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EMBRYOLOGY OF *CRAWFURDIA DELAVAYI* (GENTIANACEAE) AND ITS SYSTEMATIC VALUE

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ABSTRACT

The embryological characters of *Crawfurdia delavayi* Frabnch. are described and the systematic relationships of *Crawfurdia* discussed. Anthers are tetrasporangiate. The development of anther walls conforms to the Dicotyledonous type. The tapetum is of single origin. The development of the tapetum with uninucleate cells is of the glandular type. The tapetal cells on the connective side show radial elongation or periclinal division and intrude into the anther locule. The epidermis of anther walls persists and its cells become pillar and fibrous, and the endothecium degenerates. The ovary is bicarpellary and unilocular. The placentation is typically parietal with 8 rows of anatropous ovules. The development of embryo sac is of the polygonum type. Before fertilization, two polar nuclei fuse into a secondary nucleus. Three antipodal cells persist. Flowers are protandrous. Fertilization is porogamous. The development of the endosperm is of the nuclear type. The embryogeny corresponds to the solanad type physalis II variation. The embryological data indicate that it is better to separate *Crawfurdia* from *Gentiana* as an independent genus.

INTRODUCTION

Crawfurdia was established by Wallich in 1826 based on the two new species *Crawfurdia speciosa* and *C. fasciculata* which were different from all the known species in the genus *Gentiana*. *Crawfurdia* was reexamined by Marquand (1931, 1937), who did not accept the genus *Crawfurdia* and merged it into *Gentiana*. However, the genus is retained as an independent one by many authors (Gilg, 1895; Smith, 1965; Wu, 1984; He, 1988; He and Liu, 1990; and others). This genus has never been investigated embryologically. The objective of the present paper is to report observations on the embryology of genus *Crawfurdia* and to discuss sysematic implications of the embryological data.

MATERIALS AND METHODS

Material investigated for the present study was collected from Dali, Yunnan Province, China. Twenty specimens were examined. The voucher (LIU Jian-quan 320) is preserved in the Herbarium of Northwest Plateau Institute of Biology, Chinese Academy of Sciences, Xining, China (HNWP).

Anthers, ovules, and seeds at different stages of development were fixed in 1:3 glacial acetic acid/absolute ethanol. After being stained in Ehrlich's hematoxylin, the material was embedded in paraffin by the conventional method and sectioned at a thickness of 6–12 μm.

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Sections were stained with safranin–fast green, and then observed and photographed.

RESULTS

MICROSPORANGIA AND MICROSPOROGENSIS

Flowers of *Crawfurdia delavayi* were bisexual and protandrous. Anthers were tetrasporangiate. At an early stage of development, 4 rows of archesporial cells differentiated below the epidermis of the anthers. Archesporial cells were recognizable by their dense cytoplasm and conspicuous nuclei. These cells divided periclinally, forming outer primary parietal cells and inner primary sporogenous cells (Fig. 1). The primary parietal cells divided periclinally and anticlinally, forming two layers of secondary parietal cells. The inner secondary parietal cells gave rise to the tapetum. Thus, the tapetum was of single origin. The outer secondary parietal cells formed a subepidermal endothecium and a middle layer by periclinal and anticlinal division. The anther wall was composed of four layers: epidermis, endothecium, middle layer, and tapetum (Fig. 2). The endothecium and middle layer originated from the primary parietal cells. The development of the microsporangial wall thus conforms to the dicotylendonous type (Davis, 1966).

Cells of the tapetum on the connective side showed radial elongation or periclinal division and intruded into the anther locule. Tapetal cells were uninucleate throughout their development. At about the time of pollen tetrad formation, walls of the tapetal cells became indistinct and the tapetal cells degenerated (Figs. 2,3). The tapetal cells degenerated completely at the stage of 1-nucleate pollen grains (Fig. 4). Thus, the tapetum of *Crawfurdia delavayi* is glandular.

The middle layer was crushed during meiosis of microsporocytes.

As the anther matures, the epidermis of the anther wall persisted and the cells became pillar and fibrous. The endothecium degenerated (Fig. 7).

Simultaneous with changes taking place in the wall of microsporangia, the primary sporogenous cells underwent mitosis, forming secondary sporogenous cells, from which microsporocytes were derived. Meiosis in each microsporocyte resulted in a microspore tetrad. The cytokinesis is of the simultaneous type. Microspore tetrads were tetrahedral (Figs. 3,5).

MALE GAMETOGENESIS

Microspores were separated from the tetrad as a uninucleate pollen grain (Fig. 4). Each microspore had a dense cytoplasm with a prominent and centrally placed nucleus. As the central vacuole developed, the nucleus took a peripheral position. The first division of the microspore nucleus resulted in the formation of two unequal cells, a large vegetative one and a smaller generative one. The generative cell gave rise to two sperms by mitosis. Pollen grains were 3-celled at time of anther dehiscence (Fig. 6).

MEGASPOROGENESIS AND FEMALE GAMETOGENESIS

The ovary was superior, bicarpellary, syncarpous, and unilocular with parietal placentae. There were 8 rows of ovules in the transection of ovary (Fig. 8). The integument was initiated by periclinal and oblique division at the base of nucellus. The ovule of *Crawfurdia delavayi* is unitegmic. The integument reached the top of the nucellus and formed a micropyle by continued division. The type of ovule is anatropous (Fig. 9).

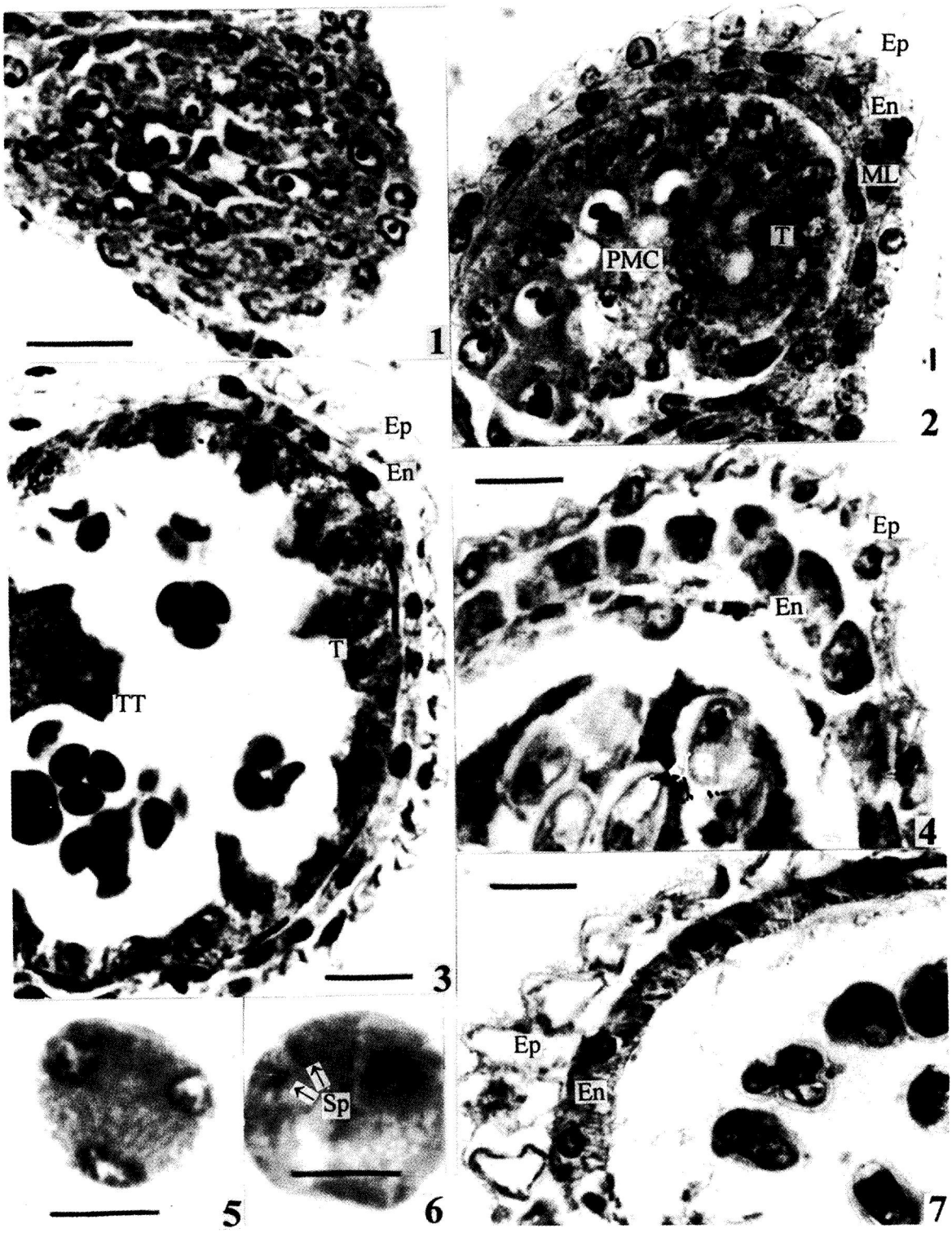
At the stage of microsporocyte, a single hypodermal archesporial cell differentiated in the young nucellus and functioned directly as the megasporocyte (Fig. 11) which was characterized by a large nucleus and dense cytoplasm. Thus, the ovule of *C. delavayi* was tenuinucellate. The megasporocyte underwent meiosis, forming a linear tetrad of megaspores (Fig. 13). The three micropylar megaspores eventually degenerated, while the chalazal one became functional (Fig. 13).

A 7- celled and 8-nucleate female gametophyte of the *Polygonum* type formed by three mitotic divisions of the functional megaspore (Figs. 14,15). The three micropylar nuclei became the egg and the two synergids. The two median nuclei became the polar nuclei. The chalazal nuclei became the three antipodals. The polar nuclei fused at the center and the resulting secondary nucleus then moved close to the egg apparatus (Figs. 16–18).

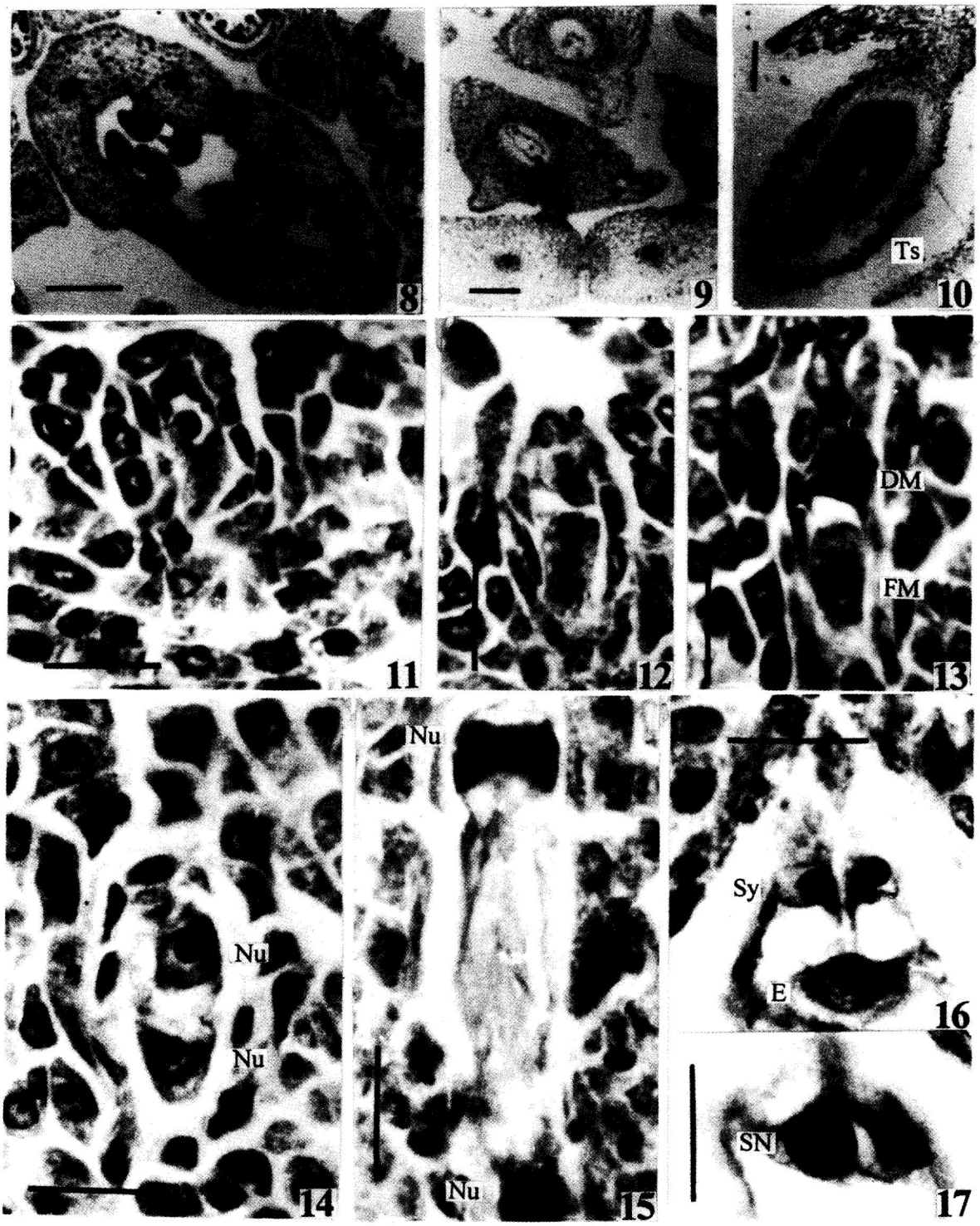
In the mature 8-nucleate embryo sac, the egg cell was recognized by the presence of a nucleus at the chalazal end and a large vacuole at the micropylar end. The two synergids had obvious filiform apparatus (Fig. 16) and were recognized by their nuclei at the micropylar end and a large vacuole at the chalazal end. The three antipodal cells were stained densely and their nuclei each divided into two (Fig. 18). The antipodal cells persisted until the stage of a 4-celled proembryo.

FERTILIZATION

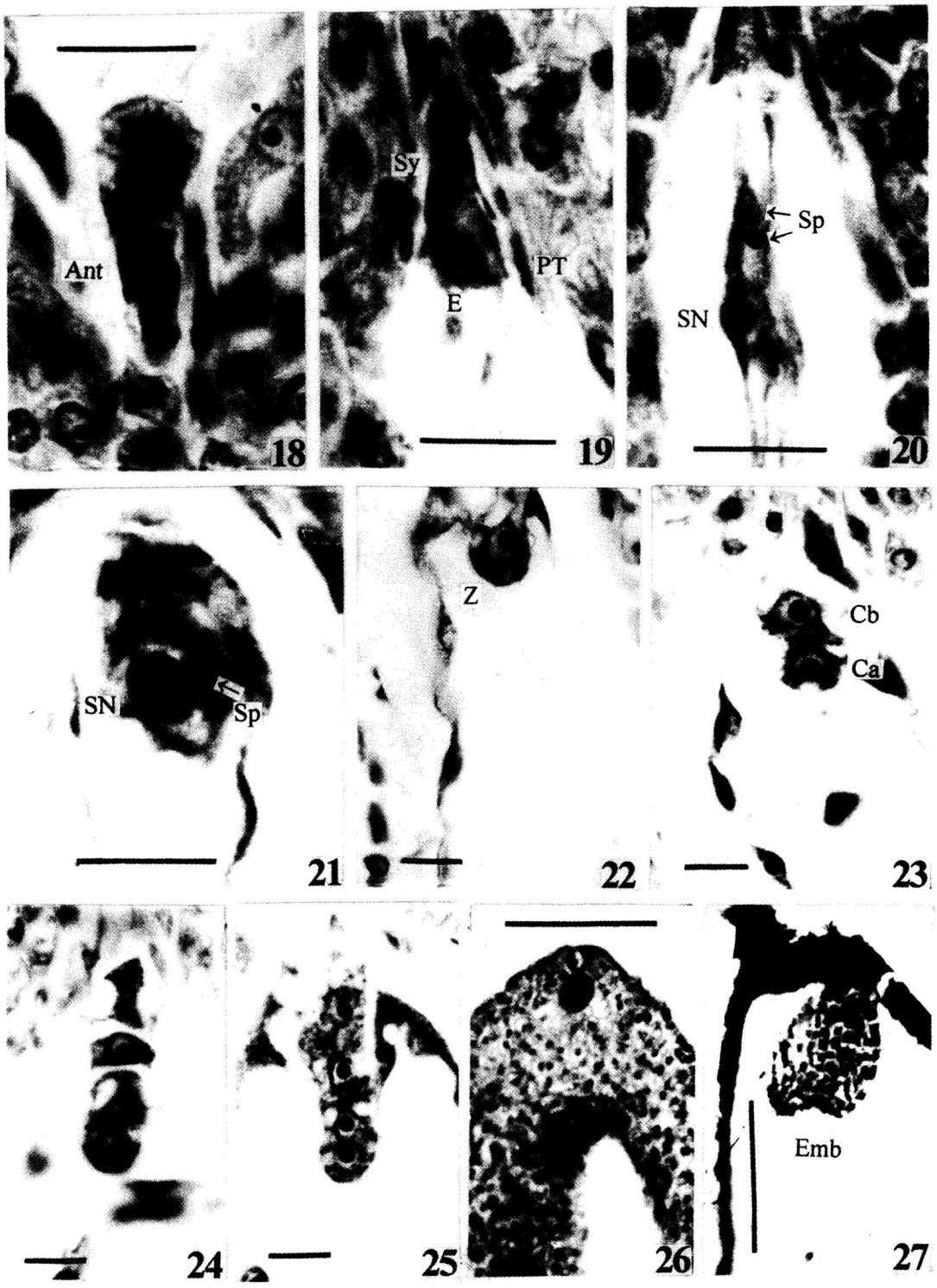
The fertilization was porogamous. The pollen tube entered the megagametophyte between the synergid and embryo sac wall (Fig. 19) and discharged two sperms between the egg and the secondary nucleus (Fig. 20). At this time the two synergids began to degenerate (Fig. 21). One of the sperms fused with the egg nucleus, forming the zygote, and the other, with the



Figs. 1–7. Anther and microsporogenesis of *Crawfurdia*. (1) Sporogenous cells stage (PMC, Pollen mother cell or Microsporocyte). (2) Four layers of anther wall cells: epidermis (Ep), endothecium (En), middle layer (ML), and tapetum (T). (3) Tetrahedral tetrad (TT) and anther wall. (4) Anaphase II of meiosis in microsporocytes. (5) 3-celled pollen grains. (6) Anther wall of 1-nucleate pollen grain and nucleus near the wall (Sp, Sperm). (7) Anther wall, showing fibrous thickening of epidermis (Ep) and pillar elongation and degenerated endothecium (En). Scale bars indicate 20 μm in Figs. 1–3,6,7, and 10 μm in Figs. 4,5.



Figs. 8–17. Ovaries, early development of the embryo sac, and mature embryo sac of *Crawfurdia.* (8) Transverse section showing eight rows of ovules. (9) Showing anatropous ovule. (10) Showing the testa (Ts) and seed wing. (11) A unitegmic ovule and a megasporocyte. (12) Anaphase II of meiosis in microsporocytes. (13) The functional chalazal megaspore (FM), with the other three degenerating (DM). A one-nucleate embryo sac and showing the other three degenerated megaspores (DM). (14) The two-nucleate (Nu) embryo sac. (15) The four-nucleate embryo sac (Nu, Nucleus). (16,17) Consecutive transections of an 8-nucleate embryo sac showing an egg (E), two synergids (Sy), and secondary nucleus (SN). Scale bars indicate 200 μm in Figs. 8–10, and 20 μm in Figs. 11–17.



Figs. 18–27. Mature embryo sac, fertilization, ovule tape, development of the embryo, and endosperm of *Crawfurdia.* (18) Consecutive transections of 16,17 showing antipodal cells (Ant). (19) Showing pollen tube (PT, Pollen tube; Sy, Synergid; E, Egg). (20) Secondary nucleus (SN) and two sperms (Sp). (21) The sperm in the secondary nucleus (Sp, Sperm; SN, Secondary nucleus). (22) Zygote (Z). (23) The terminal cell (Ca) and the basal cell (Cb), showing a two-celled proembryo. (24) A linear 4-celled proembryo. (25) A linear 6-celled proembryo. (26) Walls formed in free endosperm nuclei. (27) Embryo (Emb) at the late cardio-shaped stage when seeds are released from capsule. Scale bars indicate 20 μm in Figs. 18–25, and 200 μm in Figs. 26,27.

secondary nucleus, forming the primary endosperm nucleus (Fig. 21). The synergids degenerated completely after fertilization. The primary endosperm nucleus is larger than the zygote nucleus. The first division of the primary endosperm nucleus preceded that of the zygote.

ENDOSPERM

The development of endosperm of *C. delavayi* is of the nuclear type. The primary endosperm nucleus gave rise to two free endosperm nuclei. A large number of free nuclei formed by a series of successive divisions of the two free endosperm nuclei (Fig. 22). At the stage of multicelled proembyo, wall formation of endosperm cells initiated from the micropylar end to the chalazal end. After the formation of the endosperm cell wall, endosperm cells moved to the center of the embryo sac and surrounded the proembryo (Fig. 26). A few of the endosperm cells were absorbed by the embryo during its development.

EMBRYO AND SEED COAT

The zygote had a large nucleus, conspicuous nucleolus, dense cytoplasm, and small vacuole (Fig. 22). The zygote divided transversely, forming a terminal cell (Ca) and a basal cell (Cb). The Ca and Cb (Fig. 23) underwent transverse division forming a 4-celled linear proembryo; these cells are designated L, L′, M, Ci (Fig. 24). The L and L′ divided transversely forming a 6-celled linear proembryo (Fig. 25); these are designated L_1, L_2, L_1', L_2', M, Ci. The L_1 and L_2 divided vertically and transversely forming primordia of cotyledons (Pco), stem apex (Pvt), and hypocotyl (Phy). By vertical and transverse divisions, the cells L_1' and L_2' gave rise to the primordia of the central cylinder of the stem (Icc), central cylinder of the root (Iec), and root cap (Co). The cells M and Ci produced the suspensor (S) by vertical and transverse divisions (Fig. 25).

Thus, in this species, the cell Cb of the 2-celled proembryo did not contribute to the formation of the entire dicotyledonary embryo. The cell L of the 4-celled proembryo contributed to the development of cotyledons, stem apex, and hypocotyls. The proembryo after three cycles of divisions was composed of 6 cells. The embryogeny corresponds to the *Physalis* variation of Solanad type (Johansen, 1950).

In mature seeds, the embryo was cardio-shaped (Fig. 27). During the development of the embryo sac, integument cells at both the micropylar and chalazal ends divided, forming an outgrowth which gave rise to the wing (Fig. 10). The epidermis of the integument became the seed coat (Fig. 10). The inner layers of the integument were absorbed.

DISCUSSION

Crawfurdia was placed in the genus *Gentiana* by Marquand (1931, 1937). He and Liu (1999) summarize embryological characters of *Gentiana*: tetrasporangiate anthers; dicotyledonous type of microsporangium development; dual tapetal origin; binucleate and multinucleate tapetal cells; trabeculae and placenoids formed by division of tapetal cells; glandular tapetum, 2 middle layers; persistent epidermis in the mature anther; simultaneous cytokinesis at meiosis of the microsporocytes; tetrahedral microspore tetrads; 2- or 3-celled pollen; superior, bicarpellary, and unilocular ovary with superficial placentae; anatropous, unitegmic, and tenuinucellar, ovules 20–30 in number; *Polygonum* type of megagametophytes; porogamous fertilization; nuclear endosperm; embryogeny of the *Physalis* variation of Solanad type; globular embryo in mature seeds. Although there are numerous similar embryological characters between *Crawfurdia* and *Gentiana* (Table 1), *Crawfurdia* is different from *Gentiana* in the embryology: (1) in the anther wall, *Crawfurdia* has one middle layer, fibrous epidermis, while *Gentiana* has two middle layers, fibrous endothecium and (2) in differentiation of the tapetum, *Crawfurdia* has a single origin of tapetum which shows radial elongation or periclinal division and

Table 1
Comparison of embryological characters of *Crawfurdia* with *Gentiana*

Characters	*Crawfurdia*	*Gentiana*
Anther		
Number of sporangia	4	4
Epidermis	persistent	reduced
Endothecium	reduced	persistent
Middle layers	1	2
Origin	single	dual
Tapetum type	glandular	glandular
Placentoid	developed	well developed
Ovule		
No. of ovule rows	8	10–30
Type	anatropous	anatropous
No. of integuments	1	1
No. of archesporia	1	1
No. of antipodal cells	3	3 (4,8)
Seeds		
Type of endosperm formation	nuclear	nuclear
Type of embryogeny	solanad subtype physalis II	solanad subtype physalis II
Appendages of seed	winged	wingless (rarely winged)
Exotesta	1	1
Endotesta	absent	absent

intrusion into the anther locule, while *Gentiana* has a dual origin of tapetum, and tapetal cells form trabeculae (Eames, 1961) and placenoids (Steffen and Landmann, 1958). (3) *Crawfurdia* has 8 rows of ovules and typical parietal placentae, whereas *Gentiana* has 20–30 rows of anatropous ovules and superficial placentae (Gopal and Puri, 1962). The comparison of embryological characters between *Crawfurdia* and *Gentiana* indicates that they are significantly different in embryology, and supports the treatment of *Crawfurdia* as a distinct genus.

The evolutionary trends of embryological characters have been elucidated by many authors (Johri, 1984; Johri et al., 1992; Tobe, 1989, and others). There exist both advanced and primitive characters in a single genus, *Crawfurdia* or *Gentiana*. Thus, the systematic relationships of *Crawfurdia* and *Gentiana* can not be discussed only on the basis of embryological characters.

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