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Plant Embryology

EMBRYOLOGY AND SYSTEMATIC POSITION OF *OPHIOPOGON INTERMEDIUS*

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Ophiopogon intermedius is a scapigerous herb. The ovary is semi-inferior and adnate to the perianth tube. The fruit contains three to eight exposed, globose seeds. The anther wall consists of an epidermis, fibrous endothecium, one middle layer and a glandular tapetum. Cytokinesis in the microspore mother cells is successive, resulting in isobilateral tetrads. The mature pollen grains are elongated, monocolpate and 2-celled. The ovules are anatropous, crassinucellate and bitegmic. The embryo sac development is of the Polygonum type. The postament is present. The endosperm is of the Helobial type. The seed coat is formed by the testa alone. It is 24 to 26 layers thick and fleshy. The pericarp consists of thin-walled cells. After fertilisation, the fruit wall splits irregularly to expose the developing seeds. On the basis of morphological and embryological data it is suggested that the genus *Ophiopogon* should be placed in Liliaceae instead of Haemodoraceae.

INTRODUCTION

The genus *Ophiopogon* Ker. comprises 10 species, out of which 9 occur in India (Hooker, 1894). *Ophiopogon intermedius* is distributed in the temperate Himalayas, from Kashmir eastwards; Khasi and Manipur hills; and Ghats of Kerala and Karnataka. Divergent views have been expressed regrading the systematic position of the genus *Ophiopogon*. Bentham and Hooker (1862-1883) placed it in the family Haemodoraceae due to the presence of semi-inferior ovary. Pax (1930) transferred it to Liliaceae because of the occurrence of six stamens. Hutchinson (1973) retained it in the family Liliaceae on the basis of characters like racemose inflorescence and splitting of ovary wall to expose the seeds.

Sohnarf (1931) and Davis (1966) brought together the then existing embryological literature on Liliaceae. A thorough reading of these works has revealed that our knowledge of embryology on *Ophiopogon* is very meagre. In *O. japonicus* and *O. jaburan*, Guèrin (1927) observed a single archesporial cell in each anther lobe which

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divides to form a group of microspore mother cells. Maheshwari (1934) reported successive cytokinesis in microspore mother cells, 2-celled pollen grains, and the Polygonum type of embryo sac in *O. wallichianus*. The present investigation was undertaken to elucidate the embryology of *O. intermedius* and to discuss its systematic position.

Materials and Methods

Ophiopogon intermedius Don was collected from Summer Hill (Simla District) during July to September, 1976 and 1977. Formalin-acetic-alcohol was used for fixation. The material was dehydrated and infiltrated through alcohol-xylene series and embedded in paraffin wax of 60-62°C m.p. Longitudinal and transverse sections of buds, flowers and fruits were cut at 6 to 14 μ. Sections were stained with safranin-fastgreen combination.

Observations

1. *External morphology*

It is a perennial and scapigerous herb. The rootstock is short, and its base is covered with remains of old leaves. The leaves are tufted, linear and about 15-35 cm long. The scapes are leafless. The raceme is terminal and 5 to 12 cm long (Fig. 1 A). The flowers are bracteate, actinomorphic, trimerous, perigynous (Fig. 1 B) and white. The bracts are lanceolate. The six perianth lobes are arranged in two whorls (Fig. 1 D, E). The ovary is semi-inferior (Fig. 5 A) and adnate to the perianth tube (Fig. 1 G). It is trilocular with axile placentation. The style protrudes out of the converging stamens. The stamens are six in number and are opposite to perianth lobes. The filaments are short and erect (Fig. 1 F). The anthers are basifixed (Fig. 1 F.) The fruit is peculiar because in the early stages of its development, the ovary wall splits and exposes the developing seeds (Fig. 1 C, H). The mature seeds are globose and blue-black in colour (Fig. 1 I).

2. *Microsporangium, microsporogenesis and male gametophyte*

The anther is tetrasporangiate (Fig. 2 A-C). The anther wall consists of an epidermis, endothecium, one middle layer and glandular tapetum (Fig. 2 D-F). The tapetal cells are binucleate (Fig. 2 H-J). At the mature pollen stage the endothecium shows fibrous thickenings and the tapetum disorganises (Fig. 2 G).

Cytokinesis of the micorspore mother cells is successive (Fig. 2 K-S). The microspore tetrads are isobilateral (Fig. 2 S). The mature pollen grains are elongated and monocolpate (Fig. 2 T). They are shed at the 2-celled stage.

FIG. 1. External morphology (*b*, bract; *p*, perianth; *spl*, split; *vs*, vascular supply). *A*, plant showing rhizome, leaves and inflorescence with flowers, × 0.4; *B*, flower. × 5; *C*, flower showing splitting of ovary wall at the region adnate to the perianth to expose the developing seeds. × 5, *D*, tepal. × 5, *E*, tepal with epiopyllous stamen. × 5. *F*, stamen. × 5; *G*, gynoecium. × 5, *H*, bunch of seeds. × 1; and *I*, mature seed. × 5.

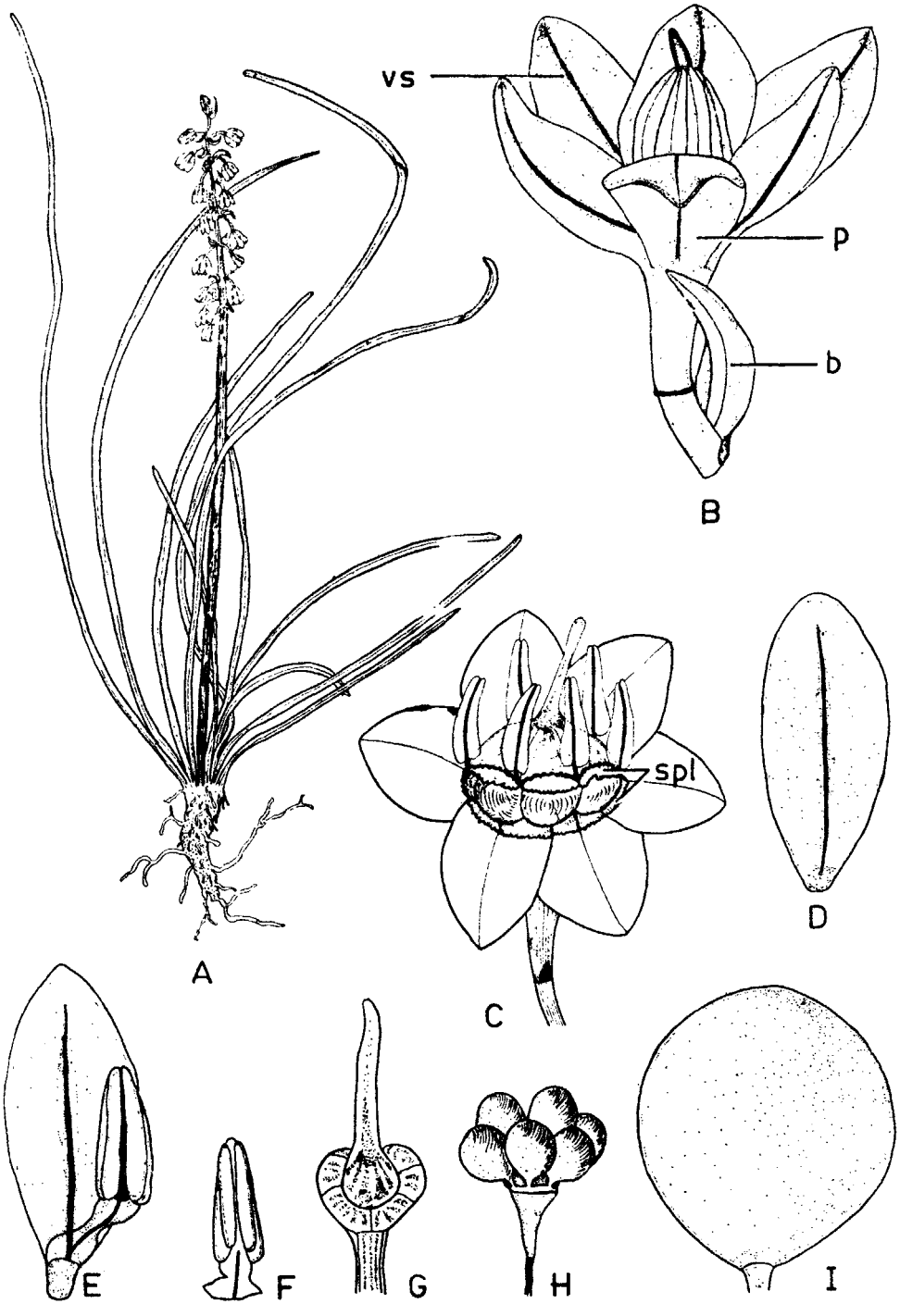

FIG. 1

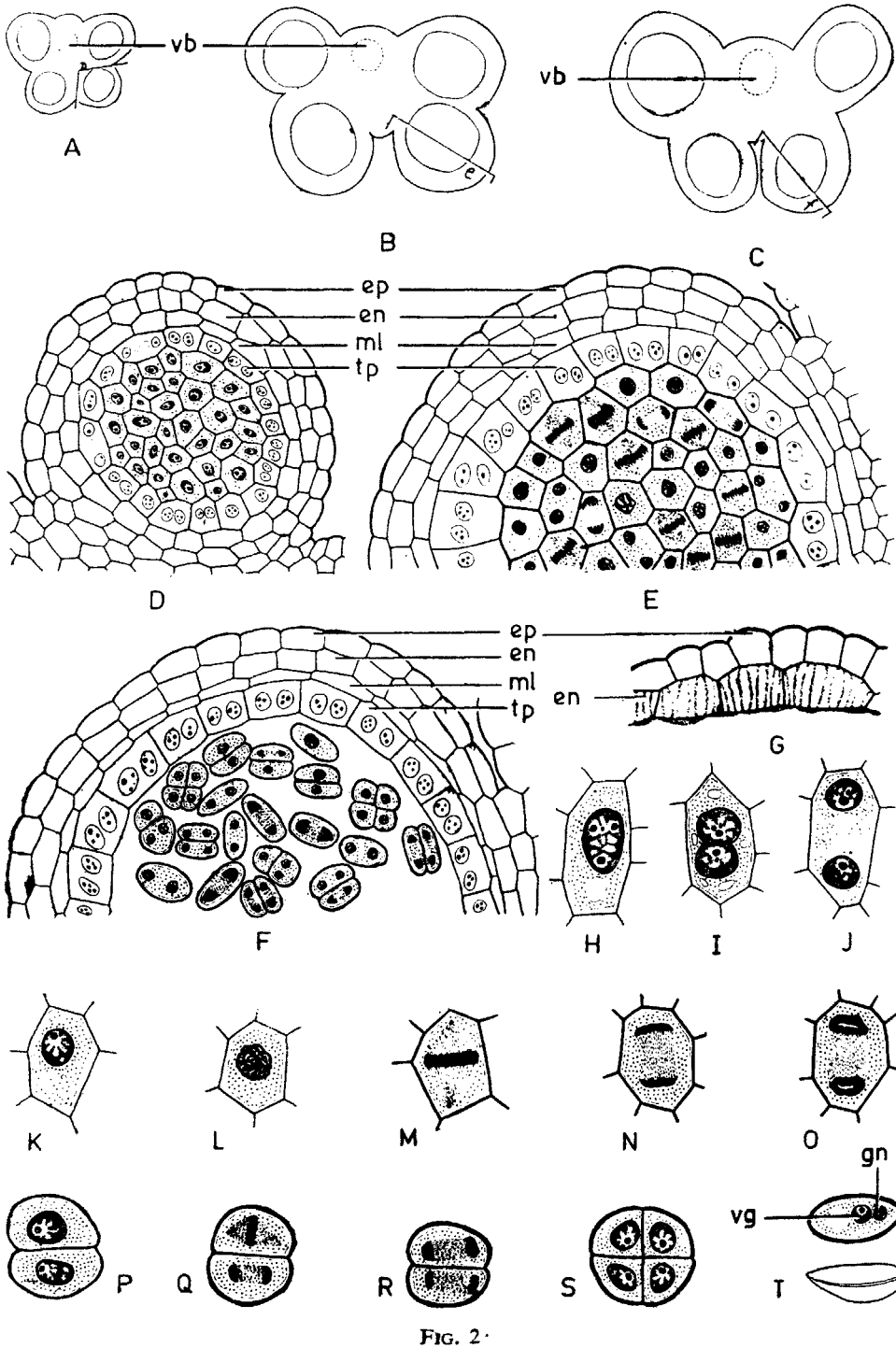

FIG. 2.

3. *Megasporangium*

The ovule arises as a small protuberance from the placenta and starts curving downwards till the micropyle comes to lie parallel to the funiculus. When mature, it is anatropous, crassinucellate and bitegmic. The micropyle is formed by the inner integument, until fertilisation (Fig. 5 B). After fertilization, however, both the integuments contribute to the formation of the micropyle (Fig. 5 C). A funicular obturator is present. The vascular supply extends up to the chalaza. The postament is present below the embryo sac and above the vascular supply (Fig. 4P). It is formed by the protrusion of the basal nucellar tissue into the embryo sac and is prominent in the young seeds. However, the postament disorganises in the mature seeds. Its cells are large, thin-walled and vacuolated (Fig. 4 Q).

4. *Megasporogenesis and female gametophyte*

The single archesporial cell (Fig. 3 A) divides periclinally to form a parietal cell and a sporogenous cell. The former undergoes anticlinal divisions forming one layer of parietal tissue. The sporogenous cell enlarges and functions as the megaspore mother cell (Fig. 3 B). Rarely the archesporial cell directly functions as the megaspore mother cell (Fig. 3 C). The megaspore mother cell undergoes meiosis resulting in the formation of a dyad (Fig. 3 D) and then a T-shaped tetrad of megaspores (Fig. 3 E). The chalazal megaspore functions whereas the others degenerate.

The nucleus of the functional megaspore undergoes three mitotic divisions and forms 2- (Fig. 3 F), 4- (Fig. 3 G) and 8-nucleate gametophytes. The organised embryo sac consists of an egg, two synergids, a secondary nucleus and three antipodal cells (Fig. 3 H). The development of embryo sac is of the Polygonum type.

In one preparation the ovule showed twin nucelli with independent embryo sacs (Fig. 3 I).

5. *Endosperm*

The primary endosperm nucleus divides just above the antipodals. The first division is followed by the laying down of a wall (Fig. 4 N), resulting in a large micropylar chamber and a small chalazal chamber (Fig. 4 N). Initially the micropylar chamber contains several free nuclei, but later, the cell walls are formed centripetally. The endosperm is of the Helobial type (Fig. 4 O).

FIG. 2. Microsporangium, microsporogenesis and male gametophyte (*en*, endothecium; *ep*, epidermis; *gn*, generative cell nucleus; *ml*, middle layer; *tp*, tapetum; *vb*, vascular bundle; *vg*, vegetative cell nucleus). *A-C*, outline diagrams of cross-sections of anthers at various stages of development. × 72; *D*, magnified view of portion marked *d* in A to show microspore mother cells, tapetum and wall layers. × 333; *E*, magnified view of portion marked *e* in Fig. B to show tapetum, wall layers and first meiotic divisions in microspore mother cells. × 333; *F*, magnified view of portion marked *f* in Fig. C to show tapetum, wall layers and second meiotic divisions in microspore mother cells. × 333; *G*, magnified view of portion of mature anther wall to show the fibrous thickenings in endothecium. × 333; *H*, tapetal cell with a polyploid nucleus. × 500; *I & J*, two-nucleate tapetal cells. × 500; *K-S*, stages of meiosis in microspore mother cells. × 500; *T*, two-celled pollen grain, sectional and surface view. × 500.

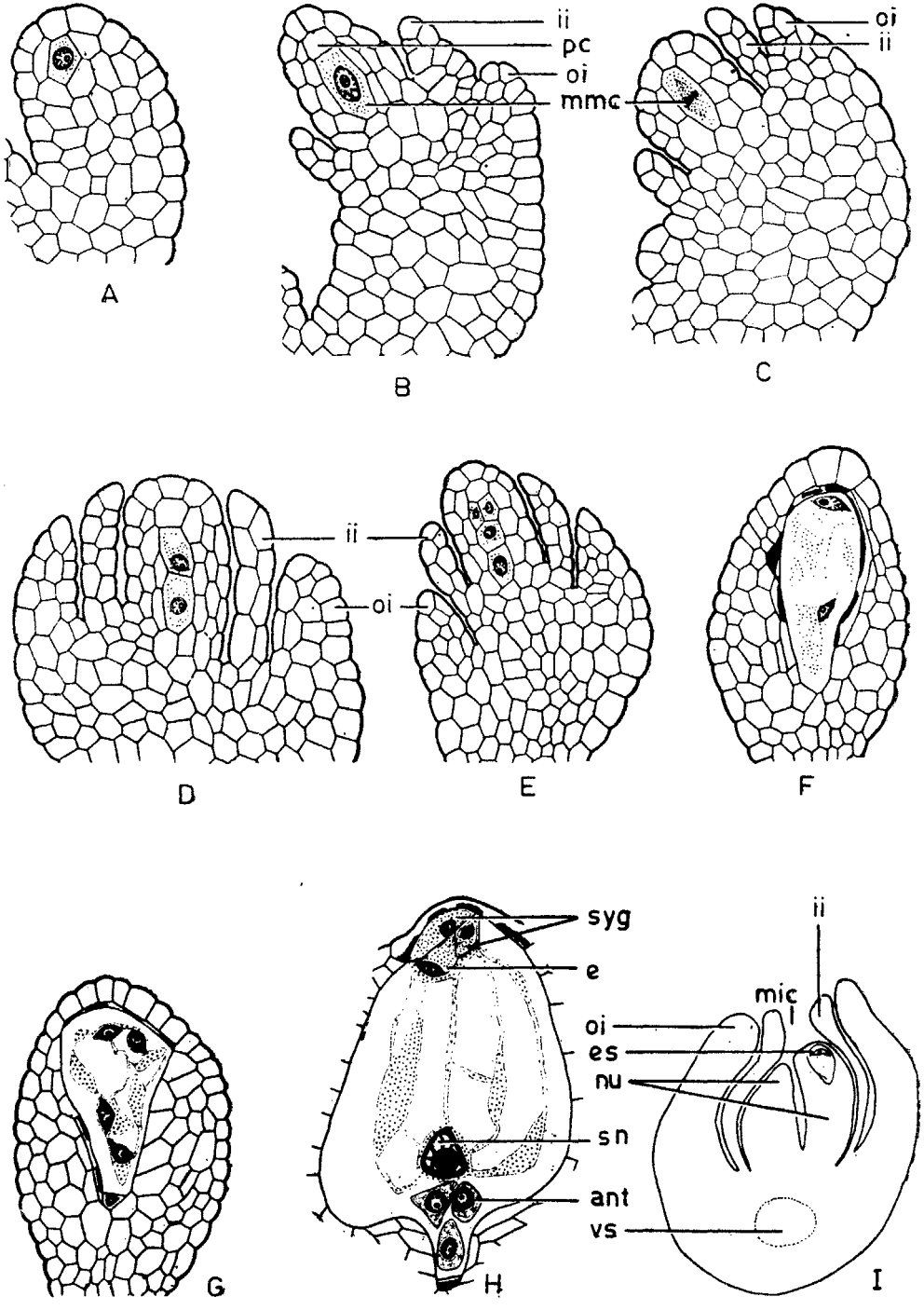

FIG. 3

6. *Embryo*

The zygote (Fig. 4 A) divides transversely to form a two-celled proembryo (Fig. 4 B). The basal cell divides in the same plane (Fig. 4 C) whereas the terminal cell divides longitudinally (Fig. 4 E). The latter undergoes transverse and longitudinal divisions and a globular embryo develops subsequently (Fig. 4 D-G). It elongates (Fig. 4 H-K), and an epicotyl apex forms at the base of the cotyledon (Fig. 4 L). The apex of the cotyledon elongates in the mature embryo (Fig. 4 M).

7. *Seed and Seed Coat*

The mature seed is blue-black in colour. Pigmentation is present in all the cells of the testa. The seed coat is fleshy. The seeds fall near the parent plant. However, bird dispersal is reported for coloured seeds of Liliaceae (*see* Pijl, 1972; Ridley, 1930).

At the megaspore mother cell stage, the inner integument is 2-cells thick, and the outer, 3-cells thick. During the subsequent development of the female gametophyte there is no change in the number of layers of the inner integument. However, the outer integument becomes 5-layered at the 4-nucleate stage of embryo sac (Fig. 5. B, F) and 12-or 13-layered at the globular embryo stage (Fig. 5 C, G). Later, the inner integument degenerates. In the mature seed, a fleshy seed coat is observed. It is formed by the testa consisting of 24 to 26 layers (Fig. 5 D, H).

8. *Pericarp*

At the megaspore mother cell stage, the ovary wall is 6-or 7- cells thick. It is composed of thin-walled cells. At the mature embryo sac stage (Fig. 5 A) it becomes 12 or 13 layered (Fig. 5 E) but the cells remain thin-walled. The epidermal cells, however, increase in size. After fertilization, the ovary wall splits and exposes the developing seeds.

Discussion

Embryology—In *Ophiopogon wallichianus, Polygonatum commutatum* and *Smilacina amplexicaulis,* the female archesporium may or may not cut off a parietal cell (Dahlgren, 1927; Maheshwari, 1934). To this list *Ophiopogon intermedius* may also be added. The funicular obturator, twin nucelli in the same ovule, and postament have not been reported previously in *Ophiopogon.*

Liriope, a member of Ophiopogonae shows the nuclear type of endosperm (Ono, 1928). However, in *O. intermedius* the type of endosperm development is Helobial.

Fig. 3. Megasporogenesis and female gametophyte (*ant*, antipodal cells; *e*, egg; *es*, embryo sac; *ii*, inner integument; *mic*, micropyle; *mmc*, megaspore mother cell; *nu*, nucellus; *oi*, outer integument; *pc*, parietal cell; *sn*, secondary nucleus; *syg*, synergid; *vs*, vascular supply. *A*, longisection of young nucleus showing archesporial cell. × 400; B, longisection of young ovule showing megaspore mother cell. × 400, *C*, same, showing dividing megaspore mother cell, note the absence of parietal cell. × 400; *D* & *E*, dyad and T-shaped megaspore tetrad. × 400, *F* & *G*, two and 4-nucleate embryo sacs. × 400 *H*, organised embryo-sac. × 400; *I*, ovule with twin nucelli. × 290.

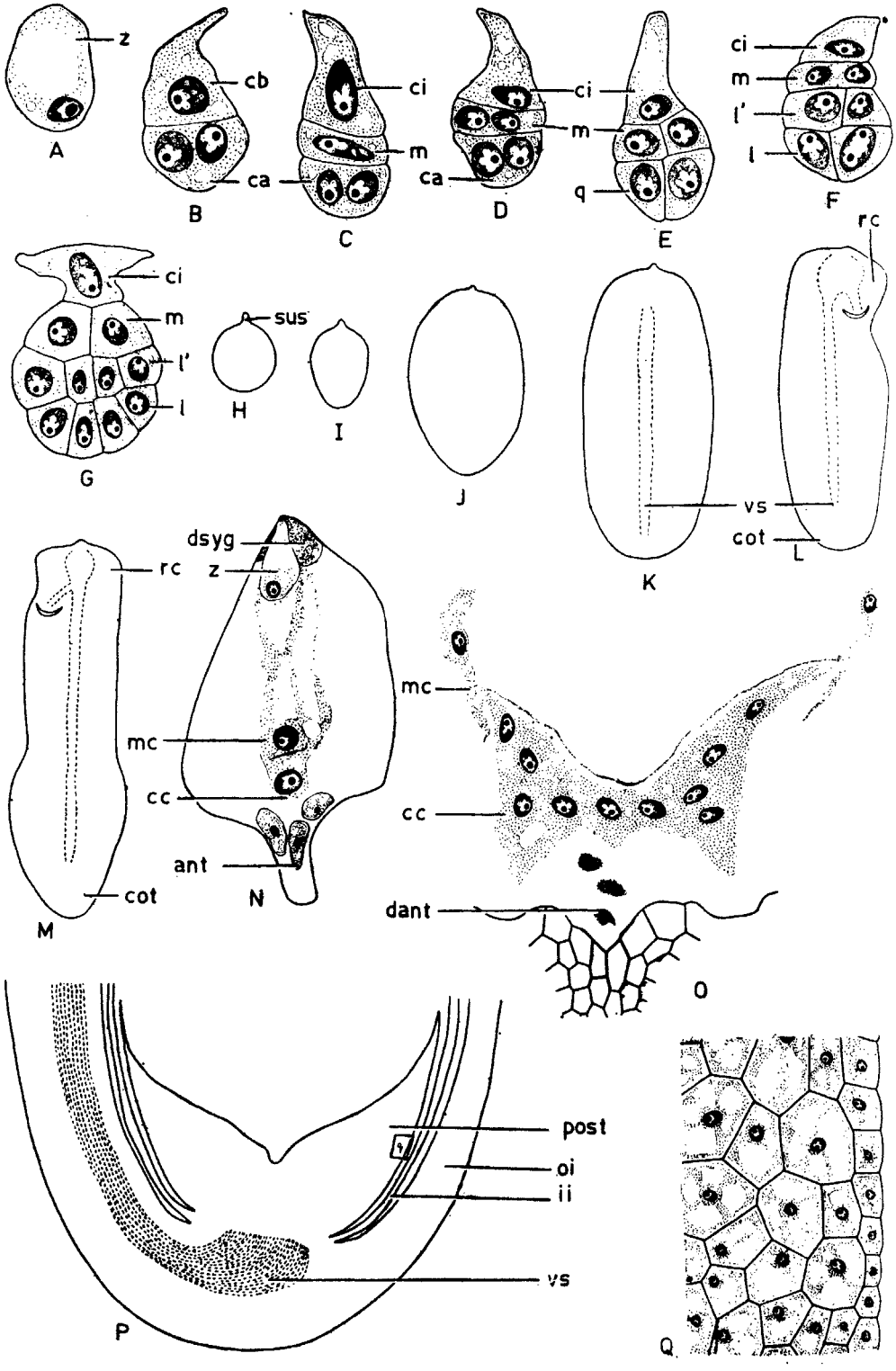
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FIG. 4

The study made by Huber (1969) of various families of Liliflorae showed several cases of free sarcotestal seeds, and a few cases where the sarcotesta is fused with pericarp. The seed coat of *O. intermedius* is not fused with pericarp. However, the testa is fleshy and contains a blue-black pigment.

Onagrad, Asterad, Caryophyllad and Chenopodiad types of Embryogeny have been reported in the Liliaceae (*see* Johansen, 1950). However, in *O. intermedius*, the embryogeny seems to be of the Onagrad type as in *Asparagus officinalis, Erythronium americanum, E. dens-canis, Fritillaria persica Gasteria verrucosa, Heleniopsis breviscapa* and *Lilium parryi* (*see* Johansen, 1950).

Systematic position of Ophiopogon—Bentham and Hooker (1862-1883) in *Genera Plantarum* placed *Ophiopogon* in the family Haemodoraceae under the tribe Ophiopogonae due to the presence of semi-inferior ovary. Krause (1930) in Engler and Prantl's '*Die Matuerlichen Pflanzenfamilien*' included *Ophiopogon* in Liliaceae under the tribe Ophiopogonoideae due to the occurrence of short rhizome, thin pericarp and fleshy seed coat. Hutchinson (1973) retained it in the tribe Ophiopogonae

	Ophiopogon	Haemodoraceae	Liliaceae
Inflorescence	Terminal raceme	Panicle, sub-umbellate cyme, raceme	Solitary, axillary or terminal raceme, cyme
Trichomes	Absent	Present on perianth, ovary, glandular	Rare
Ovary	Semi-inferior	Superior to inferior	Superior, rarely semi-inferior or inferior
Seed	Albuminous	Albuminous	Albuminous
Fruit	Ovary splits before maturity to expose developing seeds	Loculicidal capsule	Loculicidal or septicidal capsule, a fleshy berry or rupturing to expose seeds
Anther tapetum	Glandular	Amoeboid	Glandular
Pollen	Monocolpate, 2-celled	Monocolpate, 2-celled	One, two or more colpate or porate 2- or 3-celled

FIG. 4. Embryogeny, endosperm and chalaza of seed (*ant*, antipodal cell; *cc*, chalazal chamber; *cot*, cotyledon; *dant*, degenerating antipodal cell; *dsyg*, degenerating synergid; *ii*, inner integument; *mc*, micropylar chamber; *oi*, outer integument; *post*, postament; *rc*, root cap; *sus*, suspensor; *vs*, vascular supply; *z*, zygote). *A*, zygote. × 540; *B*, two-celled proembryo. × 540; *C & D*, three-celled proembryo, × 540; *E-G*, stages leading to the organization of globular embryo. × 540; *H-M*, stages leading to the organisation of mature embryo. × 165; *N*, first division of endosperm nucleus forming a small chalazal chamber and a large micropylar chamber. × 333; *O*, chalazal portion of embryo sac showing 10-nucleate chalazal chamber and a portion of micropylar chamber. × 333; *P*, chalazal portion of young seed showing postament. × 333; *Q*, magnified view of portion marked *q* in Fig. P to show the cellular details of postament. × 333.

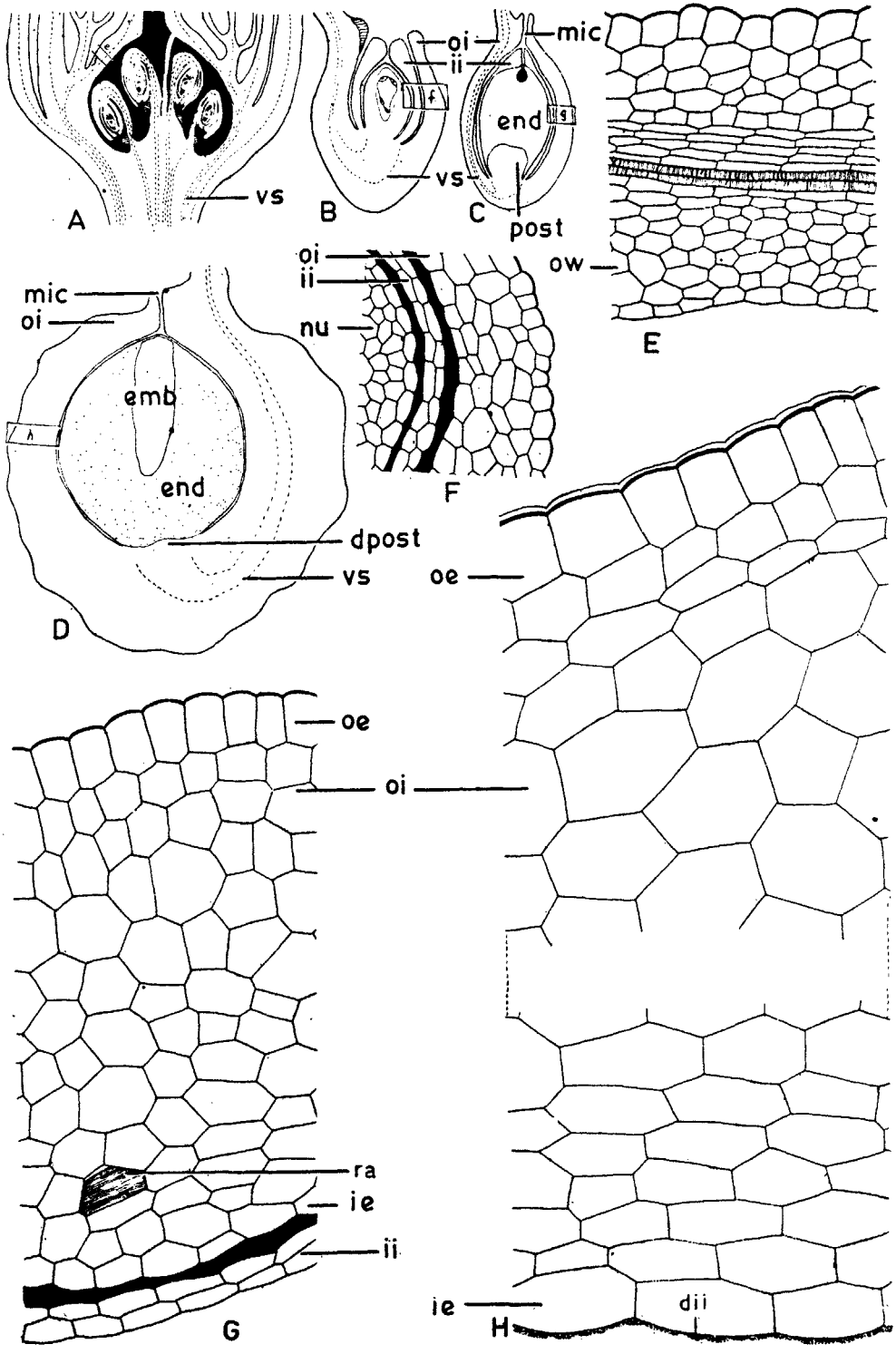
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FIG 5

of Liliaceae because of the presence of racemose inflorescence, and the splitting of ovary wall to expose the developing seeds.

The morphological and embryological characters of *Ophiopogon* are compared with those of Haemodoraceae and Liliaceae in the following Table (for literature *see* Cave, 1953, 1975; Davis, 1966; Dellert, 1933; De Vos, 1956, 1961, 1963; Erdtman, 1952; Hutchinson, 1973; Koul *et al*, 1976; Lawrence, 1951; Netolitzky, 1926; Ono, 1929; Schnarf, 1931).

Cytokinesis	Successive	Successive	Successive or simultaneous
Microspore tetrad	Isobilateral	Tetrahedral, isobilateral	Isobilateral, decussate, tetrahedral
Ovule	Anatropous bitegmic and crassinucellate	Hemianatropous, orthotropous, bitegmic and crassinucellate	Anatropous, orthotropous, hemianatropous, bitegmic and crassinucellate
Outer integument	3 to 5-layered	2-layered	3-to 5-layered
Obturator	Present	Absent	Present
Parietal cell	Present or absent	Always present	Present or absent
Megaspore tetrad	T-shaped	Linear	Linear or T-shaped
Embryo sac development	Polygonum type	Polygonum type	Polygonum, Allium, Adoxa, Drusa, Fritillaria or Scilla type
Endosperm	Helobial	Helobial	Helobial or Nuclear
Embryo	Onagrad ?	Asterad	Onagrad, Asterad, Caryophyllad or Chenopodiad

FIG. 5. Pericarp and seed coat (*dii*, degenerating inner integument; *dpost*, degenerating postament; *emb*, embryo; *end*, endosperm; *ie*, inner epidermis; *ii*, inner integument; *mic*, micropyle; *nu*, nucellus; *oe*, outer epidermis; *oi*, outer integument; *ow*, ovary wall; *post*, postaments; *ra*, raphides; *vs*, vascular supply). *A*, longisection of ovary at mature embryo-sac stage (semidiagrammatic). × 16; *B*, longisection of ovule at 4-nucleate embryo sac stage, × 94; *C & D*, young and nearly mature seeds in longisection at the globular and mature embryo stages. × 8.7; *E*, magnified view of portion marked *e* in Fig. A to show the cellular structure of ovary wall. × 300; *F*, magnified view of portion marked *f* in Fig. B to show the cellular details of integuments and nucellus. × 300. *G*, magnified view of portion marked *g* in Fig. C to show the structure of testa and tegmen; the testa is 12 or 13-layered and tegmen is 2-layered. × 300; *H*, magnified view of portion marked *h* in Fig. D to show the details of seed coat at maturity; the remnants of tegmen are discernible; testa possesses 24 to 26-cell layers. × 300.

The genus *Ophiopogon* resembles Liliaceae in several embryological characters like successive delimitation of microspores; glandular anther tapetum; monocolpate pollen grains; anatropous, crassinucellate, bitegmic ovule; thick outer integument; presence of an obturator; presence or absence of the parietal cell; T-shaped tetrad of megaspores; Polygonum type of embryo sac; Helobial type of endosperm; and copious endosperm in mature seed.

Although there are apparent resemblances between *Ophiopogon* and Haemodoraceae, the former shows marked dissimilarities like glandular anther tapetum; anatropous ovule; thick outer integument; presence of an obturator; presence or absence of parietal cell; and the T-shaped tetrad of megaspores. Contrary to these features, the Haemodoraceae show the amoeboid or plasmodial anther tapetum; absence of an obturator; presence of parietal cell and a linear tetrad of megaspores. Therefore, *Ophiopogon* does not share many common features with the Haemodoraceae, thus substantiating the opinions of Krause (1930), Pax (1930) and Hutchinson (1973). It agrees in most of its embryological characters with the genera of Liliaceae. Hence, the removal of *Ophiopogon* from the family Haemodoraceae, and placing it in Liliaceae is fully justified.

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References

Bentham, G. & Hooker, J. D. (1862-1883). *Genera Plantarum.* London.

Cave, M. S. (1953). Cytology and embryology in the delimitation of genera. *Chronica bot.*, **14** : 140-153.

———(1975). Embryological studies in *Stypandra* (Liliaceae). *Phytomorphology*, **25** : 95-99.

Dahlgren, K. V. O. (1927). Die Morphologie des Nuzellus mit besonderer Berücksichtigung der deckzellosen Typen. *Jb. wiss. Bot.*, **67** : 347-426.

Davis, G. L. (1966). *Systematic Embryology of the Angiosperms.* New York.

Dellert, R. (1933). Zur systematischen Stellung von *Wachendorfia*. *ost. bot. Z.* **82** : 335-345.

De Vos, M. P. (1956). Studies on the embryology and relationships of South African genera of the Haemodoraceae : *Dilatris* and *Wachendorfia*. *J. S. Afr. bot.*, **22** : 41-63.

———(1961). On the embryology and relationships of South African genera of the Haemodoraceae. In : *Recent Advances in Botany*, **1** : 694-698. Toronto.

———(1963). Studies on the embryology and relationships of South African genera of the Haemodoraceae : *Lanaria* Ait. *J. S. Afr. bot.*, **29** : 79-90.

Erdtman, G. (1952). *Pollen Morphology and Plant Taxonomy*, Angiosperms. Waltham, Mass., U.S.A.

Guérin, P. (1927). Le dévelopment de l'anthére et du pollen chez les Liliacées (*Sanseviera, Liriope, Ophiopogon, Peliosanthes*). *Bull. Soc. bot. Fr.*, **74** : 102-107.

Hooker, J. D. (1894). *The Flora of British India.* Vol. IV. Kent.

Huber, H. (1969). Die Samenmerkmale und Verwandtschaftsverhäeltnisse der Liliifloren. *Mitt. Bot. Muenchen.*, **8** : 219-538.

Hutchinson, J. (1973). *The Families of Flowering Plants.* London.

Johansen, D. A. (1950). *Plant Embryology.* Waltham, Mass., U.S.A.

Koul, A. K., Wafai, B. A. & Wakhlu, A. K. (1976). Studies on the genus *Gagea.* III. Sporogenesis, gametogenesis, early embryogeny and endosperm development in hexaploid *Gagea stipitata. Phytomorphology,* **26** : 255-263.

*Krause, K. (1930). Liliaceae. In : Eds. A. Engler & K. Prantl, *Die Natuerlichen Pflanzenfamilien,* **15a** : 227-386. Leipzig.

Lawrence, G. H. M. (1951). *Taxonomy of Vascular Plants.* New York.

Maheshwari, P. (1934). Contributions to the morphology of some Indian Liliaceae. I. The gametophytes of *Ophiopogon wallichianus. Proc. Indian Acad. Sci.,* **B1** : 197-204

Netolitzky, F. (1926). Anatomie der Angiospermen-Samen. Berlin.

*Ono, T. (1928). Endospermbildung von Liliaceen. *Bot. Mag., Tokyo,* **42** : 445-449.

———(1929). Embryologie der Liliaceae, mit besonderer Rücksicht auf die Endospermbildung. 1. Melanthioideae und Aletroideae. *Sci. Rep. Tohoku Univ.,* **4** : 381-393.

*Pax, F. (1930). Haemodoraceae. In: (Eds. A. Engler & K. Prantl.) *Die Natuerlichen Pflanzenfamilien* **15a** : 386-391. Leipzig.

Pijl, Vander (1972). *Principles of Dispersal in Higher Plants.* Berlin.

Ridley, H. N. (1930). *The Dispersal of Plants throughout the World.* Kent.

Schnarf, K. (1931). *Vergleichende Embryologie der Angiospermen.* Berlin.

*Original not seen.