MORPHOLOGICAL AND CYTOLOGICAL STUDIES IN THE SCROPHULARIACEÆ

III. A Contribution to the Life-History of *Ilysanthes parviflora* Benth.

By Dr. T. S. Raghavan, M.A., Ph.D. (Lond.), F.L.S.
(Head of the Department of Botany, Annamalai University)
AND
V. K. Srinivasan, M.Sc.
(Annamalai University)

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I. *Introduction*

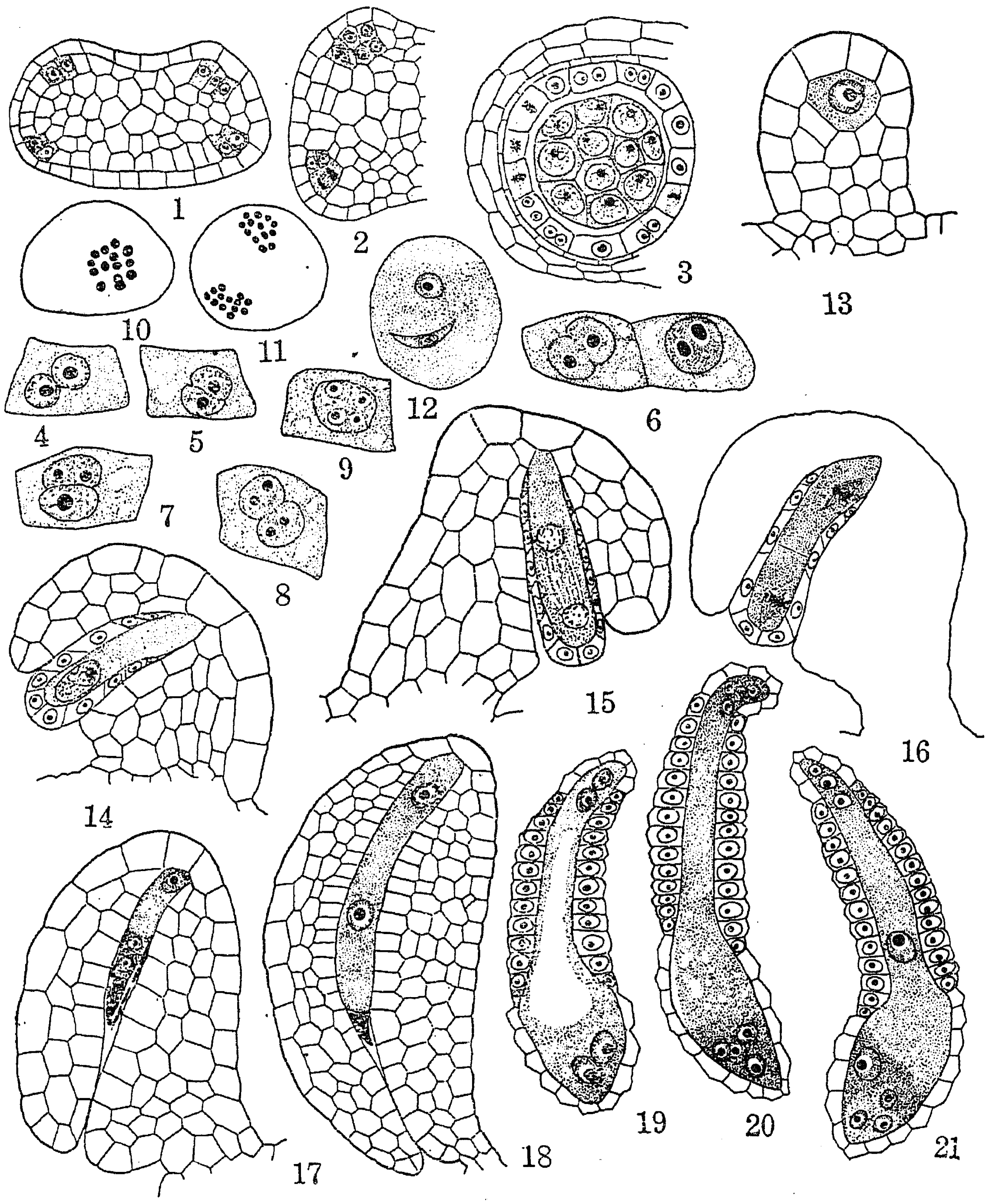
The first two papers of this series (Raghavan and Srinivasan, 1940; and Srinivasan, 1940) described the cytology of *Angelonia grandiflora*, and gave the chromosome numbers of about nine species distributed over four genera, as also the development of the anther, the embryo-sac, endosperm-haustorium and the embryo in *Angelonia grandiflora, Dopatrium lobelioides, Stemodia viscosa* and *Vandellia crustacea*. The genus *Ilysanthes* has not received any attention except for a small note by Krishna Iyengar (1929) on the development of the embryo-sac and endosperm-haustoria in an undetermined species of *Ilysanthes*. And the chromosome number of not even a single species of *Ilysanthes* has been determined so far. The haploid chromosome number of *Ilysanthes parviflora* has been recorded for the first time in the present communication. A detailed account of the ontogeny of the male and female gametophytes and of the embryo is given. The origin and development of the endosperm-haustorium is also described in detail. *Ilysanthes parviflora* is an erect branching herb. Flowers are borne in short racemes in the axils of leaves or at the ends of branches. The corolla which is bilobed is white in colour. There are two fertile and two undeveloped stamens.

II. Material and Methods

A few plants were found growing near rice-fields in Manalur, from which material for the present work was obtained. Only a few plants were available and as such sufficient anthers could not be got for any acetocarmine examination. Moreover, the anthers were so small that individual fixation was not advisable. Whole buds were therefore fixed in hot corrosive-sublimate fixative. Formalin-Acetic-Alcohol proved worthless for the purpose. Ovaries of various stages of development were fixed either in hot corrosive-sublimate or in Formalin-Acetic-Alcohol. Sections were cut at thicknesses varying from 6–14 microns and stained in Haidenhain's Iron-alum-hæmatoxylin.

III. Observations

(*a*) *Microsporogenesis.*—The primary archesporium of the anther lobe consists of a row of two or three hypodermal cells (Fig. 1) and is differentiated soon after the anther appears four-lobed in cross-section (Fig. 1). The archesporial cells are more prominent than the surrounding cells. The anther lobes become more and more prominent after the differentiation of the primary archesporium. The hypodermal archesporial cells cut off a layer of primary parietal cells (Fig. 2). The primary wall cells divide periclinally repeatedly giving rise to three layers of wall cells, the innermost of which functions as the tapetum (Fig. 3). The layer of wall cells immediately outside the tapetal cells get elongated tangentially and is finally crushed. The tapetal cells enlarge in size and small vacuoles make their appearance (Fig. 4). They are to begin with uni-nucleate. The single nucleus of the tapetal cells undergoes a division, just at the time, when the pollen mother-cells are in early prophase. This division is mitotic in nature. Fig. 3 shows various stages in the mitotic division of the tapetal nucleus. The further behaviour of the two nuclei of the tapetal cells is rather irregular. Sometimes, the two nuclei fuse to form a single large bi-nucleolated nucleus (Fig. 5). Fig. 6 shows two adjoining cells, in one of which the two nuclei are in a process of fusion, while the other shows the fused product. Often, however, the two nuclei divide again, the resulting four nuclei fusing immediately. This fusion follows so quickly upon the division, that it is hard to find them in a separate condition. Figs. 7 to 9 show the nuclei in various processes of fusion. In the nucleus which is thus organised, can be seen the four



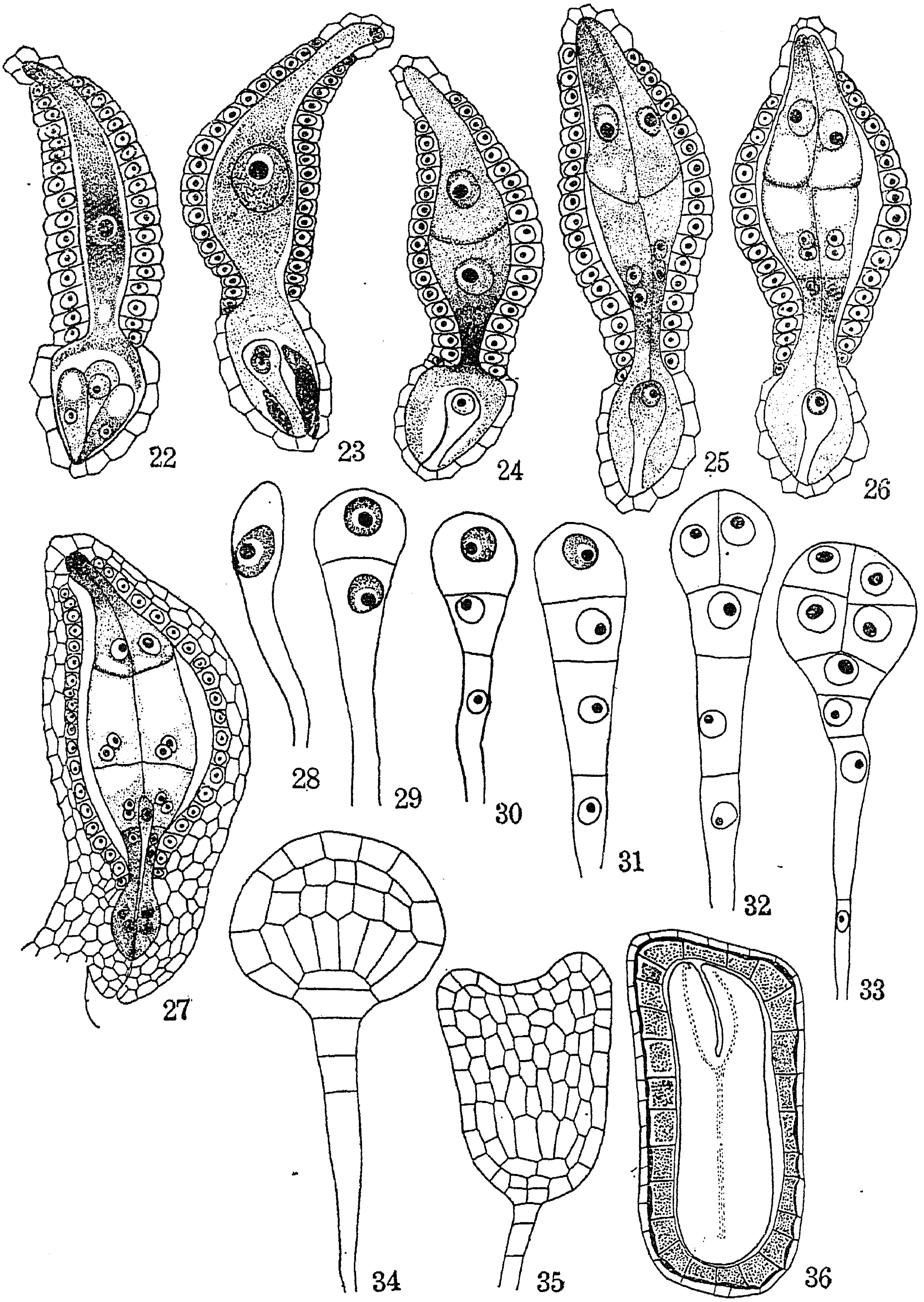
FIGS. 1–21

FIG. 1.—T. S. young anther, showing hypodermal archesporial cells. × 700. FIG. 2.—Archesporium cutting off wall cells. × 700. Fig. 3.—Shows the three layers of wall cells and the mitotic division of tapetal nuclei. × 700. FIG. 4.—Binucleate tapetal cell. × 2225. FIGS. 5–9.—Tapetal cells showing the nuclei in various processes of fusion. FIG. 10.—Metaphase I showing 13 bivalents. × 2225. FIG. 11.—Metaphase II, shows two groups of 13 univalents each. × 2225. FIG. 12.—Two-celled pollen grain at the time of shedding. × 1500. Fig. 13.—Hypodermal archesporium in the ovule. × 1500. FIG. 14.—Megaspore mother-cell invested by a single layer of nucellar cells. × 1500. FIG. 15.—Heterotypic division of the mother-cell. × 1500. FIG. 16.—Division of the dyad. × 1500. FIG. 17.—Linear tetrad. × 1500. FIGS. 18–21.—Two, four, and eight-nucleate embryo-sacs. Note the tapetum, which surrounds only the non-dilated chalazal portion. In FIG. 21.— the chalazal polar nucleus has reached the centre of the embryo-sac earlier than that of the micropylar end. × 1200.

nucleoli (Fig. 9). Very often, these various stages are represented side by side in the same tapetum. A similar behaviour of the tapetal nuclei has been recorded in *Gynandropsis pentaphylla* (Raghavan, 1938). The fused tapetal nucleus at pollen grain stage begins to disintegrate.

In metaphase, I, 13 bivalents are present in the equatorial plate (Fig. 10). Meiosis appears to be regular and disjunction is normal, there being no lagging chromosomes. Consequently, in metaphase II, the usual 13/13 distribution is the rule. Fig. 11 shows such a metaphase II showing the polar view of the two plates. Pollen tetrads are organised in the normal manner. Fig. 12 shows a pollen grain just at the time of shedding. The crescent shaped cell is the generative cell.

(*b*) *Megasporogenesis.*—The ovary is typically scrophularious being bicarpellary, and having numerous anatropous ovules arranged on an axile placenta. Early in the development of the ovule, the hypodermal archesporial cell is differentiated (Fig. 13). This takes place even before the integumental primordia have been initiated. This is a characteristic feature of not only the scrophulariaceæ but also of many other families. This archesporial cell directly functions as the megaspore mother-cell (Fig. 14) without cutting off any wall cell. By this time, the single massive integument grows rapidly accompanied by the anatropous curvature of the ovule (Fig. 15). The bulk of the ovule at this stage is made up of the integumentary tissue (Fig. 15). The megaspore mother-cell is invested almost up to its base by a single layer of nucellar cells (Fig. 14). This nucellar layer is derived from the original domed apex, which caps the archesporial cell. The megaspore mother-cell elongates considerably, after which it undergoes the heterotypic division forming a dyad (Fig. 16). The dyad by an ordinary homotypic division produces a linear tetrad of megaspores (Fig. 17). During the formation of the linear tetrad, the single layer of nucellus gets pressed against the integument and begins to degenerate, its place being taken up by the innermost layer of cells of the integument. This layer of cells is known as the tapetum, which is thus integumentary in its nature. It is composed of a regularly arranged layer of cells, which assume ultimately a more or less radially elongated configuration. The cells of this integumentary tapetum take deeper stain than the other cells of the integument and are uni-nucleate. A similar tapetal tissue of integumentary origin is characteristic of most members of the scrophulariaceæ. While the cells of this tapetum however, are all uni-nucleate, in families like Solanaceæ (Bhaduri, 1932) and Orobanchaceæ (Srivastava, 1939), binucleate tapetal cells would appear to be the rule.



FIGS. 22–36

FIG. 22.—Mature embryo-sac showing the egg-apparatus and the small-sized antipodals. × 1200. FIG. 23.—Note the male nucleus about to fuse with the egg, as also the remains of the pollen tube and synergids. × 1200. FIG. 24.—Two endospermal cells. × 1200. FIG. 25.—Shows the two uni-nucleate chalazal haustorial cells and micropylar chamber composed of two tiers of two cells each. × 1200. FIG. 26.—A micropylar tier of four cells has been cut off from the middle tier composed of four cells. × 1200. FIG. 27.—Shows the two uni-nucleate chalazal haustoria, four-nucleate micropylar haustorium and the elongated oospore, prior to division × 750. FIGS. 28-35.—Various stages in the development of the embryo. FIGS. 28–33 × 1500. FIG. 34, × 1200, FIG. 35, × 750, FIG. 36.—Longitudinal section of mature seed. × 355.

The chalazal megaspore is always the functional one, and develops into the mature 8-nucleate embryo-sac, while the three micropylar megaspores degenerate. The development of the 8-nucleate embryo-sac is quite normal and Figs. 18 to 21 show the various stages leading upto the formation of the 8-nucleate embryo-sac. Fig. 20 shows two groups of four nuclei at each end of the embryo-sac. Often the polar nucleus from the chalazal end reaches the centre of the embryo-sac earlier than the polar nucleus from the micropylar end (Fig. 21). The polar nuclei are distinctly larger than the other nuclei of the embryo-sac. In the mature embryo-sac, the micropylar part is enlarged and contains the egg-apparatus (Fig. 22). The tapetum surrounds only the non-dilated chalazal portion of the embryo-sac (Fig. 22).

(*c*) *Fertilization.*—Fig. 23 shows the male nucleus about to fertilize the egg. The male nucleus would appear to be spherical. The remains of the pollen tube as well as the degenerated synergids can be seen. It is said that vermiform and spiral-shaped male cells are by far the commonest in Angiosperms. Spherical nuclei, have, however, been occasionally reported. Weinstein (1926) in *Phaseolus vulgaris*, Madge (1929) in *Viola odorata*, Newman (1934) in *Acacia Baileyana* and Raghavan (1937) in *Cleome Chelidonii* are some of the records we have of spherical male nuclei. The male nuclei as well as the egg nuclei appear to be in a resting condition at the time of contact. Such a condition appears to be common not only in Angiospermous families, but also in the Coniferales (Guilliermond, 1933) and some Cycadales (Lawson, 1926). This may not have any phylogenetic significance, since this phenomenon is found in such widely separated families as Oenotheraceæ (Ishikawa, 1918), Hydrocharitaceæ (Wylie, 1923), Orchidaceæ (Pace, 1907) and Capparidaceæ (Raghavan, 1937). The antipodals are ephemeral.

(*d*) *Endosperm.*—The first division of the fusion endosperm nucleus is followed by the formation of a transverse wall, which divides the embryo-sac into two more or less equal chambers, a chalazal one and a micropylar one (Fig. 24). The micropylar chamber now divides followed by a longitudinal wall. In the meanwhile, the nucleus of the chalazal chamber has divided longitudinally to form two cells. These become the two chalazal haustoria and are the first to be differentiated (Fig. 25). The two micropylar endosperm cells undergo a second longitudinal division, the plane of this division being at right angles to the first longitudinal division. As a result, the micropylar chamber consists of two tiers of two cells each. These four cells divide transversely, thereby separating a micropylar tier of four cells from a similar middle tier (Fig. 26). The micropylar tier of four cells form the four micropylar haustoria, which soon fuse into a single tetranucleate haustorial body (Fig. 27). The micropylar haustorium is not very active and most of the integumentary cells in this region are left undigested. The two uni-nucleate chalazal haustorial cells (Fig. 27) are more aggressive than the micropylar haustorium.

(*e*) *Embryo.*—The development of the embryo commences only after a considerable amount of endosperm tissue has been formed. The first sign of the development of the embryo is the commencement of a marked elongation of the oospore (Fig. 28). After the oospore has elongated considerably, the first division of the oospore takes place, which is followed by the formation of a transverse wall (Fig. 29). The apical or the lower cell forms the embryo proper, while the basal cells form the suspensor. Fig. 31 shows the four-celled linear proembryo. Such a linear arrangement of the four-cells of the proembryo is characteristic of the Scrophulariaceæ. A longitudinal wall formed in the lowermost cell of the four-celled proembryo gives rise to the quadrant stage (Fig. 32). A transverse wall across the quadrants results in the octant stage (Fig. 33). Figs. 34 and 35 show later stages, the differentiation of the primary meristems, and the lobing of the cotyledons. The two cotyledonary lobes grow with the plumule between them. On the opposite end the radicle is differentiated. The vascular supply of the radicle and the hypocotyl become gradually continuous with those of the cotyledons (Fig. 36). Fig. 36 shows the mature seed in longitudinal section. There is no endosperm, the entire seed being occupied by the two cotyledons. The testa is composed of two layers of cells. During the growth of the seed, the tapetum is disorganised and the remaining two layers would appear to persist and make up the testa. The inner layer is composed of large thick-walled cells, while the outer layer is composed of thin walled cells tangentially elongated.

IV. Discussion

Haustoria, chalazal and micropylar, appear to be a constant feature of the Scrophulariaceæ. The form and the extent to which they are developed may vary considerably. In all the genera investigated so far, haustoria have been recorded. Only in *Angelonia* the absence of it was reported (Srinivasan, 1940). Schertz (1919) in the course of his work on *Scrophularia marylandica* observes incidentally that in the " Rhinanthaceæ and other members of the Scrophulariaceæ, micropylar and chalazal haustoria appear to be quite constant characteristics. In *Melampyrnum memorosa*, they are very arborescent, while in some other species only rudimentary haustoria occur. In *Scoparia* no haustoria are noticeable ". We have not been able to investigate *Scoparia* yet. But the suggestion made in the previous paper seems to be justified from the observations made in this genus also. It was suggested that the absence of haustoria in *Angelonia*—the only genus where haustoria was found wanting—was correlated to the persistence of the synergids. In other words to some extent the synergids took the place of the micropylar haustorium. They increased in size and form after fertilization instead of shrivelling and disappearing as they usually do. In forms where haustoria, micropylar and chalazal, are the rule, their nature is now beyond doubt. They are all endospermal in origin. But there was a time when the micropylar haustoria were regarded as transformed synergids. While therefore the true haustorium cannot be anything but endospermal, the idea of synergids functioning as haustoria cannot be considered new. Thus it is quite likely that in such of those few forms, where true endospermal haustoria do not occur, the synergids come forward and take up the role. This can, however, be generalised only by an examination of other forms like *Scoparia* which are said to possess no endospermal haustoria. The present investigation corroborates the previous finding to this extent, namely, that endospermal haustoria being a feature of constant occurrence, there is nothing abnormal in the behaviour of the synergids. Positive corroboration must, however, await further investigation especially of the genus *Scoparia.*

V. Summary

The haploid chromosome number of *Ilysanthes parviflora* has been determined for the first time, to be thirteen. The development of the anther is described. Meiosis of the pollen mother-cells is normal.

The development of the embryo-sac is described. The integumental tapetum surrounds only the non-dilated chalazal portion of the embryo-sac.

There are two uni-nucleate chalazal haustorial cells, which are more aggressive than the four-nucleate micropylar haustorium.

The development of the embryo is normal.

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