EMBRYOLOGICAL STUDIES IN GENTIANACEAE
(Gentianoidae and Menyanthoidae)

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Received May 25, 1962
(Communicated by Dr. J. Vonkateswarlu, F.A.Sc.)

INTRODUCTION

The family Gentianaceae which comprises 80 genera and 800 species (Willis, 1948) enjoys a world-wide distribution from highest Northern regions to equatorial tropical regions. Arctic and Alpine plants, halophytes, saprophytes (Voyria, Voyriella, Cotylanthera, etc.), marshy plants like Menyanthes and water plants like Limnanthemum occur in this family. Bentham and Hooker (1862-1883) and Rendle (1938) recognised two subfamilies, namely, (1) Menyanthoideae including all marshy plants and (2) Gentianoideae including the rest, while Wettstein (1935), Don (1838) and Lindsey (1938) raised the rank of Menyanthoideae to that of a separate family. Evidence from the study of vegetative anatomy (Perrot, 1897) and floral anatomy (Lindsey, 1938) was adduced in support of the treatment by Wettstein (1935) and Don (1838).

The saprophytic members seem to have received close attention from a long time and from about the beginning of this century, the rest of the Gentianaceae were studied from the point of view and certain opinions have been expressed on the systematic status of the two subfamilies mentioned above.

A close study of the embryological literature in the family shows that not even a single plant has been worked out in detail, covering all aspects of embryology. Further, no uniformity is seen in the structure and development of the anther, and the accounts of different authors, on the embryo development in Limnanthemum are contradictory. Therefore there appeared to be scope for further detailed study with reference to representatives of the two subfamilies.
The present paper deals with the life-history of the following members of the two subfamilies of Gentianaceae:

(i) **Gentanoideae**:
2. *E. pumilum* Griseb.
5. *C. diffusa* R. Br.

(ii) **Menyanthoideae**:

**Review of Previous Work**

Schnarf (1931) has given a brief summary of the embryological work done in the family Gentianaceae, up to 1930 in his book *Vergleichende embryologie der Angiospermen*. Stover (1932) reported Normal type of embryo-sac development in *Limnanthemum nymphoides*. His account of embryo development is meagre and contains only a few figures. Srinivasan (1941) investigated a few stages of male and female gametophytes in *Enicostemma littorale* and *Limnanthemum cristatum*.

Souèges (1943) reported Senecio variation of Asterad type of embryo development in *Menyanthes trifoliata*. Créte (1949 a and 1949 b) studied Solanad type of embryo development in *Gentiana asclepiadea* and *Erythraea centaurium*. Mecoy (1949) studied the development of male and female gametophytes in *Swertia carolinsis*. He reported a peculiar type of sterile tissue intruding into the interior of the anther lobe. The embryo-sac development is of the Polygonum type. Créte (1955 and 1956) reported Solanad type of embryo development in *Chlora perfoliata* and Senecio variation of Asterad type in *Limnanthemum nymphoides* respectively.

**Materials and Methods**

The material of *Limnanthemum indicum* was collected at Vizianagaram by Sri. J. V. Pantulu, Maharaja's College, Vizianagaram, and the rest of the species were collected by the author at various places; *Canscora diffusa* and *C. decussata* at Anantagiri; *Exacum bicolor* at Sunkarametta hills; *E. pumilum* at Muchkund; *E. petiolare* at Jeypore and *Limnanthemum*
cristatum at Kondakarla lake. The materials were fixed either in Formalin-acetic-alcohol or in Acetic-alcohol. Customary methods of dehydration and infiltration were followed. The sections were stained with Delafield’s haematoxylin.

MICROSPOROGENESIS AND MALE GAMETOPHYTE

The andrcœcum consists of four epipetalous stamens in Canscora diffusa, C. decussata, Exacum petiolare, E. bicolor and five stamens in Limnanthemum indicum and L. cristatum. In Canscora decussata, one of the four stamens is larger than the rest of the three (Fig. 10).

The primary archesporium in the anther lobe consists of a plate of 5–10 rows of hypodermal cells (Fig. 16). Each row is 6–16 cells deep (Fig. 11). The archesporial cells undergo periclinal divisions resulting in the formation of an outer primary parietal layer and an inner sporogenous layer (Figs. 1, 11, 12, 16 and 17). The parietal layer undergoes further divisions forming three wall layers in all the species investigated except in Exacum bicolor where it is five-layered. The innermost wall layer develops into the anther tapetum (Figs. 2–4 and 13).

In none of the species of Exacum a fibrous layer is differentiated and the dehiscence of the anther occurs by apical pores. Similar observations were made by Oehler (1927) in Cotylanthera tenuis. In C. diffusa, C. decussata, L. indicum and L. cristatum the hypodermal wall layer develops into the fibrous endothecium. Towards the connective side of the anther it is multi-layered. The middle layers become crushed in all the species studied except in E. bicolor and E. petiolare where the wall layers remain as crushed cells (Fig. 6).

The parietal tapetum is of the secretory type in the species of Limnanthemum and Exacum (Figs. 2–5, 18 and 19) and is of the amœboid type in the species of Canscora (Fig. 14). In the three species of Exacum the tapetal cells remain uninucleate and uniseriate throughout (Figs. 4 and 5). In L. cristatum the tapetal cells become biseriate towards the connective side of the anther. In L. indicum nuclear fusions of tapetal cells occur (Fig. 19).

In E. bicolor and E. pumilum, small granular thickenings are present on the inner walls of the tapetal cells (Fig. 5). In Canscora diffusa and C. decussata, the tapetal cells, towards the connective side, become multi-layered. At the time of the formation of the pollen tetrads in the anther lobe, the walls of the tapetal cells become disorganised and the cytoplasm flows into the anther lobe forming the periplasmodium (Fig. 14). The tapetal nuclei remain in a healthy state for a long time.
FIGS. 1-31
The primary sporogenous tissue divides in all planes and forms a fairly extensive sporogenous tissue in all species studied except in *E. bicolor* where it forms an extensive tissue. In *E. bicolor* the sporogenous tissue is half-moon shaped. The pollen mother cells divide in a simultaneous manner and both bilateral and tetrahedral types of pollen tetrads are formed (Figs. 7, 24 and 25). Cytokinesis takes place by furrowing. The mature pollen grains are three-celled (Figs. 9, 23 and 31). In *Canscora diffusa*, the generative nucleus divides either in the pollen grain or in the pollen tube. Thus both three-celled and two-celled pollen grains occur within the same pollen sac (Fig. 15).

The exine, in the three species of *Exacum* and in the two species of *Canscora*, shows minute projections (Figs. 8, 9, 22, 23 and 27–29). In the two species of *Limnanthemum*, the surface of the exine shows reticulate bands of thickenings (Figs. 30 and 31). The pollen grains are triporate. They are spherical in the species of *Exacum* and *Limnanthemum* and spindle-shaped in *Canscora* (Fig. 26).

During germination of the pollen grain, the position of the vegetative nucleus is not constant in the two species of *Canscora*. In some cases, it remains within the pollen grain and the generative cell travels into the pollen tube (Fig. 28), while in other cases, the vegetative nucleus travels into the distal end of the pollen tube and the generative cells remain in the pollen grain (Fig. 27). Regular metaphase plate and spindle fibres are seen in *Canscora diffusa*, during the divisions of the generative cell into two male cells in the pollen tube.

Degenerations of pollen sacs and pollen grains have been observed in all the species studied in the present investigation (Figs. 7, 20, 21 and 25).

The epidermal cells of the wall layers, in the three species of *Exacum*, contain fan-shaped crystals. In the two species of *Canscora*, the outer walls of the anther epidermis develop minute projections (Fig. 15).

**Ovary and Ovule**

The ovary is superior and bicarpellary syncarpous. It is unilocular in *L. indicum*, *L. cristatum*, *C. diffusa* and *C. decussata* and bilocular in the three species of *Exacum*. In the species of *Limnanthemum* the ovules are borne on 2–4 suppressed parietal placentas, while in the two species of *Canscora* there are two parietal placentas which fork at the base each into two branches (Figs. 32 and 33). In the three species of *Exacum* the parietal placentas grow deep into the ovary so as to meet in the centre to form an axile structure and make the ovary bilocular (Figs. 34 and 35).
The ovule is anatropous, unitegmic and tenuinucellate. The integument is 3–4 celled thick towards sides and 4–6 cells thick at the base1 region (Fig. 40). In *Exacum pumilum*, *L. cristatum* and *L. indicum* an integumentary tapetum is differentiated (Figs. 41–47). In later stages, in all the species studied, but for the epidermis, all the wall layers of the integument become thickened and used up by the developing endosperm and embryo.

In *E. petiolare*, about half of the ovules of an ovary degenerate either before or after formation of an eight-nucleate embryo-sac in them.

**Abnormal Ovule**

In the present study, in one case of *L. cristatum* fusion of two ovules is seen. The integumentary tapetum is differentiated in the two ovules and two micropyles are formed. However, formation of archesporium and megaspore mother cell, etc., are not seen in the fused structure.

**Megasporogenesis and Female Gametophyte**

The archesporium consists of a single hypodermal cell which directly functions as the megaspore mother cell (Figs. 36 and 40). In *C. decussata*, sometimes it consists of two or three cells (Fig. 37). The megaspore mother cell undergoes the two usual meiotic divisions resulting in the formation of a linear tetrad of megaspores of which the chalazal one develops into the eight-nucleate embryo-sac according to the Polygonum type (Figs. 38, 39, 40–43 and 45–52). The egg apparatus shows normal structures. Three uninucleate antipodals are formed. They are ephemeral in the two species of *Canscora* and degenerate after fertilisation in the rest of the species studied (Fig. 53).

In *Exacum petiolare* and *Canscora diffusa*, sometimes the upper dyad cell divides in a belated manner and the division is not followed by the formation of cell-wall (Figs. 38, 49 and 50). Sometimes, in *L. cristatum*, both the chalazal and the second megaspores of the tetrad develop further (Fig. 46). But no ovule with two embryo-sacs has been observed.

**Fertilisation and Endosperm**

Usually, the pollen grains in all forms studied are monosiphonous. But in 10% of cases of *Canscora diffusa*, however, two pollen tubes are formed per pollen grain.

In *L. indicum* and *L. cristatum*, the epidermal cells of the stigmatic head become papillate, by the time the pollen grains are deposited on it. The
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Figs. 32–65
cells of the stylar tissue, differ from the rest of the cells in size, shape and containing abundant cytoplasm (Fig. 57).

Fertilisation is porogamous. The pollen tube attacks one of the synergids and discharges its contents into it through a terminal pore (Figs. 54-56). A large number of starch grains accumulate in the fertilisable embryo-sacs of the two species of *Canscora* (Figs. 54-56). Syngamy and triple fusion take place more or less simultaneously (Fig. 56). The pollen tube persists until the primary endosperm nucleus undergoes the first division.

The endosperm is of the nuclear type in *Canscora diffusa*, *C. decussata*, *Exacum petiolare*, *E. pumilum* and *E. bicolor* and is of the cellular type in *Limnanthemum cristatum* and *L. indicum*.

In the species of *Exacum* and *Canscora* the first division of the endosperm nucleus is unaccompanied by wall formation (Fig. 58). After a few free endosperm nuclei are formed in the embryo-sac wall formation commences from the periphery to the centre, ultimately filling the whole embryo-sac with cellular tissue (Figs. 59-61). In the two species of *Limnanthemum* the first division of the endosperm nucleus is accompanied by cell-wall formation. Gradually the whole of the embryo-sac is filled with cellular tissue. In all the species studied starch and albumen crystals appear as reserve food (Figs. 61-65). The seeds are endospermic.

**EMBRYO**

The course of events in the development of the embryo in the genus *Limnanthemum* is different from the other two genera and hence a separate account is given for the species of *Limnanthemum*.

*Canscora decussata*, *C. diffusa*, *Exacum pumilum* and *E. petiolare*

The fertilised egg divides transversely resulting in a two-celled pro-embryo (Figs. 66, 82, 95 and 96). The terminal cell *ca* and the basal cell *cb* undergo one more transverse division resulting in a four-celled linear pro-embryo (Figs. 67, 83 and 97-102). They are termed as *l, l', m* and *ci* starting from the base. The two tiers *l* and *l'* undergo each one more transverse division resulting in four cells, which are arranged in a linear row, above the two basal cells *m* and *ci*. The two daughter cells of the tier *l* are designated as *l_1* and *l_2* and that of *l'* as *l'_1* and *l'_2*. Thus a six-celled linear embryo is formed (Figs. 68, 84, 85 and 103-105).

Sometimes, in the two species of *Canscora*, in the four-celled linear pro-embryo, transverse divisions either in the cell *m* or in the cell *ci* may also
In all the species studied, in each of the three tiers of cells $l_1$, $l_2$ and $l_1'$ two vertical divisions take place at right angles to one another resulting in the formation of four cells in each tier (Figs. 70–75, 86–88 and 107–10). The sequence of vertical divisions is as follows: first the cell $l_2$ divides vertically followed by vertical divisions in the cells $l_1$ and $l_1'$. Sometimes in the two species of *Canscora*, a deviation is seen from the normal course of events in the vertical divisions of the cells $l_1$, $l_2$ and $l_1'$. The vertical division in the terminal
cell 1 takes place followed by vertical division either in the cell 2 or in the cell 1' (Figs. 116 and 117).

Periclinal divisions take place in the tiers 1 and 2, as a consequence of which dermatogen layer is differentiated in these tiers (Figs. 76-80, 89-93 and 111-13). The inner layer of cells divide in all directions and periblem and plerome are differentiated. The derivatives of the tier 1 contribute to the formation of cotyledons and the derivatives of the tier 2 to the formation of hypocotyledonary region (Figs. 78-81, 90-94 and 112-15).

In the two species of *Exacum*, in the tier 1' further divisions take place in all directions and their derivatives contribute to the formation of all parts of root-root cortex, root-tip and root-cap. In the two species of *Canscora*, both the cells 1' and 2' divide and their derivatives contribute to the formation of root cortex, root-tip and root-cap (Figs. 78-81, 90-94 and 110-15).

In the genus *Exacum*, 2' forms a part of suspensor. In both the genera, the two cells m and c1 divide transversely resulting in the formation of an uniseriate suspensor.

The relation of the individual cells of the proembryo to the organs of the mature embryo is shown in the following schematic representation:

$$
\text{Exacum}
\begin{array}{c}
\text{Fertilised egg} \\
\text{l}_1 \\
\text{c}_a \\
\text{l}_2 \\
\text{m} \\
\text{e}_b \\
\text{f} \\
\text{c}_1 \\
\text{n'} \\
\text{n} \\
\text{d} \\
\text{l}_3' \\
\text{l}_2' \\
\text{l}_1' \\
\text{l}_1 \\
\text{.. Cotyledons} \\
\text{.. Hypocotyledonary region} \\
\text{.. All parts of root} \\
\text{.. Suspensor}
\end{array}
$$
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Figs. 95-117
From the above, it can be made out that the four-celled proembryo is linear and the cell \textit{ca} contributes to the development of the embryo proper and the basal cell \textit{cb} contributes to the formation of the suspensor. This is characteristic of the Solanad type. Further, in the destination of the cells of the tetrad it closely resembles Physalis 11 variation. Thus the embryo development conforms to the Physalis 11 variation of Solanad type.

\textit{Limnanthemum indicum} and \textit{L. cristatum}

The fertilised egg divides transversely resulting in the formation of two superposed cells, the terminal cell \textit{ca} and the basal cell \textit{cb} (Fig. 118). The basal cell \textit{cb} divides transversely and produces two superposed cells \textit{m} and \textit{ci}. The cell \textit{ci} again divides transversely and produces two superposed cells \textit{n} and \textit{n’} (Figs. 119–22). The cell \textit{n’} undergoes a transverse division giving rise to two superposed cells \textit{p} and \textit{o} (Figs. 123 and 124).

The terminal cell \textit{ca} of the two-celled proembryo undergoes two vertical divisions at right angles to each other resulting in the formation of quadrants. The four cells of the tier \textit{q} divide by obliquely oriented walls (Figs. 121–25) and form octants. Due to periclinal divisions in the octants dermatogen layer is demarcated in the apical tier. The inner cells divide in all directions
and give rise to periblem and plerome initials. The derivatives of this tier contribute to the formation of stem tip and cotyledons (Figs. 126–29). The cell \( m \) undergoes two vertical divisions at right angles to each other resulting four cells (Fig. 120). In this tier also dermatogen periblem and plerome initials are differentiated and the derivatives of the tier contribute to the formation of the hypocotyledonary region and plerome initials of root.

The cells \( n \) and \( o \) undergo two vertical divisions at right angles to each other (Figs. 125 and 126). The derivatives give rise to the root-tip, root-cap and dermatogen of the root.

The cell \( q \) undergoes one or two transverse divisions to form a short uniseriate suspensor.

The relation of the individual cells of the proembryo to the organs of the mature embryo is shown in the following schematic representation:

\[
\begin{align*}
\text{Fertilised egg} & \quad \text{.. Cotyledons and stem tip} \\
\text{.. Entire hypocotyledonary region and plerome initials of root} \\
\{ \quad \text{.. Root-tip, root-cap and dermatogen of root} \\
\text{.. Suspensor}
\end{align*}
\]

The four-celled proembryo is ‘T’-shaped and is arranged in three tiers, and the derivatives of both \( ca \) and \( cb \) contribute to the formation of the embryo proper. These features are characteristic of Asterad type of development. The walls laid during the second division of the cells in the tier \( ca \) are oblique and the cell \( m \) gives rise to the entire hypocotyledonary region and plerome initials of root. Thus the embryo development keys out to the Senecio variation of Asterad type.

**Polyembryony**

In one case of *Limnanthemum indicum*, however, two embryos were seen in addition to the zygotic embryo (Fig. 130). Judging from their position, they seem to be developed from the two synergids.
SEEDCOAT AND FRUIT WALL

In all the plants studied, the outer epidermis of the integument alone forms the seedcoat. In the species of *Exacum* the inner and radial walls of the epidermis become enormously thickened (Fig. 139). Figure 138 shows surface view of the seedcoat. In the two species of *Canscora* the walls of the epidermal cells become stratified to form the seedcoat. In the post-fertilisation stages of the ovules, in the two species of *Limnanthemum* the epidermal cells of the integument become elongated and are thin-walled (Fig. 131). By the time the octants are formed in the embryo, most of the layers of the integument including the integumentary tapetum become crushed and absorbed by the endosperm. At this stage the radial walls of the epidermal cells become zigzag and get thickened (Fig. 132). The surface of the seedcoat shows reticulate ornamentation and develops spinous projections (Figs. 133 and 134). In the meanwhile usually, two of the epidermal cells at regular intervals project out and thus the seedcoat in the final stages of its formation becomes irregular in outline.

The fruit wall, in the three species of *Exacum*, consists of the epidermis, sub-epidermal layer with thick-walled cells and a few layers of parenchymatous cells (Fig. 136). In *Canscora decussata* and *C. diffusa* the fruit wall consists of four or five layers of cells below the epidermis (Figs. 135 and 137).

DISCUSSION

The anther tapetum in the family shows great variation. An uninucleate condition is seen in *Exacum petiolare, E. pumilum* and *E. bicolor* (present study), *Enicostemma littorale* (Srinivasan, 1941) and in *Swertia carolinsis* (Mecoy, 1949). On the other hand, in *Limnanthemum cristatum* (Srinivasan, 1941 and present study) multi-nucleate tapetal cells and in *L. indicum* (present study) tapetal cells with nuclear fusions were observed.

Secretory type of anther tapetum has been recorded in all species of Gentianaceae so far investigated. In the present study in *Canscora diffusa* and *C. decussata*, however, a periplasmodial type of anther tapetum was observed. Since the tapetal nuclei remain in a healthy condition for a long time, it can be classified as true periplasmodial tapetum of Tischler (1915).

Guérin (1924, 1925 and 1926) reported three types of anther tapetum in this family. In the first type, according to him, the anther tapetum is sporogenous in origin as in *Gentiana* and *Swertia perennis*. Mecoy (1949) recorded in *Swertia carolinsis* an anther tapetum of parietal origin which shows normal behaviour. All the members, investigated in the present
study, show an anther tapetum which is parietal in origin and resembles that of *Swertia carolinsis*.

Guérin (1926) recognised the second and third types of anther tapetum on the basis of the shape of the tapetum. In the second type, it is half-moon shaped as in *Erythrea centaurium* and *Chlora perfoliata* and in the third type it is spherical as in *Menyanthes trifoliata* and *Limnanthemum nymphoides*. I am of the opinion, that the shape of the tapetum changes with the shape of the sporogenous tissue and hence cannot be taken, as a criterion for the classification of the tapetum.

Usually, the pollen grains are three-celled, but in *Canscora diffusa*, both two- and three-celled pollen grains occur. Occurrence of both two- and three-celled pollen grains is known in a few species of *Dionaea* (Smith, 1929), *Viola* (West, 1930), *Epimedium* and *Iris* (Schnarf, 1937). Maheshwari (1950) says, that this is probably due to environmental changes. But in *C. diffusa* occurrence of these two conditions is seen even in the same anther of the flower and in plants growing together in the same locality.

Poddubnaja-Arnoldi (1936), on the basis of position of the vegetative nucleus in only one of the branches of the pollen tube (in *Aconitum*, *Cucurbita* and *Papaver*), considered it as a vestigial structure which has no role in the growth of the pollen tube. In *Canscora diffusa*, however, the vegetative nucleus is not always at the distal end of the pollen tube. In some cases, it travels to the distal end of the pollen tube and in other cases it remains behind the male cells. Thus, my observations support the view of Poddubnaja-Arnoldi (1936) on the nature and function of the vegetative nucleus.

The aquatic forms, *Limnanthemum cristatum* and *L. indicum*, are characterised by the presence of an integumentary tapetum, a character reported by Stolt (1921) in other aquatic members of the family, namely, *L. nymphoides*, *Menyanthes trifoliata* and *Villarsia reniformis*. In the terrestrial forms, no integumentary tapetum is differentiated. However, in *Exacum pumilum*, which is a terrestrial form, an integumentary tapetum is found during the present study. In this respect this resembles the aquatic forms.

The endosperm, in the aquatic forms, develops according to the cellular type and in the terrestrial forms according to the nuclear type. Similar observations were made by Stolt (1921) both in the aquatic and terrestrial forms. However, the saprophytic forms show a deviation from this feature. In *Voyria* and *Voyriella*, the development of the endosperm takes place according to the cellular type and in *Cotylanthera* and *Leiphaimos*, it develops according to the nuclear type.
Except for the works of Souèges (1943) and Créte (1949 a, 1949 b, 1955 and 1956), a detailed information about the development of the embryo is not available in this family. Johansen (1950) based on a few observations made by Paley (1920) on Swertia longifolia and by Stover (1932) on Limnanthemum nymphoides, says probably that the embryo development in Swertia longifolia is of the Senecio variation of Asterad type. The observations cannot be taken as final, because essential details are lacking in the accounts on the development of the embryo, on which Johansen (1950) based his interpretations. Further, Paley (1920) stated that in Swertia longifolia, the basal cell of the two-celled proembryo does not contribute to the formation of the embryo proper, while in the Asterad type both the terminal and basal cells take part in the development of the embryo proper. Créte (1956) reported Senecio variation of Asterad type of embryo development in Limnanthemum nymphoides. Johansen (1950) concluded, that in Limnanthemum nymphoides (Stover, 1932) the embryo development, probably conforms to the Caryopyllad type. But Créte (1956) reported Senecio variation of Asterad type of embryo development in Limnanthemum nymphoides. In the present study, the embryo development in Limnanthemum cristatum and L. indicum resembles that in Menyanthes trifoliata (Souèges, 1943) and L. nymphoides (Crété, 1956).

Judging from the available knowledge on the development of the embryo in this family, it can be seen that the aquatic forms are characterised by the Senecio variation of Asterad type and the terrestrial forms by Solanad type.

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<tr>
<th>Aquatic forms (Asterad type)</th>
<th>Terrestrial forms (Solanad type)</th>
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<tbody>
<tr>
<td>Menyanthes trifoliata</td>
<td>Chlora perfoliata</td>
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<tr>
<td></td>
<td>Canscora diffusa</td>
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<tr>
<td>Limnanthemum nymphoides</td>
<td>Canscora decussata</td>
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<tr>
<td>Limnanthemum cristatum</td>
<td>Exacum petiolare</td>
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<tr>
<td>Limnanthemum indicum</td>
<td>Exacum pumilum</td>
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Morphologically Menyanthoideae is characterised by the presence of alternate leaves, valvate or imbricate corolla and either aquatic or marshy habit. The Gentianoideae are characterised by opposite decussate leaves, twisted corolla lobes and terrestrial habit. Both vegetative (Perrot, 1897
and 1898 and Metcalfe and Chalk, 1950) and floral anatomical (Lindsey, 1938) characters are in support of raising the Menyanthoideae to a separate family rank. Anatomically Menyanthoideae differs from Gentianoideae in the absence of sieve tubes outside the normal phloem, presence of collateral bundles and the presence of discrete vascular bundles of the stem (Perrot, 1897 and 1898), while the members of Gentianoideae are characterised by the presence of sieve tubes outside the phloem, typical siphonostele in the stem and bicolateral or amphicribial vascular bundles.

In the floral anatomy, the members of the Menyanthoideae are distinguished from the Gentianoideae in the presence of ovule traces, fusion of adjacent corolla laterals, bilaterally symmetrical vascular pattern and epigynous insertion of floral parts in certain genera accompanied by extreme vascular fusion (Lindsey, 1938).

My observations on the embryology of the aquatic and terrestrial forms of the family Gentianaceae, also support the splitting up of the family into two independent families, namely, Gentianaceae and Menyanthaceae, as proposed by Souèges (1943) and Créte (1949 a, 1949 b, 1955 and 1956).

Embryologically the members of Menyanthaceae are characterised by the presence of bi- or multi-nucleate cells of anther tapetum with nuclear fusions, suppressed parietal, placentas, integumentary tapetum, constant occurrence of three uninucleate antipodal cells, cellular endosperm, Senecio variation of Asterad type of embryo development and the ornamentation of seedcoat, while the members of Gentianaceae are characterised by the uninucleate cells of the anther tapetum, well-developed placentas sometimes fusing at the centre to form a bilocular ovary, the absence of integumentary tapetum, great variation of antipodal cells and nuclei in them, nuclear endosperm and Solanad type of embryo development.

Thus, on the basis of morphological, anatomical (both vegetative and floral) and embryological evidence the two subfamilies of the family Gentianaceae may be treated as two independent families, namely, the Gentianaceae and Menyanthaceae.

**SUMMARY**

The present paper deals with the structure and development of anther and pollen, ovary, ovule, megasporogenesis, embryo-sac, fertilisation, endosperm, embryo and seedcoat in *Exacum petiolare*, *E. pumilum*, *E. bicolor*, *Canscora decussata*, *C. diffusa*, *Limnanthemum indicum* and *L. cristatum*.
The archesporium in the anther lobe consists of a plate of 5–10 rows of cells and each row is 6–16 cells deep. Two wall layers occur in *L. indicum*, *C. diffusa*, *C. decussata* and *E. pumilum*, three in *E. petiolare* and four in *E. bicolor* and *L. cristatum*. The sub-epidermal layer develops into the fibrous endothecium in all the species studied except in the three species of *Exacum*. The tapetum is of the secretory type in the two species of *Limnanthemum* and in the three species of *Exacum*. It is of the amoeboid type in the two species of *Canscora*. In the two species of *Limnanthemum*, nuclear divisions and fusions of tapetal cells are seen. Small granular thickenings are present on the inner walls of tapetal cells in *E. pumilum* and *E. bicolor*. Division of pollen mother cells is of simultaneous type. Cytokinesis is by furrowing. Both bilateral and tetrahedral tetrads are formed. The pollen grains are three-celled and triporate. The exine shows minute projections in the three species of *Exacum* and in the two species of *Canscora*. In *L. cristatum* and *L. indicum* the exine shows rod-like thickenings in it. Degenerations of pollen and pollen sacs are observed in *E. petiolare* and *C. decussata*.

The ovary is superior and bicarpellary syncarpous. It is unilocular in *L. cristatum*, *L. indicum*, *C. decussata* and *C. diffusa* and is bilocular in *E. petiolare*, *E. pumilum* and *E. bicolor*. The ovule is unitegmic, anatropous and tenuinucellate.

The primary archesporium is unicellular. A linear tetrad of megaspores is formed. The chalazal megaspore of the tetrad develops into the eight-nucleate embryo-sac according to the Polygonum type. Three uninucleate antipodal cells are formed.

Fertilisation is porogamous. Usually, the pollen grains are monosiphonous, but in about 10% of cases of *C. diffusa* two pollen tubes are formed per pollen grain.

Endosperm is of the nuclear type in *E. pumilum*, *E. petiolare*, *C. diffusa* and *C. decussata* and is of the cellular type in *L. cristatum* and *L. indicum*.

Embryo development keys out to the Senecio variation of Asterad type in *L. cristatum* and *L. indicum* and Physalis 11 variation of Solanad type in the species of *Exacum* and *Canscora*.

The outer epidermis of the integument forms the seedcoat.

On the basis of morphological, anatomical (both vegetative and floral) and embryological characters, the two subfamilies, *Gentianoideæ* and *Menyanthoideæ* of the family *Gentianaceaæ*, may be raised to the rank of independent families, namely, the *Gentianaceaæ* and *Menyanthaceæ*. 
ACKNOWLEDGEMENTS

The writer wishes to express her sincere thanks to Prof. J. Venkateswarlu for his guidance and constructive criticism throughout the course of the investigation and in the preparation of the manuscript. She is also thankful to Sri. J. V. Pantulu, Maharaja’s College, Vizianagaram, for kindly collecting the material of *Limnanthemum indicum*.

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EXPLANATION OF FIGURES


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