EPIDERMAL STRUCTURE AND STOMATAL ONTOGENY IN SOME MEMBERS OF MENISPERMACEAE

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ABSTRACT

The details of the epidermal structure and stomatal ontogeny in five members of Menispermaceae viz., *Tiliacora acuminata* Miers., *Tinospora cordifolia* Miers., *Cissampelos pareira* Linn., *Cocculus hirsutus* (Linn.) Diels. and *Stephania rotunda* Lour. are described. The development of stomata is generally perigenous in *Stephania* and *Cissampelos* and mesoperigenous in *Tiliacora* but the development of stomata in *Tinospora* and *Cocculus* may either be perigenous or mesoperigenous. Stomatal ontogeny suggests that the Menispermaceae may be related to Berberidaceae, Ranunculaceae, Lardizabalaceae and Nymphacaceae.

INTRODUCTION

The characters of the epidermis and stomata of Menispermaceae are briefly mentioned by Solereder (1908) and Metcalfe and Chalk (1950), but neither the details of epidermal structure nor the ontogeny of stomata in the family are described. Accordingly, we took up the development of stomata in some members of the family whose living material was locally available.

MATERIAL AND METHODS

Young and mature leaves of *Tiliacora acuminata* Miers., *Tinospora cordifolia* Miers., *Cissampelos pareira* Linn., and *Cocculus hirsutus* (Linn.) Diels., were collected from Allahabad and its adjoining areas. Leaves of *Stephania rotunda* Lour., were collected from plants cultivated in the Garden of the Botany Department from tubers of wild plants, growing at Champawat and Naini Tal or from leaves of wild plants. The identifications have been authenticated by comparison with previously identified herbarium sheets in the Duthie Herbarium of the Botany Department, Allahabad University. In addition, fresh herbarium sheets of plants studied by us have been deposited in the Duthie Herbarium for record. Young and mature leaves were fixed in Farmers fluid and F. A. A. Peels were stained in acetocarmine and haemotoxylin. Slides of cuticles were prepared by maceration of leaves in Schulze's fluid and by mounting the residual cuticles in glycerine jelly. The terminology used here is according to Pant (1965).

OBSERVATIONS

Epidermis

Leaves of all species are hypostomatic and the stomata are confined to mesh areas (Fig. 1 A, C, E, G, I). The cells of either epidermis are elongated and straight-walled over midrib and veins (Fig. 1 D), and polygonal over meshes. While the cells of mesh areas in upper epidermis of *Tiliacora*, *Tinospora* and *Cocculus* are straight-walled (Fig. 1 B, D, F) those of *Cissampelos* and *Stephania* are sinuous-walled (Fig. 1 H, J). Depending on the character of the sides of cells of mesh areas in the lower epidermis, the various forms investigated by us can be arranged in a series beginning with *Tiliacora* which has straight

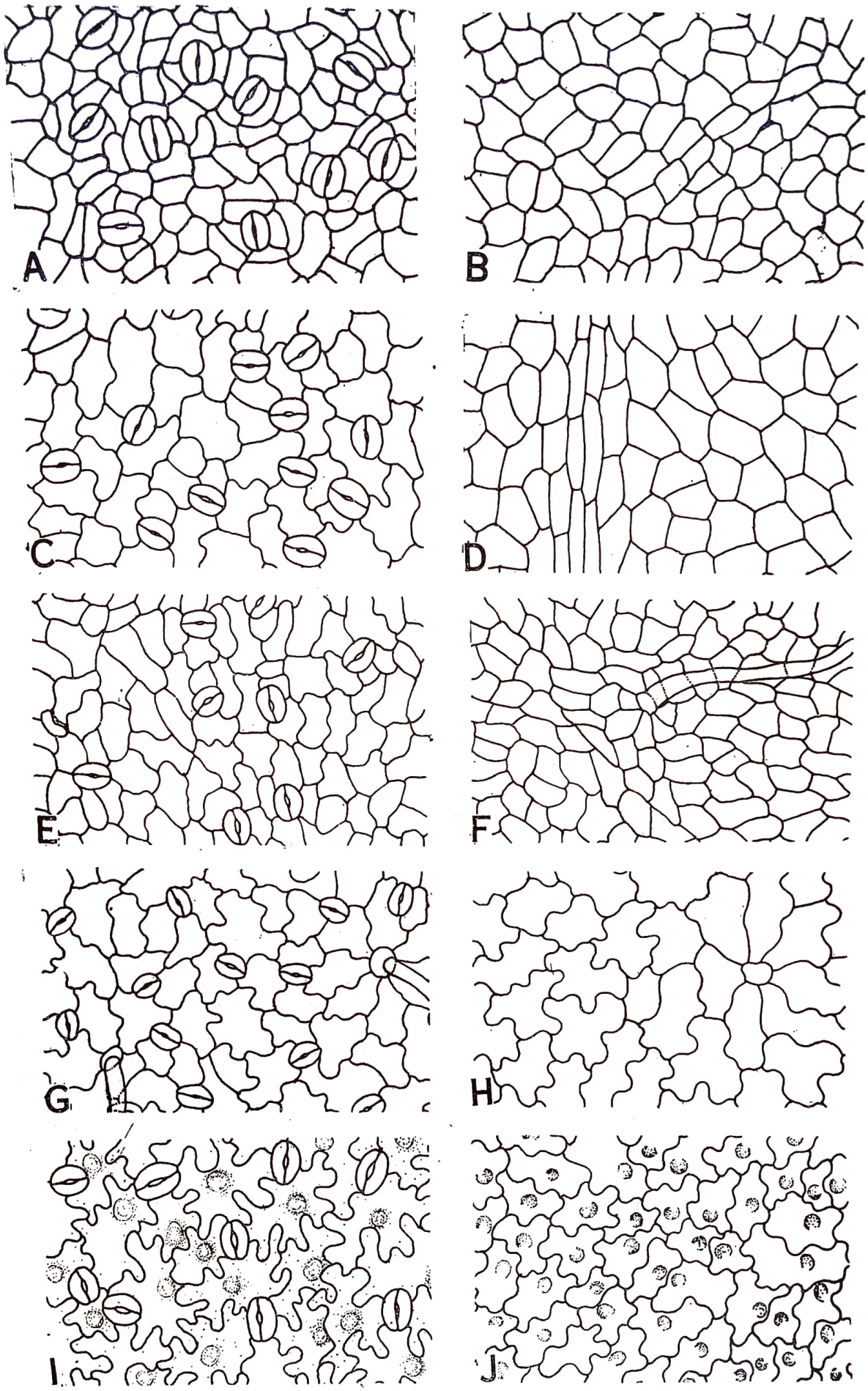


Fig. 1. A, B. *Tiliacora;* C, D. *Tinospora;* E, F. *Cocculus;* G, H. *Cissampelos;* I, J. *Stephania*
A, C, E, G, I. Lower epidermis showing form and arrangement of epidermal cells and stomata in mesh areas. In I every cell shows a rounded papilla. B, D, F, H, J. Upper epidermis, D showing elongated cells in vein areas and J showing median papilla in each cell. (all × 200, I × 300)

walled cells (Fig. 1 A). Then comes a succession of forms with mesh area cells having more and more sinuous walled sides, viz., *Tinospora*, *Cocculus* and *Cissampelos* (Fig. 1 C, E, G), the series thereafter ending in *Stephania* whose mesh-area cells have deeply sinuous walls (Fig. 1 I).

Stomata

The stomata of all species are surrounded by 3-7 scarcely differentiated neighbouring cells (Fig. 2 D-I). However, one of the guard cells in many stomata of *Tiliacora* and a few in *Tinospora* and *Cocculus* may be flanked by a more or less clearly differentiated parallel subsidiary cell (Fig. 2 B, E). The number of neighbouring cells is mostly 4-5 in *Tiliacora*, *Tinospora* and *Stephania*, 5-6 in *Cocculus* and 3-4 in *Cissampelos*.

The guard cells are flush with the epidermal cells and their walls lack any discernible thickenings.

Contiguous stomata were observed in *Tiliacora* and *Tinospora* (Fig. 2 J, K). A number of stomata in *Cocculus* and also a few in *Tiliacora* showed only one normal guard cell with or without a pore on its concave side (Fig. 2 Q, Plate 1 G). Careful study of a number of such unicelled stomata suggests that they arise by the abortion of one of the guard cells in normal bicelled stomata (Fig. 2 L). The abortion of a normal guard cell starts with the degeneration of its nucleus, thereafter, its cytoplasm also disappears, and this is followed by the collapse of the lumen which finally disappears and the guard cell takes the form of a linear wall-like structure (Fig. 2 M-Q, Plate 1 D-G).

Hairs

Hairs are a common feature of all the species except *Stephania* where each epidermal cell shows a short median papilla (Fig. 1 I, J). They are present only on the lower side of the midrib of *Tiliacora* but in the other species they may occur anywhere on either face of a leaf. The frequency of hairs is highest in *Cissampelos* and *Cocculus*.

The hairs of *Tinospora* are glandular and four-celled, the basal cell is small and the three distal cells are arranged in a row to form a club-shaped body (Fig. 2 A). The hairs of other species are bicelled with a short basal cell and an elongated tapering thick-walled apical cell (Fig. 2 C).

Cuticle

The leaves of all species yield thin cuticles usually with obscure cell outlines. The hairs are also cutinised.

Stomatal development

The development of stomata in *Stephania* and *Cissampelos* is invariably of perigenous type. Most of the stomata of *Tiliacora* develop mesoperigenously, while a large majority of stomata in *Tinospora* and *Cocculus* are perigenous. The guard cell mother cells (meristemoids) of perigenous stomata divide only once to give rise to the two guard cells (Fig. 3 K), but in mesoperigenous stomata the first division of the stomatal meristemoid results in the formation of a mesogene subsidiary cell and thereafter the meristemoid (guard cell mother cell) divides by a wall parallel to the first wall to cut off the two guard cells (Fig. 3 A—G, J, Plate 1 A, B). The subsidiary cell may divide again by a wall which may be parallel to the first wall to form a parallel encircling cell or it may divide by a wall at right angles to the first wall to form two mesogene subsidiary or neighbouring cells (Fig. 3 H, I, Plate 1 C), one by the side of the other. Such mesoperigenous stomata have one or two meso-

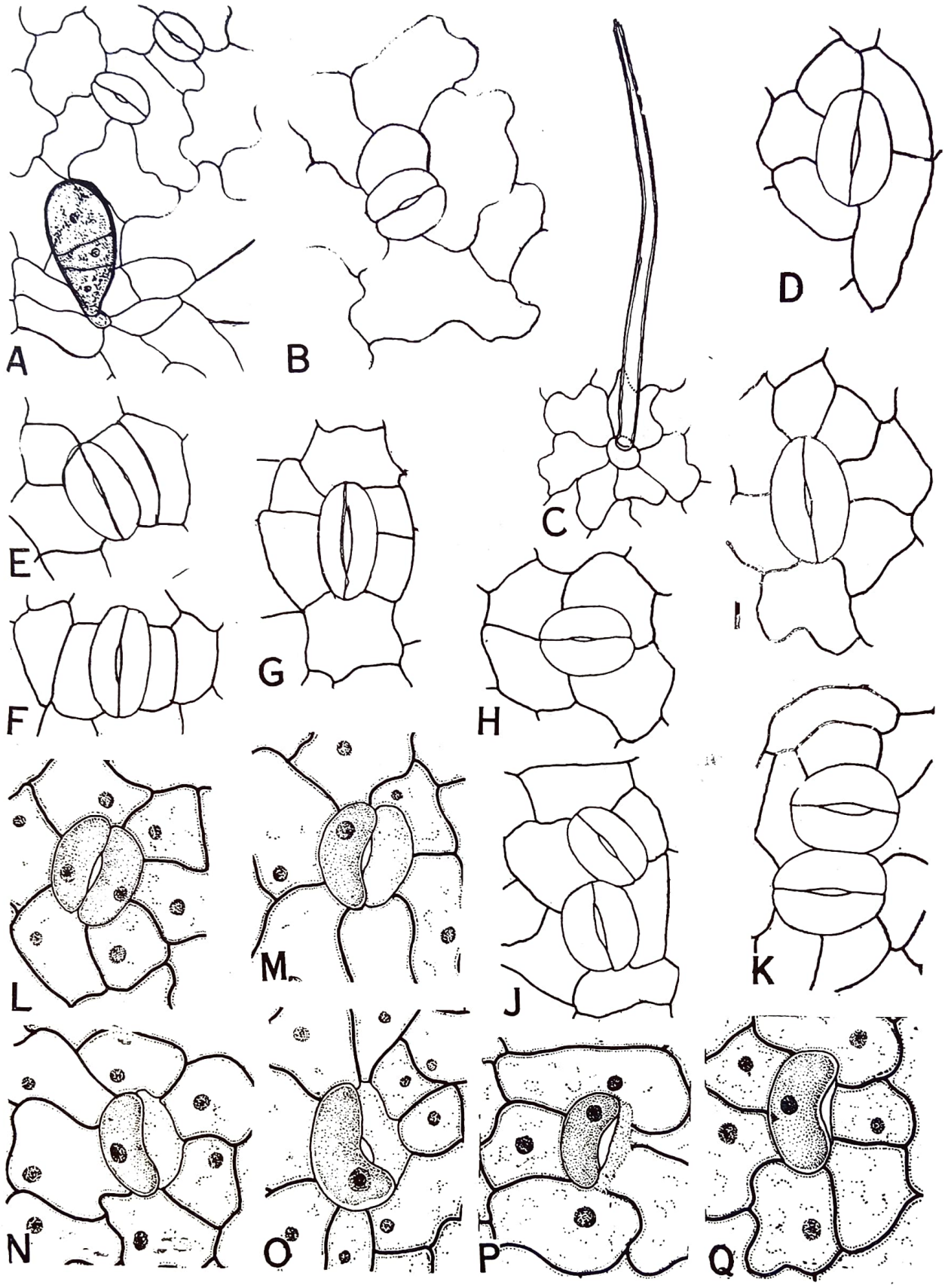


Fig. 2. A, B. *Tinospora;* C. *Cissampelos;* D, K. *Tiliacora;* L-Q. *Cocculus.*

A, Lower epidermis showing a club-shaped hair over a vein (× 300). B, Stoma flanked unilaterally by parallel subsidiary cell (× 400). C, A bicelled hair (× 200). D-I, Different types of stomata showing number and arrangement of subsidiary cells (× 500). J, K, Contiguous stomata (× 500). L, Normal stoma. M-P, Different stages in abortion of one of the guard cells. Q, Unicelled stoma. (L-Q × 600)

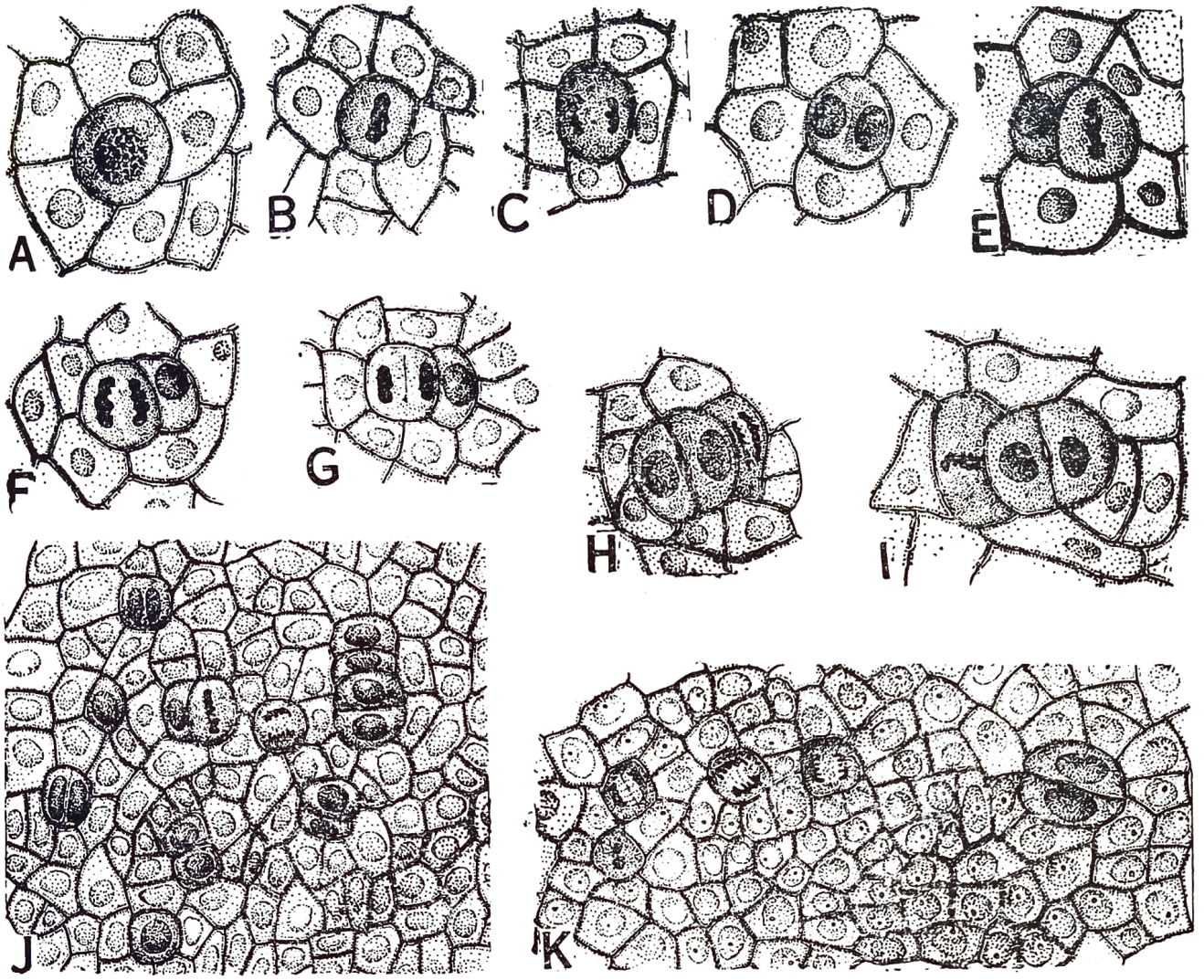


Fig. 3. A-J. *Tiliacora;* K. *Stephania.*

A, Stomatal meristemoid. B-D, Meristemoid at different stages of division. E-G, Dividing guard cell mother cell and a parallel subsidiary cell. H, I, Stomata with dividing subsidiary cells. J, K, Epidermis of young leaves showing meristemoids in different stages of development. (A-I × 800, J, K × 600)

gene subsidiary or neighbouring cells on one side of the guard cells and the other neighbouring cells of the stomata are all perigene.

DISCUSSION

A common character of the leaves in the investigated species of Menispermaceae is their hypostomatic nature and confinement of the stomata to mesh areas. According to Solereder (1908) and Metcalfe and Chalk (1950) the stomata of Menispermaceae are in general surrounded by ordinary neighbouring cells. The only exception to this rule found in the presently investigated plants is *Tiliacora*, some of whose stomata are unilaterally flanked by clearly differentiated parallel subsidiary cells. The differences between the epidermal characters of the different species are depicted in Table 1.

Three types of trichomes are found in various species, (1) bicelled hairs, (2) club-shaped four-celled glandular hairs and (3) papillae. Out of the three kinds of trichomes, bicelled hairs are of more common occurrence being found in *Cissampelos*, *Cocculus* and *Tiliacora*, club-shaped hairs occur only in *Tinospora* and papillae only in *Stephania*. Occurrence of bicelled hairs in *Cissampelos* and *Cocculus* was earlier reported by Metcalfe and Chalk (1950), but these are also found in *Tiliacora* although only over the lower surface of midrib.

Table 1. Size of epidermal cells, stomatal frequency and index in five genera of Menispermaceae. (Figures given in the parentheses are the mean value)

Name of genus	Upper epidermis		Lower epidermis					
	Size of cell in μm Length	Breadth	Size of cell in μm Length	Breadth	Size of guard cell in μm Length	Breadth	Frequency of stomata per mm²	Stomatal index
1. *Cissampelos*	73—43 (60)	40—23 (31)	66—33 (49)	36—20 (29)	23—20 (22)	10—7 (9)	207	19.2
2. *Cocculus*	56—30 (40)	33—17 (24)	50—26 (36)	23—10 (19)	33—20 (26)	13—7 (10)	153	7.6
3. *Stephania*	66—33 (50)	50—23 (32)	66—40 (55)	40—23 (32)	23—17 (20)	10—7 (8)	227	20.5
4. *Tiliacora*	46—23 (33)	23—17 (20)	59—20 (37)	30—13 (18)	33—23 (28)	13—7 (10)	200	9.9
5. *Tinospora*	66—36 (47)	46—23 (31)	69—26 (48)	43—20 (29)	33—23 (27)	13—10 (11)	183	17.6

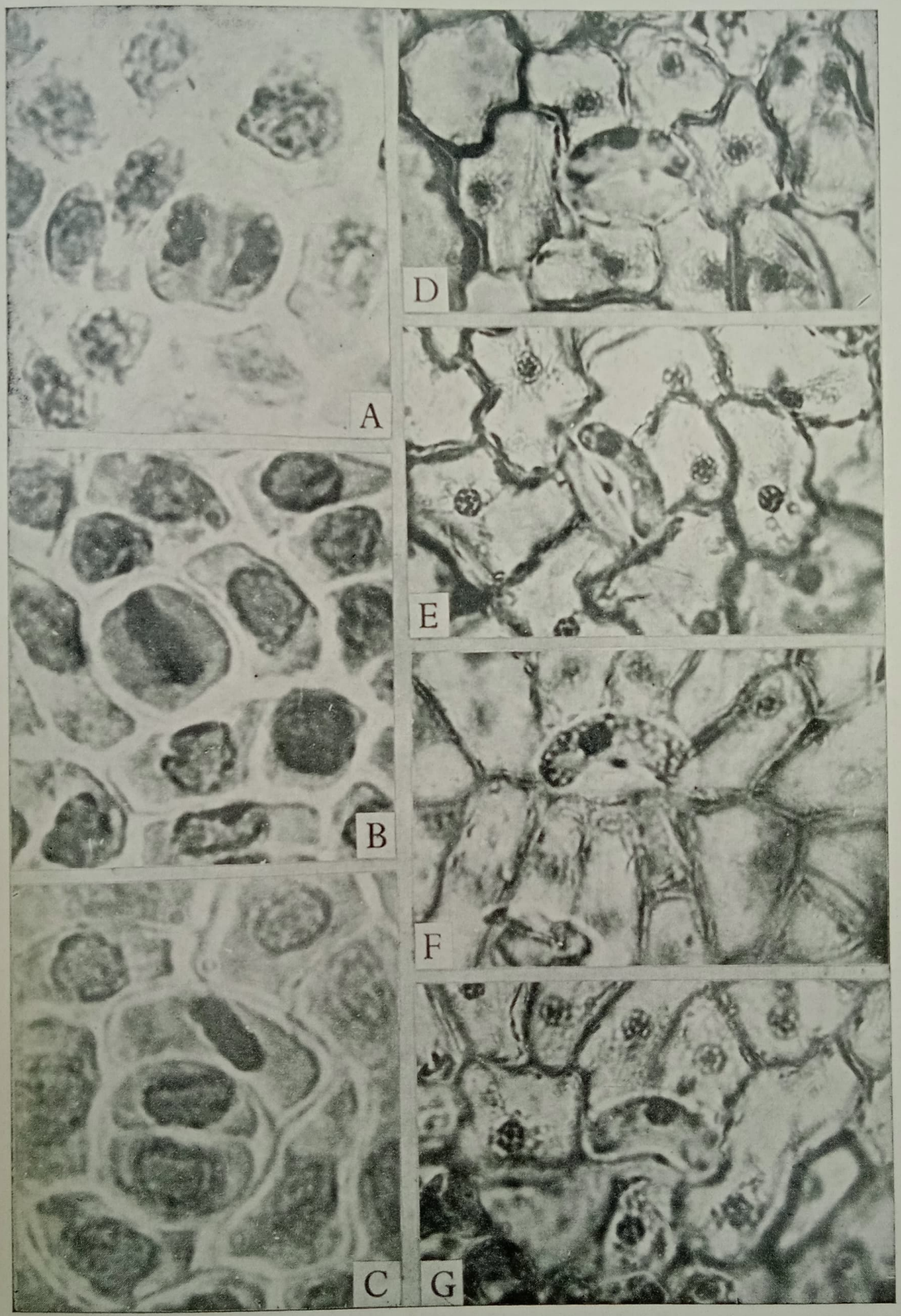
Development of normal stomata in members of Menispermaceae and of peculiar unicelled abortive stomata in *Cocculus* is being reported here for the first time.

Development of stomata is of two kinds, perigenous and mesoperigenous. Out of these, perigenous development would appear to be more widespread in the family since all stomata in *Stephania* and *Cissampelos* and at least a few stomata in all other plants develop perigenously. However, most of the stomata of *Tiliacora* are mesoperigenous while a majority of stomata in *Tinospora* and *Cocculus* are perigenous.

According to Pant (1965) mesogenous stomata, whose meristemoids undergo a greater number of divisions, are more primitive than mesoperigenous or perigenous stomata, whose meristemoids undergo fewer divisions and such stomata could be regarded as reduced. If this conclusion is right then the Menispermaceae could be regarded as more specialised than the plants of Magnoliales—Ranales complex where the stomatal meristemoid undergoes at least three divisions.

Unicelled stomata have been reported previously by Dehnel (1961), Ahmad (1964), Inamdar (1968a, b ; 1969a, b), Inamdar and Chohan (1969), Inamdar, Gopal and Chohan (1969), Pant and Kidwai (1964), Pant and Banerji (1965a, b, c) and many others in diverse plants but no one has reported such stomata and their development in members of this family.

The Menispermaceae are differently classified in various systems of classifications. They are placed, (i) between the Annonaceae and Berberidaceae by Bentham and Hooker (1862—1867), (ii) next to the Berberidaceae in the Ranales by Engler (1897—1915), (iii) between Lardizabalaceae and Nymphaeaceae by Rendle (1952), (iv) in the Berberidales between Lardizabalaceae and Nandinaceae by Hutchinson (1959), (v) in the Ranunculales between Lardizabalaceae and Coriariaceae by Cronquist (1968) and (vi) in the Ranunculales between Sargentodoxaceae (which comes next to Lardizabalaceae) and Ranunculaceae by Takhtajan (1969). As far as the stomatal ontogeny is concerned, the perigenous and mesoperigenous ranunculaceous stomata of the Menispermaceae do not support its being placed next to Annonaceae, as suggested by Engler (1897—1915), which has mesoperigenous tetracytic stomata. However, the stomatal ontogeny of Menispermaceae is similar to that of the Ranunculaceae, Berberidaceae, Nymphaeaceae, Lardizabala-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ceae and this may be used as an additional argument in support of the views of other taxonomists, without offering any grounds in favour of any one of these systems. Nevertheless, if we cousider the floral characters of the above families, a preference for closer ties of Menispermaceae, with Lardizabalaceae is clearly indicated as suggested by PANT, NAUTIYAL AND SINGH (1978).

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EXPLANATION OF PLATE 1

A—C. *Tiliacora;* A. Dividing stomatal meristemoid. B. Guard cell mother cell in metaphase. A parallel subsidiary cell is seen on one side. C. Stomata with dividing subsidiary cell (all X 2250). D—G. *Cocculus.* D—F. Stomata showing different stages in the abortion of one of their guard cells. G. Unicelled stoma (all × 800).