum nigroplacium*.

**Sporangia and spores:** The sporangia of *Polysticum nigroplacium* are lens shaped, stalk consist of 3 rows of cells. The capsule 250  $\mu\text{m}$  long, 175  $\mu\text{m}$  wide and 125  $\mu\text{m}$  thick. The main cells are thin walled, variously shaped and backwardly oriented from the stomium. Stomium transverse 50  $\mu\text{m}$  wide, 60  $\mu\text{m}$  deep and 50  $\mu\text{m}$  when dehisced. Annulus is vertically placed like a hood having full length starting from upper cell of stomium and ending at first cell attached to the stalk. There are 18-20 cells in the annulus (Fig. 5). The spores are bilateral, monoete, laesurae proximal, sinus bounded by well marked labrum, proximal surface smooth, distal surface ornamented with verrucae. Exospores accompanied with perine which is warty at the distal region. Dimensions of the spore based on 25 specimens are 48 (52) 55 x 38 (43) 45  $\mu\text{m}$  (Fig. 6). Tschudy & Tschudy (1965) described the spores of *Polysticum platyphyllum* having similar size range as that of the spores of *Polysticum nigroplacium* but differ greatly in its proximal surface.

*Woodwardia unigemmata* has stalked sporangia. The stalk is long and composed of 4 variable rows of cells into 2-3 tiers. The sporangial capsule is lens shaped. Average length of the capsule is 326  $\mu\text{m}$  with a maximum breadth 260  $\mu\text{m}$ . The wall of the sporangium consists of single layer of cells which are variously shaped thin walled and flat. Annulus is horizontally placed with a transversely formed stomium. Cells of annulus are 25  $\mu\text{m}$  deep outwardly wedge shaped and plastic in appearance. These cells are 25  $\mu\text{m}$  wide and 50  $\mu\text{m}$  long on the outer tangential layer. Annulus from stomium to stalk is composed of 25-28 cells (Fig. 7). The spores are all similar i.e. isospores produced in bilateral tetrads, reniform, medium brown, polpodiaceous, proximal surface smooth, distal surface subverrucate with perine hyaline. Spores monoete, laesurae distinct to discernable, faintly developed less then the length of the polar axis. Exine 2  $\mu\text{m}$  thick, perine 2  $\mu\text{m}$  and intine 0.5  $\mu\text{m}$ . Dimensions of the spore based on 25 specimens are 45 (50) 55 x 28 (30) 34  $\mu\text{m}$  (Fig. 8). It is the only species distributed in Pakistan in Himalayas from Kashmir to Bhutan which has not been studied palynologically.

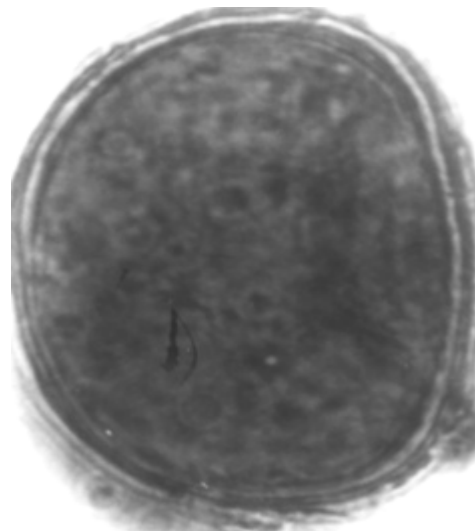


Fig. 7. Sporangium of *Woodwardia unigemmata* with long stalk

Fig. 8. Bilateral spore of *Woodwardia unigemmata*

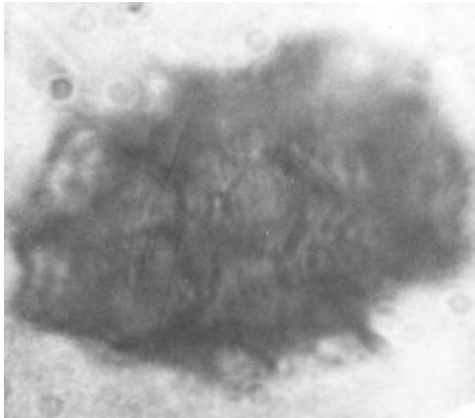
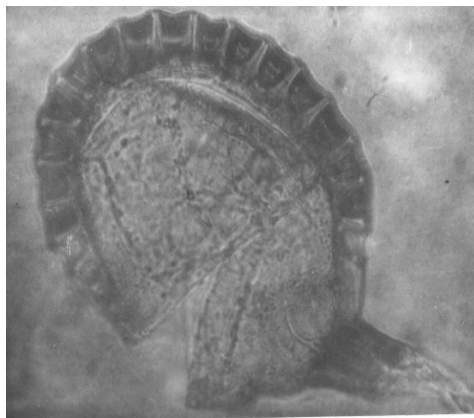


Fig. 9. Dehiscent sporangium of *Asplenium adiantum nigrum*

Fig. 10. Spore of *Asplenium adiantum nigrum* with ornamentation.

In *Asplenium adiantum nigrum* the sporangia are small sized with long stalk. The stalk is formed of 1-3 rows of cells. The capsule is lens shaped and is 150-165  $\mu\text{m}$  long, 115-120  $\mu\text{m}$  wide and 50  $\mu\text{m}$  in thickness. The annulus is composed of 16-20 cells which run above the stalk and reaching up to the neighboring cells of stomium. The annulus cells are wedge shaped. Average size of the annulus cell is 12  $\mu\text{m}$  thick, 10  $\mu\text{m}$  in height and 10  $\mu\text{m}$  wide and located straight around the sporangium. Stomium smooth lined, transverse, bearing spores (Fig. 9). Spores are isomorphic, bilateral, distal region patinate, intermixed with granules, perine 3-5  $\mu\text{m}$ , exine warty, laesurae 20  $\mu\text{m}$  long, laesurae faintly developed. Dimensions of the spore based on 25 specimens are 33 (37) 45 x 27 (31) 35  $\mu\text{m}$  (Fig. 10).

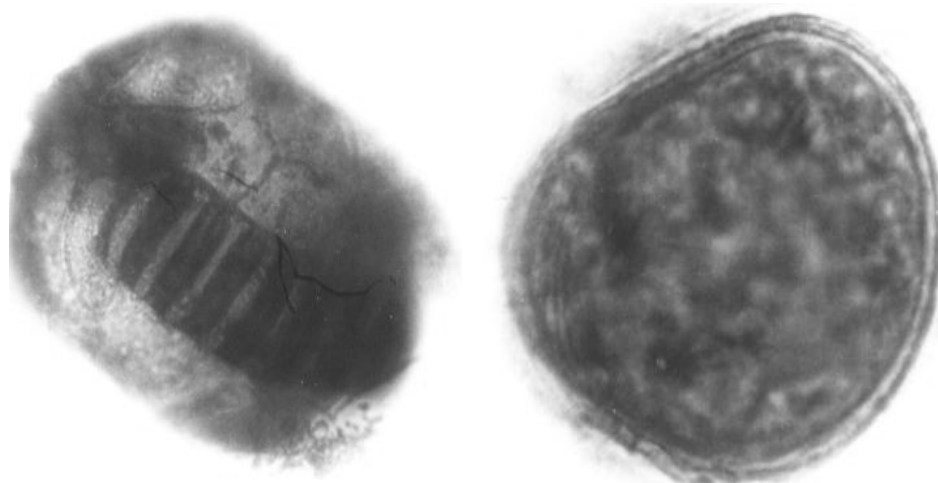


Fig. 11. Proximal view of indehiscent sporangium of *Adiantum capillus veneris*. Fig. 12. Distal view of trilete spore of *Adiantum capillus veneris*.

Sporangia of *Adiantum capillus veneris* are basically globular but are variously shaped when seen from different angles. The sporangia are stalked. The capsule being 200  $\mu\text{m}$  thick, 250  $\mu\text{m}$  in height and 150  $\mu\text{m}$  broad. The cells around the stomium are brick shaped and horizontally placed while the marginal cells are inwardly oriented. The annulus is placed obliquely starting from the stomium making a slight twist and reaching towards the stalk is unique feature of the sporangia of the plant. The annulus is composed of 18-25 cells. The cells of the annulus are heavily thickened. The annulus cells just on the upper region of the stomium are 100  $\mu\text{m}$  wide while the rest of the cells are less broader and more thicker having 40  $\mu\text{m}$  width, 13  $\mu\text{m}$  thickness and 3-5 depth (Fig. 11). Spores are isospores, contour sub-spherical, proximal surface raised, pyramidal, trilete, pyramid inwardly compressed, contact area indistinct, trilete marking distinct to discernable, sinuous two third of the length of spore radius, proximal area are smaller than distal region; line of contact above the equatorial line, exine project inwardly at the contact of proximal region, distal region thicker, reticuloid, muri 3  $\mu\text{m}$ , laecunae variable 5-8  $\mu\text{m}$ , perine translucent and thin, exine 3.5  $\mu\text{m}$  thick. Equatorial diameter of the spores based on 25 specimens is 37 (42) 45 x 47  $\mu\text{m}$  (Fig. 12). These results are in agreement with earlier paleontologists (Erdtman & Sorsa, 1971). Spores of *Adiantum capillus veneris* are similar with *Adiantum reniformis* as far as its size and other morphological features are concerned, but are greatly differentiated by its heavily constructed reticuloid ornamentation. The ornamentation pattern of the spore is not provided by Erdtman & Sorsa (1971) and this pattern seems to be unique and can be used as means of identification of the species.

The anatomy of the rachis and production of bilateral spores of *Polystichum*, *Woodwardia* and *Asplenium* showed di-tri-tetrarch stele in the rachis which support the classification proposed by Holttum (1949) to include these genera in the family Dennstaedtiaceae. The members of Dennstaedtiaceae like *Polystichum nigropaleaceum* either have umbrella shaped indusium or no indusium at all. The sori are horse shoe shaped in *Dryopteris*, a close associate of the *Polystichum*. It would appear that all the

four ferns species described are distinct anatomically and palynologically. Morphology of sori, morphology of meristele in the rachis, shape of sporangia, placement of annulus and morphology of the spores are useful characters for the identification of these species.

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