

THE USE OF STIPE CHARACTERS IN FERN TAXONOMY I⁽¹⁾

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Abstract: Stipes of ferns are of considerable taxonomic interest. Based on the study of stipe characters a multiple choice key has been prepared for the Taiwan ferns. Characters used include the number and shape of vascular bundles, distribution of the sclerenchyma, the presence or absence of grooves, aeration structures and hairs or scales.

INTRODUCTION

Students of ferns have devoted much study to fern spores, gametophytes, venation, stomata, biochemical and cytological characters. Stipe characters have been recognized to be very important for some time, and have been used to distinguish between difficult taxonomic groups, for instance, between *Athyrium* and *Asplenium* (Milde, 1866), *Thelypteris* and *Dryopteris* (Ching, 1936), and the subdivision of the Polypodiaceae *sensu lato* (Ching, 1940). Stipe anatomy has proved to be helpful in the discussion of family, genus, or species relationships (Kato, 1972, 1974, 1975; Keating, 1968; Lucansky and White, 1974; Smith, 1976). Ogata (1928-40) figured the cross sections of the rhizomes and stipes in his eight volumes of drawings of Japanese ferns, and others (Bower, 1923-28; Holttum, 1974; Li *et al.*, 1975; Wagner, 1952) have occasionally included the drawings of the cross sections of stipes in their works. Waters (1903) prepared a key to the ferns of Northeastern United States based on the characters of the stipe, but few have continued what he started. In this paper the authors are presenting a study of fern stipes based on Taiwan ferns, using both internal and external characters of the stipe for taxonomic purposes.

Based on the study of stipe characters of ferns belonging to 22 families, 80 genera and 170 species we have prepared a multiple choice key to the genera and or families of most of our Taiwan ferns (Li *et al.*, 1975). The small aquatic ferns, the eusporangiate ferns, and a few other groups such as the Hymenophyllaceae have not been included in the key. This study is being continued and we hope to publish further findings at a later time.

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MATERIALS AND METHODS

Fresh materials were collected for examining the stipe anatomy of ferns. The stipes were cut into about 4 cm lengths then fixed in 50%-70% alcohol and stored in vials in a refrigerator where they can be preserved for years. Vouchers are kept in the Herbarium of the Department of Botany of National Taiwan University (TAI). Permanent slides are stored in the same department.

Several methods of sectioning were employed for cutting cross sections depending on the degree of hardness of the materials. In most cases, free hand sections were made with razor blades. Very hard stipes were boiled in water and softened with glycerol or triethylene glycol,

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then sectioned with a sliding microtome into 30-50 μ sections. For delicate stipes, the usual paraffin method was used to obtain 10 μ sections. Unstained sections are usually satisfactory except for those made by the paraffin method. To make permanent slides, sections were stained with safranin O and fast green (Johansen, 1940) or toluidine blue O (Sakai, 1973).

Drawings were made with the Nikon microscope projection screen or with the Reichert Visopan.

GENERAL CHARACTERISTICS OF THE FERN STIPE

The stipe of a fern frond corresponds to the petiole of the leaf of a seed plant.

Most ferns have modified stipe bases which usually differ in color, size, shape, the presence or absence of a groove (grooves), indumentum and internal anatomy from the upper portions of the stipe. The stipe base is transitional between the rhizome and the frond, and so is its vascular structure. The descriptions of stipe characters in this paper refer to portions other than the base unless particularly noted.

The division of the stipe into three nearly equal parts is made for convenience. The lower, middle, and upper portions usually differ in minor aspects and these changes are always gradual.

I. External characters

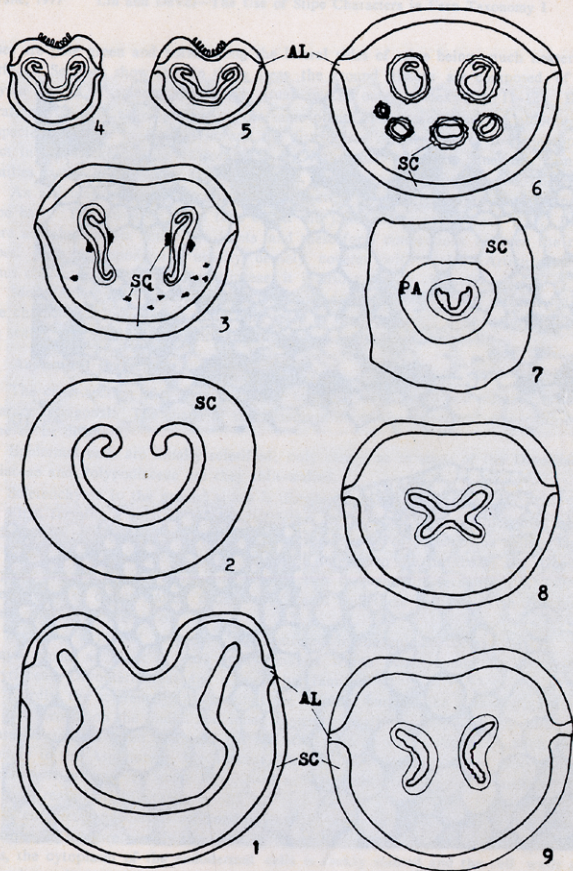
Stipes are usually tufted on the short creeping rhizomes and on erect stems, but are either approximate or distant on the long creeping rhizomes. This is usually a genus character.

The color of the stipe may be green, brown or purple, and the brown or purple stipes may be polished. The green color may persist but usually turns to a brownish or stramineous color when specimens are dried or when they get old. Some stipes are green on one side, which usually is the adaxial side, and brown or purple on the other side, others are green on both sides but with brown streaks. The brown color may be associated either with the brown cell walls of the epidermis or cortex, and these can be easily seen in cross sections of the stipes.

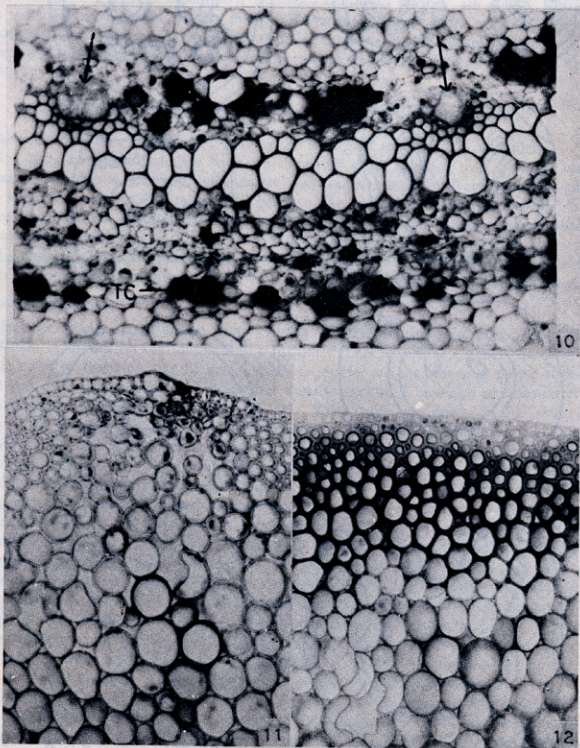
Grooves are common in the stipes of most Taiwan ferns except the Schizaeaceae (*Lygodium*), Plagiogyriaceae, Cheiroleuriaceae, Grammitidaceae, and some of the Hymenophyllaceae, Polypodiaceae *sensu stricto* and Lindsaeaceae. Grooves usually occur on the adaxial side or they may be found on the lateral sides as well. Stipes may have from one to three grooves, more than three grooves are rarely found. The groove may start in any portion of the stipe and run on into the rachis. The rachis and rachilla may both be grooved, and sometimes the rachis groove is open to receive the rachilla grooves. Grooves may appear on dried stipes but not in fresh material; or they may appear deep and conspicuous from the outside but do not show up in the prepared cross sections. Sometimes continuous lines of aeration tissue which appear on the lateral sides of stipe may become sunken when dried and may be mistaken for grooves. The groove is a good genus or family character but requires careful observation.

Wings do not usually occur on stipes with grooves but are often present on the ungrooved stipes. The wings may either be of parenchyma or of sclerenchyma tissue. These are raised on the adaxial surface as in the Davalliaceae or are lateral and extending from the angles of the stipe as in the Plagiogyriaceae. The wing is a species character in *Asplenium*, a genus character in *Plagiogyria*, a family character in the Davalliaceae, and also occurs on the stipes of some other ferns. Some ferns as *Vittaria* and *Lepisorus* have decurrent leaf bases but these are not considered wings. The difference between a leaf base and a wing can not always be clearly determined by examining the cross section of a stipe but is easily distinguished by observing the whole frond.

Two kinds of aerating structures appear on the external surfaces of stipes: the aerophore and the aeration line (Keating, 1968), or these may be collectively called pneumatophores (Bower, 1923-28). The aerophores are globose or linear and occur as pale patches. The aeration



Figs 1-9. Cross section of stipe 1. *Pteris excelsa* Gaud. upper $\times 8.75$ Perg 2410. 2. *Dipteris conjugata* Reinw. lower $\times 8.5$ Lin 45. 3-5. *Diplazium donianum* (Mett.) Tard.-Blot; 3. lower; 4. middle; 5. upper $\times 9.75$ Lin 21. 6. *Dryopteris varia* (L.) O. Ktze. lower $\times 18.75$ Lin 41. 7. *Lindsaea orbiculata* (Lam.) Mett. ex Kuhn lower $\times 32.75$ Lin 34. 8-9. *Asplenium cuneatum* Lam.; 8. upper; 9. lower $\times 17.5$ Liu 1793. AL=region of aeration line, PA=parenchyma, SC=sclerenchyma.



Figs. 10-12. Cross section of stipe 10. *Osmunda banksiaefolia* (Pr.) Kuhn showing the proxylem cavity (arrow) and tannin cell (TC) $\times 155$ Liu 1546. 11-12. *Coniogramme intermedia* Hieron.; 11. showing parenchyma and air space in the region of aeration line. 12. the ordinary sclerenchyma of outer cortex $\times 145$ Liu 1504.

lines are pale green and borne along the lateral sides of stipe being much nearer the adaxial surface. Sections show that in these areas the ground tissues are composed of loose parenchyma instead of sclerenchyma as is usual in the outer cortex (fig. 11-12). Both types of aeration tissue are never present on the same plant. The position of the globose aerophore is a species character in *Plagiogyria*, and the distribution of the rows of linear aerophores is distinct in the Dicksoniaceae (*Cibotium*) and Cyatheaceae. Aeration lines are common in such families as the Blechnaceae, Dryopteridaceae, Aspidiaceae, Athyriaceae and Thelypteridaceae.

An articulation may occur at the base of stipe or rachilla, or at some distance above the stipe base. The frond or pinna falls off at the joint and leaves a clear scar. In some ferns, there are phyllopodia below the joints and these form connections between the rhizomes and stipes. The phyllopodium is usually densely covered with scales. Articulation is always a genus character, and in the Davalliaceae, it is also a family character.

Most ferns have various kinds of hairs and scales borne on the rhizome, stipe or frond. The characters noted in this study only refer to their presence, their color, and the shape of the scales, and whether the hairs are unicellular, multicellular or stellate.

II. Anatomical characters

The cross section of a stipe is usually terete or oval. In a few cases it is oblong, triangular or tetragonal. The shape of the cross section of the upper and lower stipe may be the same or be different.

Epidermal cells are usually sclerified; only rarely, as in some of the Davalliaceae, Plagiogyriaceae and Polypodiaceae are they thin-walled.

Sclerenchyma in the ground tissue is distributed in three ways:

First, forming a zone of several cell layers in the outer cortex, which may be next to the epidermis or may be separated from it by several layers of parenchyma cells. The cell walls are yellow-brown or orange in the unstained state when the surface of the stipe is brown or purple, and transparent when green. This occurs frequently in ferns (figs. 1, 3-9, 12). Sometimes groups of brown-walled cells are scattered and these coincide with the brown streaks on the surface of the stipe.

Secondly, arranged in groups and scattered in the middle cortex (fig. 3). These areas always appear as black or dark brown masses which occur commonly in the Aspidiaceae, Athyriaceae, Dryopteridaceae and a few other families.

Thirdly, the sclerenchyma surrounds the vascular bundle like a band (fig. 6). These cells are dark brown on the radial and inner tangential walls because of being filled with tannin, or are transparent when unstained but red after staining with safranin O. Such bands are very common in the Aspidiaceae, Blechnaceae, Davalliaceae, Dryopteridaceae, Polypodiaceae, Oleanthaceae and others. In some ferns, such as *Ctenopteris*, the unstained cells of the band are for some reason bright orange.

A complete endodermis surrounding each vascular bundle is found in the cross sections of all stipes. They are usually modified by Casparian strips and are very easily seen. In a few ferns, the cytoplasm of the endodermal cells is darkly stained and the cell walls do not have Casparian strips.

The vascular bundles are clearly distinct in the cross section of a stipe. They can even be seen by the unaided eye. We do not intend to name the bundle types in this paper, as this work has been previously done by Thomae (1886), Colomb (1888), Bertrand and Cornaille (1902), Pelourde (1909) and others. The classification of bundles into various types has not been very satisfactory. Ogura's (1972) revised and rather complete work is quite elaborate, the types are numerous, but they seem impractical for taxonomic purposes.

The number, shape, arrangement of the bundles and the configuration of the xylem strand

are of definite importance in identifying ferns. Using the bundle characters, different criteria are needed under the different groupings.

The number of bundles in the cross sections of stipes varies from one to over twenty. Many ferns have only one bundle at the stipe base. In *Cheiropleuria* and *Dipteris* this bundle divides into two or more bundles upwards, but in almost all others as far as we know, this single bundle runs throughout the entire length of the stipe. There are other ferns with two bundles at stipe base, which may unite into one bundle in the upper portion of stipe or may remain two until crossing into the rachis. There are also a large number of ferns which have three or more bundles in their stipes. The number of bundles is not a reliable character in these ferns. But their arrangement and the configuration of their xylem strands are distinctive. There is often a different number of bundles between the younger and more mature stipes, between the larger and smaller ones, and in different portions of the same stipe. The differences found along the stipe length are due to the uniting and dividing of the vascular strands in their process of running from the rhizome to the rachis. This may occur several times in one stipe.

The bundle or bundles are arranged to form an arc opening towards the adaxial surface of the stipe. When the arc is a continuous line, it may be C-, U-, or V-shaped (figs. 1, 2, 4, 5) having its ends curved inwards (fig. 2) or extending towards the lateral sides (fig. 1). When there are two bundles, the arc can be considered as being interrupted in the middle, each bundle being oval or oblong with its long axis parallel to the lateral side (fig. 3). In many ferns such as in the Blechnaceae and Dryopteridaceae, the arc is a broken line, *i. e.*, it is constituted of several terete bundles, with the larger ones located at either end and with the smaller ones between (fig. 6). The basic curve is sometimes very complicated, being curled and broken in such ferns as *Cibotium*, *Pteridium*, and the Cyatheaceae and this condition is supposed to be due to the complex nature of the formation of the leaf traces. In addition to the basic arc-like bundle, there are also terete (fig. 7) or X-shaped (fig. 8) bundles in stipes having a single bundle.

There are two main types of the configuration of the xylem strand. One is curved and hooked at one or both ends, which appears as a sea-horse and so is called the hippocampus shape by Ogura (1972) (fig. 3). The non-hippocampus type is linear or slightly curved but never hooked at the ends (fig. 9).

Many ferns have a cavity in connection with each protoxylem group (fig. 10). These cavities are considered to have secreting functions because the stipes of these ferns usually excrete a mucilaginous substance when they are cut. In *Osmunda*, tannin-filled cells are found in the phloem and pericycle (fig. 10).

A MULTIPLE CHOICE KEY TO THE GENERA OR FAMILIES OF TAIWAN FERNS BASED ON STIPE CHARACTERS

- | | |
|---|--------------------|
| 1. Upper stipe with one vascular bundle..... | 2 |
| 1. Upper stipe with two or more vascular bundles..... | 24a-24e |
| 2. Lower stipe with one vascular bundle..... | 3 |
| 2. Lower stipe with two or more vascular bundles..... | 13 |
| 3. Lower stipe winged and cross section triangular or tetragonal..... | <i>Plagiogyria</i> |
| 3. Lower stipe not winged and cross section terete or oval..... | 4a-4d |
| 4a. Vascular bundle terete or oval..... | 5a-5c |
| 4b. Vascular bundle C-shaped opening towards adaxial surface, with inrolled ends..... | 6 |
| 4c. Vascular bundle C-shaped abaxially and the adaxial ends extended..... | 7 |
| 4d. Vascular bundle U-, V-, or heart-shaped..... | 8 |

- 5a. Xylem core round or angular *Lygodium*
- 5b. Xylem strand V-shaped *Lindsaea*
- 5c. Xylem strand 2-stranded at base of stipe, united into an X-shaped strand above *Asplenium*
6. Tannin cells scattered within the bundle (these cells orange when unstained and black after staining) *Osmunda*
6. Tannin cells absent (but the walls of sclerenchyma tissue containing tannin) *Gleicheniaceae*
7. Xylem strand wavy, the ends folded back for 1/3 of the arm length *Coniogramme*
7. Xylem strand not wavy, the ends not folded back or only slightly so *Pteris*
8. Stipe grooved on adaxial side 9
8. Stipe not grooved; upper stipe slightly winged on adaxial side 12
9. Sclerenchyma of the outer cortex of 1-3 cell layers; cortical parenchyma brown-walled *Cheilanthes*
9. Without the combination of the above two characters 10
10. Hairs multicellular 11
10. Hairs absent *Pteris*
11. Sclerenchyma forming black masses scattered in middle cortex, somewhat along the bundle *Pteris*
11. No sclerenchyma scattered in middle cortex *Microlepia*
12. Vascular bundle of lower stipe V-shaped *Sphenomeris*
12. Vascular bundle of lower stipe distinctly U-shaped *Pteris*
13. Stipe with three or more bundles at base; articulate to rhizome 14
13. Stipe with two bundles at base; not articulate to rhizome 15a-15d
14. Stipe terete, not winged below the decurrent leaf base of simple frond *Polypodiaceae* (in part)
14. Stipe oval, winged at least in upper part *Davalliaceae* (in part)
- 15a. Vascular bundle in upper stipe terete or oval 16a-16d
- 15b. Vascular bundle in upper stipe oblong 18
- 15c. Vascular bundle in upper stipe U-, or V-shaped, with or without modification 19
- 15d. Vascular bundle in upper stipe X-shaped 23
- 16a. Xylem strand flattened; with a thick zone of sclerenchyma surrounding the bundle *Ctenopteris*
- 16b. Xylem strand X-shaped or divided *Asplenium*
- 16c. Xylem strand V-shaped; cortical parenchyma brown-walled *Adiantum*
- 16d. Xylem strand curled 17
17. Stipe articulate *Woodsia*
17. Stipe not articulate *Onychium*
18. Xylem strand distinctly V-shaped or flattened; parenchyma of cortex with brown walls *Cryptogramma*
18. Xylem strand U-shaped or divided; parenchyma of cortex not brown-walled *Lindsaea*
19. Upper stipe winged 20
19. Upper stipe not winged 21a-21c
20. Sclerenchyma scattered in middle cortex of lower stipe *Monachosorum* (*M. henryi*)
20. Not as above *Pteris*

- 21a. Hairs whitish, unicellular or if multicellular, not glandular Thelypteridaceae (in part)
- 21b. Hairs multicellular with rounded tips Athyriaceae (in part)
- 21c. Hairs absent 22
22. Vascular bundles united in the uppermost stipe, U-shaped with extended ends *Matteuccia*
22. Vascular bundles united in the middle stipe, V-shaped *Woodwardia*
23. Lower stipe grooved on adaxial side *Asplenium*
23. Lower stipe not grooved *Histiopteris*
- 24a. Stipe with a terete bundle at base, then with 2 bundles running to near the top where it divides into 3 and then into 4 bundles *Cheiropleuria*
- 24b. Stipe with a C-shaped bundle inrolled at ends from the base to the middle of the stipe, then dividing into 2-8 bundles above *Dipteris*
- 24c. Stipes with two oval bundles towards base, uniting into a single C-shaped bundle in middle stipe, dividing again into two oval bundles in upper stipe. *Blechnum* (*B. eburneum*)
- 24d. Stipe with two vascular bundles throughout the entire length 25
- 24e. Stipe with three or more bundles in lower part 29
25. Xylem strands of hippocampus shape, *i. e.*, hooked at one or both ends. 26
25. Xylem strands not of hippocampus shape, linear or slightly curved *Asplenium*
26. Parenchyma of cortex with brown walls *Pityrogramma*
26. Not as above 27
27. Sclerenchyma of outer cortex homogeneously brown-walled *Monachosorum* (*M. maximowiczii*)
27. Sclerenchyma of outer cortex not brown-walled or not homogeneously so 28
28. Hairs whitish, unicellular or if multicellular, not glandular. Thelypteridaceae (in part)
28. Hairs multicellular with rounded tips. Athyriaceae (in part)
29. Vascular bundles form an arc, with large bundles at both adaxial ends 30
29. Not as above 35a-35c
30. The large adaxial bundles boot-shaped. *Diplazium* (*D. pseudo-doederleinii*)
30. The large adaxial bundles terete, oval or oblong. 31
31. Xylem strand of the large adaxial bundle of hippocampus shape, *i. e.*, hooked at one or both ends. 32
31. Xylem strand of the large adaxial bundle not of hippocampus shape, linear or slightly curved. 33
32. Stipe base abruptly swollen, its anatomy quite different from the above portions. *Aspidiaceae* & *Dryopteridaceae*
32. Not as above. *Blechnaceae* (in part)
33. Stipe winged at least in upper parts. *Davalliaceae* (in part)
33. Stipe not winged below the decurrent leaf base. 34
34. Stipe grooved on adaxial side *Nephrolepis*
34. Stipe not grooved. *Polypodiaceae* (in part)
- 35a. Vascular bundles terete, polycyclic *Pleocnemia*
- 35b. Vascular bundles with wavy xylem strands in three or four series; one or two abaxial and two adaxial which are mirror images of each other 36
- 35c. Vascular bundles irregular in shapes and arrangement *Pteridium*
36. Vascular bundles united into a curved line from the middle stipe upwards *Cibotium*

36. Vascular bundles united into three curved lines, one abaxial and two adaxial in upper stipe; or the stipe only with three or four vascular bundles with wavy xylem strand Cyatheaceae

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