EPIDERMAL STRUCTURE AND DEVELOPMENT OF STOMATA AND TRICHOMES IN *HEMIONITIS ARIFOLIA* (BURM.) MOORE

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ABSTRACT

Cuticle, mature epidermis and development of stomata and trichomes in leaves of *Hemionitis arifolia* have been studied. Smooth and delicate cuticle and sinuous walled irregularly distributed epidermal cells are typically fern-like. Mature stomata are anomocytic, diacytic and anisocytic. Ontogenetically, these stomatal types are perigenous or mesoperigenous.

INTRODUCTION

In recent years, the studies of PANT AND MEHRA (1965), PANT AND KHARE (1969, 1971), THURSTON (1969), COTTHEM (1970), INAMDAR *et al.* (1971a, 1971b), PROBST (1971) and others have shown that the epidermal features are of great help in solving some of the taxonomic problems of diverse taxa of pteridophytes. Moreover, the mode of development of stomata is particularly helpful in such considerations as sometimes two similar looking mature stomata may have a dissimilar mode of development. Accordingly, various features of mature epidermis and the ontogeny of stomata and trichomes in leaves of *Hemionitis arifolia* (Burm.) Moore are described. While studying the development of stomata mitotic division figures of the stomatal meristemoid were particularly observed right from its inception upto the formation of the two guard cells—the need of which has been already emphasized by PANT (1965).

MATERIAL AND METHODS

Fresh material of *Hemionitis arifolia* was obtained from the fern house of the Botany Department of Allahabad University, the plants of which were originally brought from South India. Pieces of young and mature leaves were fixed in Farmer's fluid and later stored in ethyl alcohol. Cuticles were prepared by macerating pieces of mature leaves in Schulze's fluid. General orientation and distribution of stomata and epidermal cells were studied in leaf transparencies made by Foster's technique (FOSTER, 1966). Development of stomata was studied in temporary acetocarmine mounts of the epidermal peels of young leaves, the slides of which were later made permanent by passing through n-butyl alcohol grades and subsequently mounting them in euparal. Microtome sections of young and mature leaves were cut at 5-8 μm thickness.

OBSERVATIONS

Maceration of pieces of mature leaves in Schulze's fluid yield a smooth textured thin and delicate cuticle showing faint impressions of guard and neighbouring cells. Epidermal cell imprints are generally obscure.

Epidermal cells on both faces of leaves are irregularly arranged and have deeply sinuous, thick anticlinal walls (Fig. 1, A B). Over the main veins, the cells are narrow and elongated with comparatively thicker and less sinuous walls, whereas, over the lateral

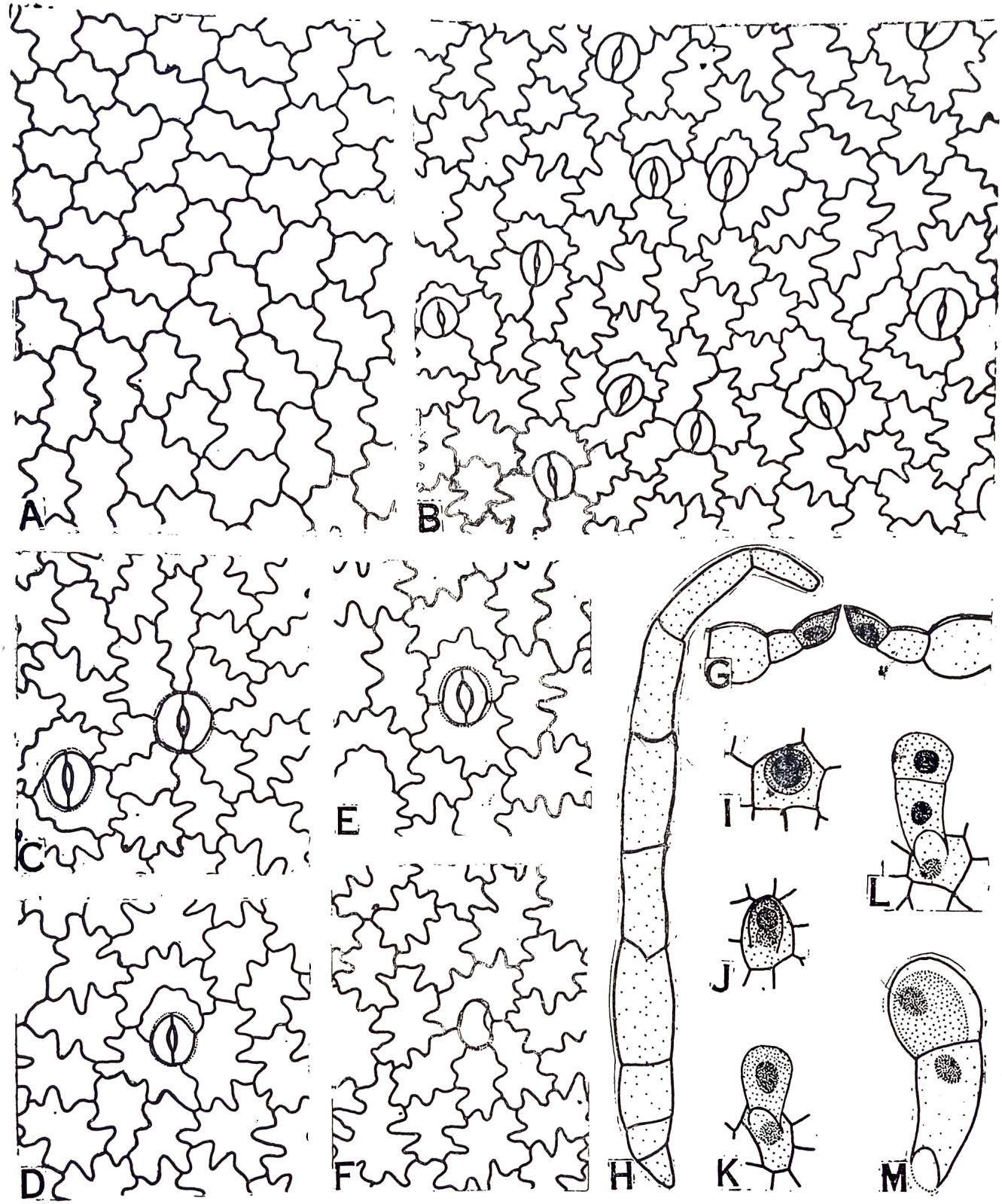

Fig. 1. *Hemionitis arifolia.* A, B : Portions of upper and lower epidermis respectively showing distribution of stomata and epidermal cells. C : Showing an anomocytic stoma in the centre surrounded by 8 neighbouring cells. D : A stoma surrounded by 3 neighbouring cells. E : A diacytic stoma. F : Single guard cell. G : Cross section of a stoma showing raised guard cells (stippled area of the beak is lignified). H : A simple hair. I-L : Various stages in the development of a trichome. M : A glandular hair. (A, B×125 ; C-F, H×150 ; G, I-M ×300)

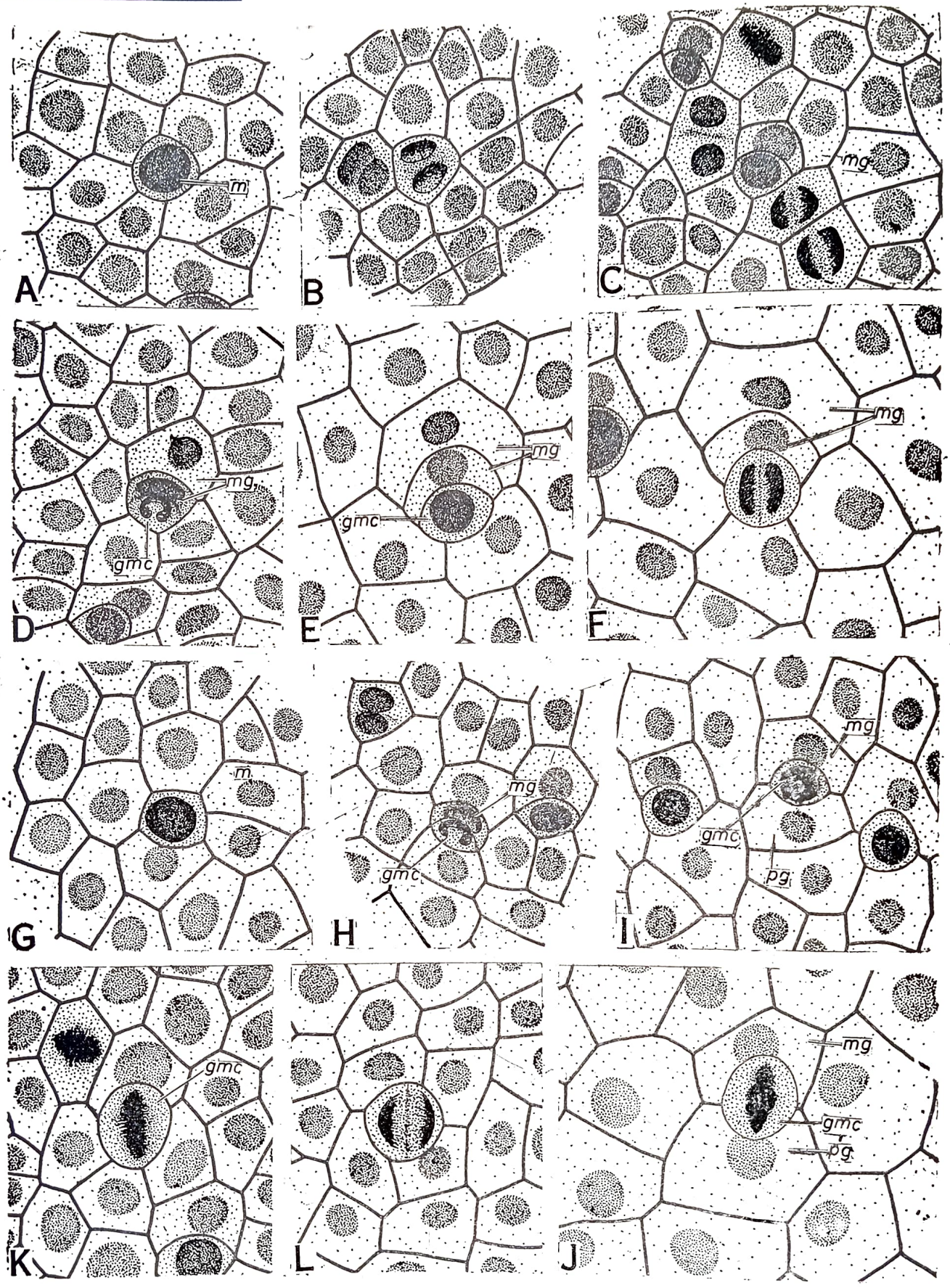

Fig. 2. *Hemionitis arifolia*, A : A stomatal meristemoid. B : 1st division of the stomatal meristemoid. C : Two cells have been formed as a result of division in 'B'. D : Second division of the meristemoid. E : Division in 'D' has resulted two mesogene cells placed one above the other and a guard cell mother cell. F : Division in guard cell mother cell. G : a stomatal meristemoid. H : Division in the stomatal meristemoid. I : Guard cell mother cell surrounded by two cells one mesogene and the other perigene. J : Division in guard cell mother cell. K, L : Stomatal meristemoid directly dividing into two guard cells (all ×600). *m*-stomatal meristemoid, *mg*— mesogene cell, *Pg*—perigene cell, *gmc*—guard cell mother cell.

veins which form polygonal meshes, the cells are like elsewhere. Stomata are confined only to the lower surface of leaves and are irregularly orientated (Fig. 1B). The number of stomata ranges from 1 4 to 48 per mm^2 (average 32 per mm^2) and the length and breadth of guard cells between 14.1 to 54.6 μm (average 50.2 μm) and 14.7 to 21 μm (average 17.4 μm), respectively. The stomatal index is about 9.8. In a sectional view guard cells appear slightly raised above the surface of the epidermal cells (Fig. 1G). Mature stomata are surrounded by 2-8 neighbouring cells which are like ordinary epidermal cells (Fig. 1B—E). Generally the stomata are surrounded by three neighbouring cells out of which one is cap-like covering almost half of the stoma towards one of its poles and the rest two are on the lateral sides (Fig. 1D). The cap cell being always towards the mid rib of the leaf and the stomata appear anisocytic. Stomata surrounded by two neighbouring cells are diacytic (Fig. 1E). Anomocytic stomata are seen surrounded by up to 8 radially arranged neighbouring cells (Fig. 1C). Statistical observations show that out of the total population of stomata about 72 per cent are anisocytic, 19 per cent are anomocytic while diacytic constitute only about 9 per cent. The wall of the guard cells near the pore are lignified and appear in a cross section as lignified beaks taking red colour when stained with phloroglucinol (Fig. 1G). Among the abnormalities, single guard cells are sometimes observed (Fig. 1F).

Thin-walled many cell long simple hairs (Fig. 1H) and three cell long glandular hairs with bulbous apical cell having densely filled dark contents (Fig. 1M) are abundantly present on both the surfaces of leaves but they fall off during maturity. In a mature epidermis, their bases may be recognised as hyaline rounded structures in the epidermal cells.

Development of stomata is perigenous or mesoperigenous. In the epidermis of a young leaf, stomatal meristemoids may be recognised by their deeply stained larger nuclei and dense cytoplasm (Fig. 2A, G). Formation of a perigenous stoma takes place by a stomatal meristemoid which directly functions as the guard cell mother cell. It enlarges, corners round off and divides into two equal halves (Fig. 2K, L) each of which later on become kidney shaped and develop a pore along their adjacent walls. During the development of a mesoperigenous stoma the stomatal meristemoid divides once or twice by slightly curved walls parallel to each other to cut off one or two mesogene cells placed in a row towards the mid rib and a lenticular guard cell mother cell towards the margin of the leaf (Fig. 2A-E). The guard cell mother cell enlarges and divides by a wall perpendicular to the previous one cutting off two guard cells (Fig. 2F) which in the usual course of development become bean shaped and developed a pore. The surrounding epidermal cells may sometimes simultaneously divide in different planes and their number may increase as a result of anticlinal divisions (Fig. 2B, C). Such mesoperigenous stomata can hardly be distinguished from the perigenous ones and in the mature epidermis appear anomocytic. Sometimes, the curved wall dividing the stomatal meristemoid into a mesogene and the guard cell mother cell is laid down in such a way that the latter becomes surrounded by only two cells (Fig. 2G—J). Out of which one is the mesogenous and the other being perigenous in origin. Such guard cell mother cells after division produce diacytic stomata (Fig. 1E). During the present study, it was observed that at the time the stomatal meristemoid undergoes final division by a curved wall resulting into the formation of the mesogene cell and the guard cell mother cell, the young nucleus of the latter usually attains a characteristic 'C' shape in the beginning with the hollow of 'C' facing opposite to the newly laid wall (Fig. 2D, H). However, it may not be of any significance. Gradually the 'C' shaped nucleus becomes round like the nuclei of other cells and prepares to divide into two guard cells.

The development of simple and glandular hairs is similar. Initiation of a trichome takes place as a small bulge (Fig. 1 I) on the surface of an ordinary epidermal cell at the time when the leaf is very young. The nucleus of the cell divides into two and one of them migrates into the bulge (Fig. 1 J). The nucleus of the bulge divides several times resulting in the formation of a several cell long simple trichomes (Fig. 1 K, L). However, in case of glandular hairs, the division stops after two and the apical cell becomes swollen and densely filled with dark contents (Fig. 1M).

SUMMARY AND DISCUSSION

The occurrence of a smooth and delicate cuticle showing only faint impressions and sinuous walled epidermal cells on both faces of leaves is a typical fern feature (unpublished observation of PANT AND KHARE). KONDO AND TODA (1956) and KONDO (1962) have reported the development of stomata in *H. arifolia* and *H. palmata* to be of their '3A' type and PROBST (1971) in *H. palmata* to be of "Pteris type", i.e. the stomatal meristemoid undergoing two successive divisions parallel to each other before dividing into two guard cells and thus resulting into the formation of two mesogene subsidiary cells placed one above the other towards one pole of a stoma. However, during the present observations, it has been found that this sequence of division is not strictly followed. Even in the same leaf, a stomatal meristemoid may directly divide into two guard cells or it may divide once before cutting off the two guard cells to produce a single mesogene subsidiary cell towards the pole of the stoma (mesoperigenous—"Plagiogyria type" of PANT, 1965) or it may divide twice as already described by the above authors (copole-mesoperigenous type of FRYNS-CLAESSENS AND COTTHEM, 1973). Thus the ontogeny of stomata is not only mesoperigenous but also perigenous. However, at maturity the surrounding cells which are up to 8 are so modified that they are generally indistinguishable from the mesogene subsidiary cells and a mesoperigenous stoma may appear anomocytic, or actinocytic (see METCALFE AND CHALK, 1950). Sometimes during the development a stoma gets surrounded by one mesogene and a single perigene cell in such a way that their common wall is at right angles to the guard cells and the stoma appear diacytic. Similar diacytic stomata of mesoperigenous origin are also reported in *Schizaea* (MAROTI, 1961), *Alsophila*, *Cheilanthes*, *Cyathea*, *Davallia*, *Marsilea*, *Nephrolepis* and *Pteris* (INAMDAR *et al.* 1971a, 1971b). A fairly large number of mesoperigenous stomata are surrounded by three neighbouring cells out of which one is the mesogene, being cap-like and smaller at one pole and the two perigene cells markedly larger on the lateral sides making the appearance of the stomata anisocytic (aniso-mesoperigenous type of FRYNS-CLAESSENS AND COTTHEM, 1973).

Present observations and also a number of previous reports on the structure and development of stomata (KONDO, 1962 ; PANT AND MEHRA, 1965 ; PANT AND KIDWAI, 1965, 1968 ; PANT AND KHARE, 1969, 1971 ; INAMDAR, 1970 ; INAMDAR *et al.*, 1971a, 1971b ; COTTHEM, 1970 ; PROBST, 1971, PATEL *et al.*, 1975) in diverse pteridophytes show that in advanced ferns like those belogning to families Pteridaceae, Davalliaceae, Aspidiaceae, Aspleniaceae, Polypodiaceae, water ferns and others the development of stomata and their organisation at maturity in relation to the surrounding cells is perhaps not strictly constant as compared to primitive pteridophytes like *Psilotum*, *Tmesipteris*, *Lycopodium*, *Phylloglossum*, *Isoetes*, *Selaginella*, *Equisetum*, Ophioglossaceae, Marattiaceae, Osmundaceae, Gleicheniaceae and others where this feature appears to be usually constant. However, a definite conclusion in this respect can be drawn only when more details are available for various genera and species of different ferns.

Recently PANT AND CHOWDHURY (1977) have compared the vegetative leaves of

H. arifolia with those of the fossil genus *Belemnopteris* and found that except the external morphology of the leaf and venation pattern, the two are quite different. Details of the epidermal features also appear to be unrelated.

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