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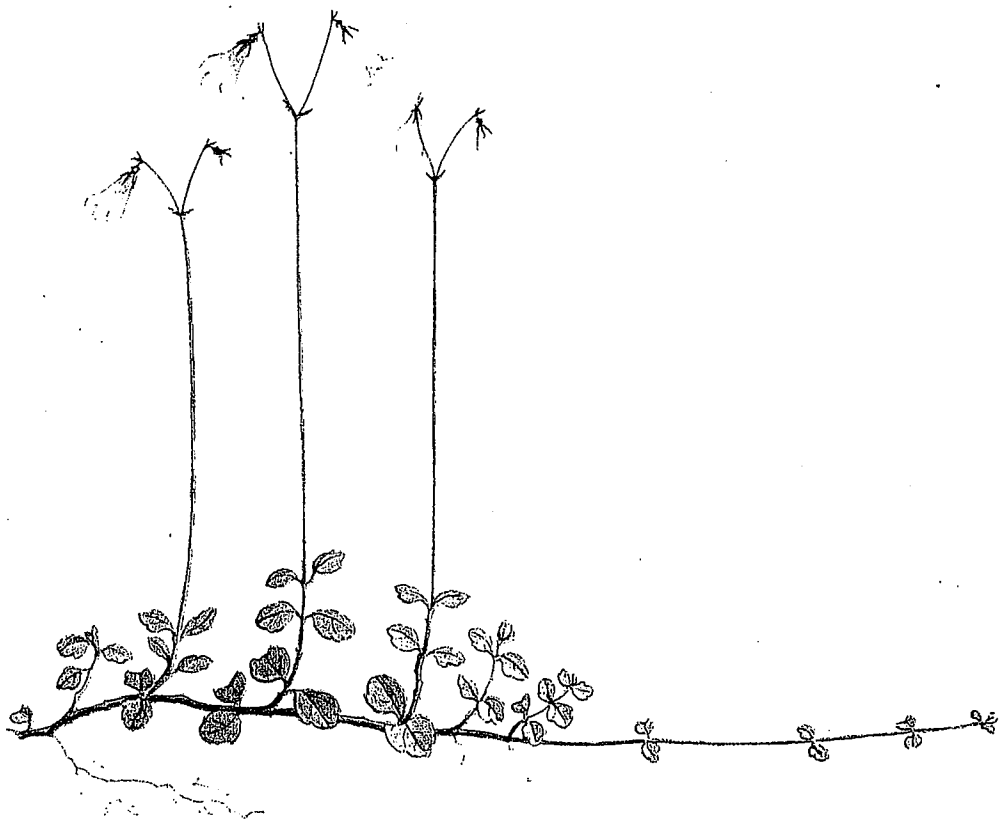

Nordic Journal of Botany

Embryological studies in Sanango racemosum (Loganiaceae s.l.)

S. Maldonado, M. Otegui and J. P. Bozini

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An embryological study was undertaken in the Amazonian genus *Sanango* in an attempt to help clarify the relationship of the genus. Detailed description of microsporangium, microsporogenesis, microspore, ovule, megasporogenesis, embryo sac and development of the endosperm are presented. The results confirm that *Sanango* is undoubtedly related to the Scrophulariales but it remains to be determined what the most satisfactory assignment of the genus should be. On the basis of early endosperm development, *Sanango* appears as closely related to the Gesneriaceae as well to the Scrophulariaceae. Further studies of embryonic stages and seed coat development would be helpful in clarifying this relationship. The other embryological features studied here seem not to be of taxonomic importance. A closer relationship with the Loganiaceae (Gentianales) is improbable.

S. Maldonado, Instituto de Recursos Biológicos. INTA. 1712. Villa Udaondo. Castelar. Argentina. – M. Otegui, Facultad de C. Nat. y Museo. Universidad Nacional de La Plata. 1900. La Plata. Argentina. – J. P. Bozini, Instituto Nacional de Microbiología. Vélez Sarsfield 563. 1281. Buenos Aires. Argentina.

Introduction

The monotypic genus *Sanango* has a restricted distribution in South America having been found in Peru (Bunting & Duke 1961) and Ecuador (Norman 1994). It is classed in the tribe Buddlejeae of the Loganiaceae when the family is considered in the broad sense (Bunting & Duke 1961; Leeuwenberg 1980) and in the Buddlejaceae when the tribe is given family status in the Scrophulariales (Cronquist 1981). *Sanango* has also been included in Scrophulariaceae (Takhtajan 1966) and more recently - after studies on anatomy (Dickison 1994), morphology (Norman 1994) and chemotaxonomy (Jensen 1994) - in Gesneriaceae (Wiehler 1994).

Loganiaceae s. l. exhibits remarkable variation in some of its embryological features (Table 1). The division into four families, Loganiaceae s. str., Desfontainiaceae, Buddlejaceae and Retziaceae, appears to be justified on the presence or absence of an integumentary tapetum (endothelium), endosperm haustoria and the pattern of endosperm and embryo development (Engell 1987; Maldonado de Magnano 1986a, b; 1987; Maldonado 1989)

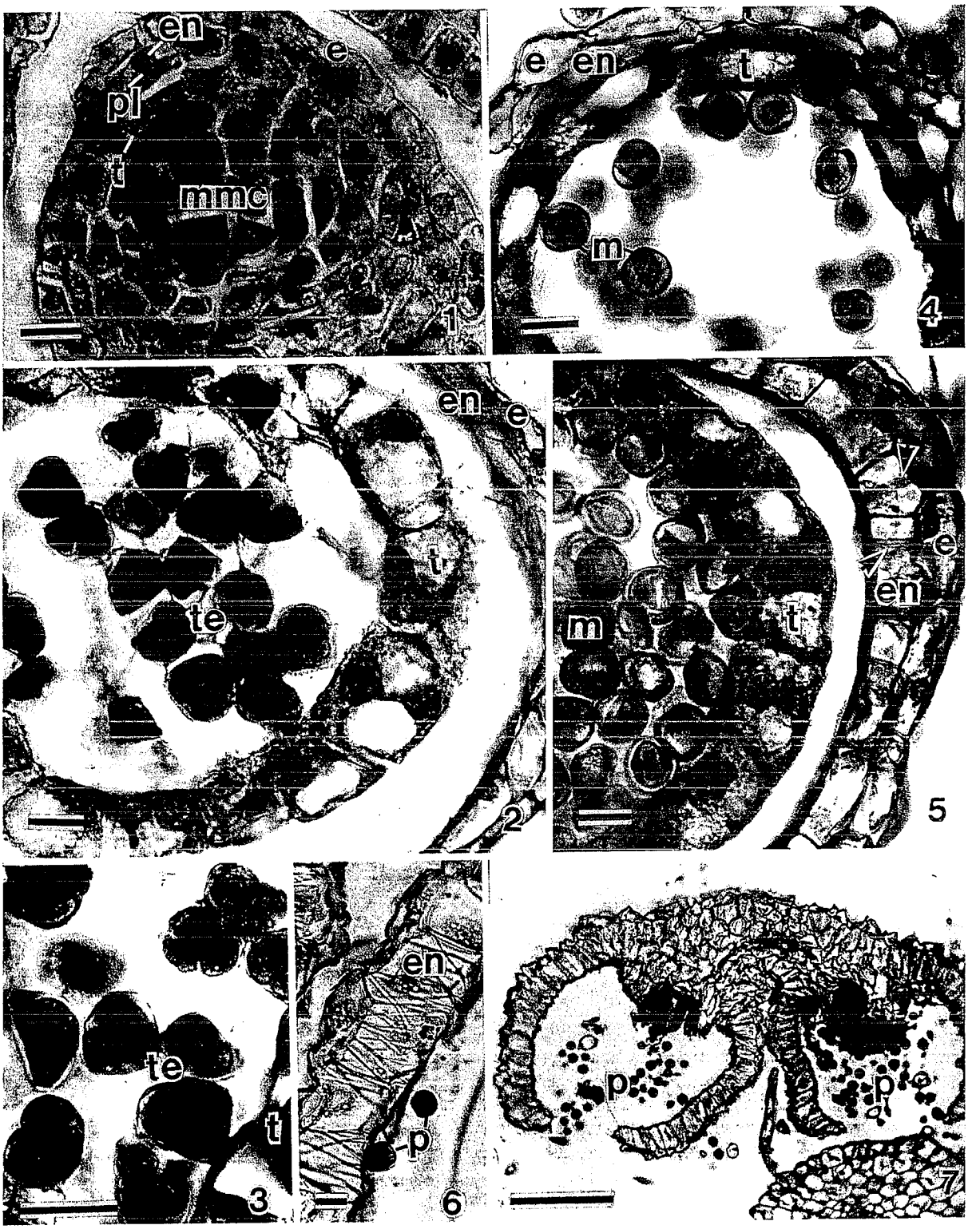
The present communication is the first report on the embryology of *Sanango racemosum* (Ruiz & Pav.) Barringer. It describes the structure of anther and ovule, megasporogenesis, microsporogenesis, development of gametophytes and the first steps of endosperm development. Some other aspects related to the embryology, including ultrastructural studies, will be publisher elsewhere.

Material and methods

Material

Sanango racemosum Ecuador. Napo: Jatun Sacha, 21 Sep 1990 Neill 9458 (DLF, MO, US)

Accepted 14-9-1997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Specimen preparation

Inflorescences in different stages of development were collected and fixed in FAA. Flowers were partially dissected, dehydrated and embedded in Paraplast. Longitudinal and transverse sections were cut at 6-8 µm. Some flowers were embedded in Spurr's resin and sectioned at 1-2 µm. The staining procedures also included: acid fuchsin and toluidine blue (Pearse 1985); fast green FCF (Fulcher et al. 1972); iodine potassium iodide and periodic acid-Schiff (PAS) (O'Brien & McCully 1981).

Results

Microsporangium, microsporogenesis and microspore

The anther is tetrasporangiate. The archesporium is hypodermic and extends throughout the entire length of a young four-lobed anther. The initial archesporial cells divide and produce the sporogenous tissue after cutting off the primary parietal layer. The cells of the primary parietal layer divide and form two secondary parietal layers. The wall formation conforms to the Dicotyledonous type of Davis (1966), i. e. the outer secondary layer divides to give rise to the endothecium and one middle layer and the inner layer functions directly as the tapetum (Fig. 1). The tapetum is glandular and its cells become two to four nucleate (Figs 2-5). The middle layer is ephemeral. U-shaped thickenings occur on the walls of the endothecial cells (Figs 5-7). Weak thickenings occur also in the connective endothecial cells adjacent to the pollen sacs.

Meiosis in the microspore mother cell (mmc) is accompanied by simultaneous cytokinesis giving rise to the tetrahedral tetrads (Figs 2, 3). The microspore has a very dense cytoplasm with a centrally situated nucleus. Then the cell increase in volume and a big vacuole is formed. The vacuolation is followed by a displacement of the nucleus from the centre to a place adjacent to the wall. Almost immediately the nucleus begins to divide. Division produces the vegetative and generative cells. Before anthesis the generative cell is located entirely within the cytoplasm of the first one. Pollen is 2-celled when shed.

Ovule

Numerous ovular primordia arise on the placenta and mature into anatropous, unitegmic and tenuinucellate ovules. An undifferentiated provascular strand runs through the middle of the raphe tissue. The single integument is both dermal and subdermal in origin (Figs 8-10) and consists of three cell layers, becoming more massive toward the micropyle, where there may be five or six cell layers (Fig. 11). An endothelium differentiates from the innermost layer of the integument at about the tetrad stage (Fig. 13). Its cells are radially elongated and the number of these (seven or eight) in transverse section remains constant from the initial differentiation (Fig. 20). Divisions occur in the other anticlinal plane, just increasing longitudinally the number of cells of the endothelium. This layer surrounds the chalazal two-thirds of the embryo sac (chalazal portion of the embryo sac, Fig. 16) and later, the endosperm (chalazal haustoria and endosperm proper, Figs 18, 19, 22, 23). Around the micropyle one third of the embryo sac (micropylar portion) the cells of the inner layer are flattened radially and get a dense cytoplasm as they mature (Figs 16, 17). The underlying cells in the micropylar region are also dense and starch storing (Figs 19, 22). At this time, a hypostase formed of thicker walled cells, usually starch storing, is formed in the chalaza (Fig. 16).

Megasporogenesis, megagametogenesis and fertilization

A single hypodermic cell differentiates in the tip of the young ovular primordium. It enlarges to become megaspore mother cell (Fig. 12) and has dense cytoplasm, a central nucleus and no vacuoles. The first meiotic division results in the production of two daughter cells, approximately equal in size. Often the micropylar dyad cell degenerates prior to the onset of meiosis II resulting in a triad of cells at the completion of the meiosis. The triad is composed of the degenerate micropylar dyad cell and two megaspores derived from the second division of the chalazal dyad cell. The megaspore at the chalazal end of the lineal triad or tetrad is the functional one; the other three megaspores soon degenerate (Fig. 13). Degeneration of the nucellar epidermis coincides with that of the micropylar megaspores.

Figs 1-7. Development of the anther and microspore in *Sanango racemosum*. – Fig. 1. A late sporogenous cell stage. – Fig. 2. Tetrad stage. – Fig. 3. Enlarged detail of Fig. 2. – Fig. 4. Early microspore stage. – Fig. 5. Middle microspore stage; endothecial cells with wall thickenings (arrows). – Fig. 6. Late microspore stage. Note that the anther wall is constituted only of epidermis and endothecium at this time. – Fig. 7. Transverse section of the anther after dehiscence. – Scale bars = 10 µm in Figs 1-6; = 100 µm in Fig. 7. – e, epidermis; en, endothecium; m, microspore; mmc, microspore mother cell; p, pollen; pl, parietal layer; t, tapetum: tc, tapetal cell; te, tetrads.

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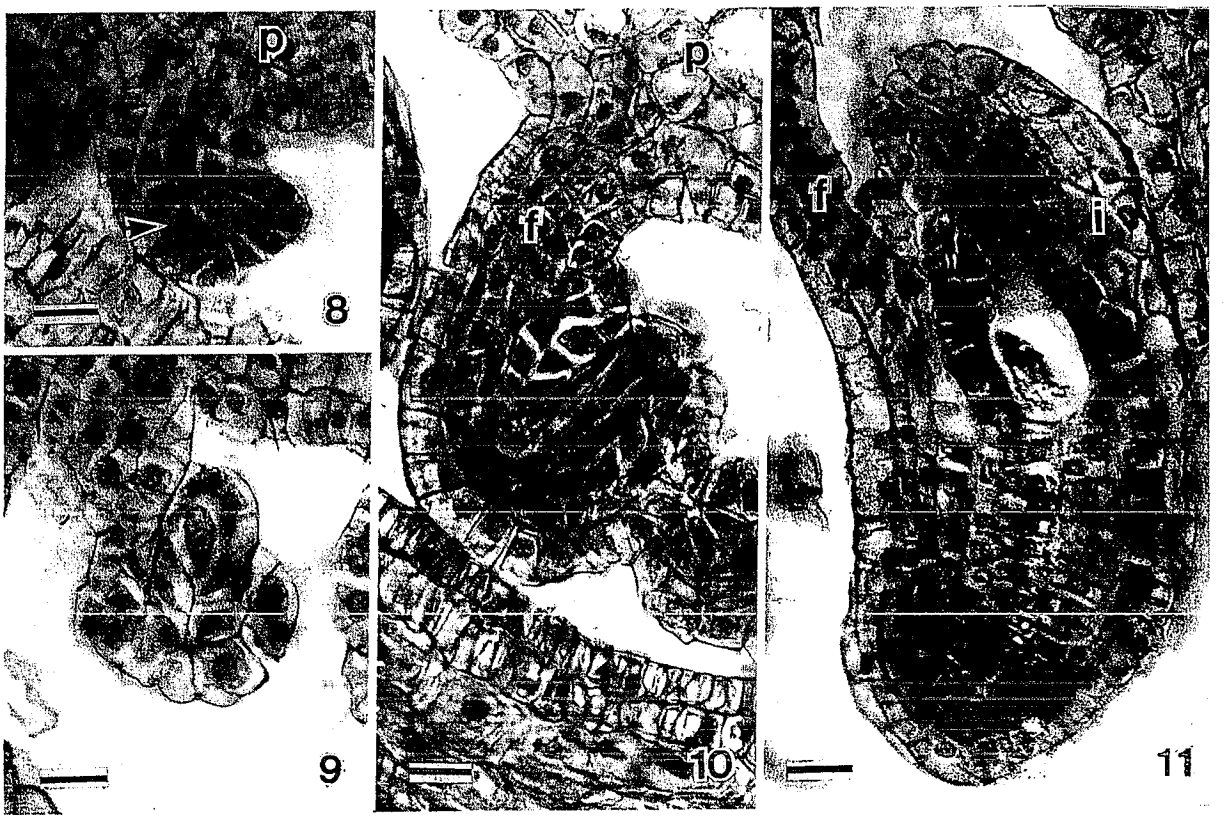
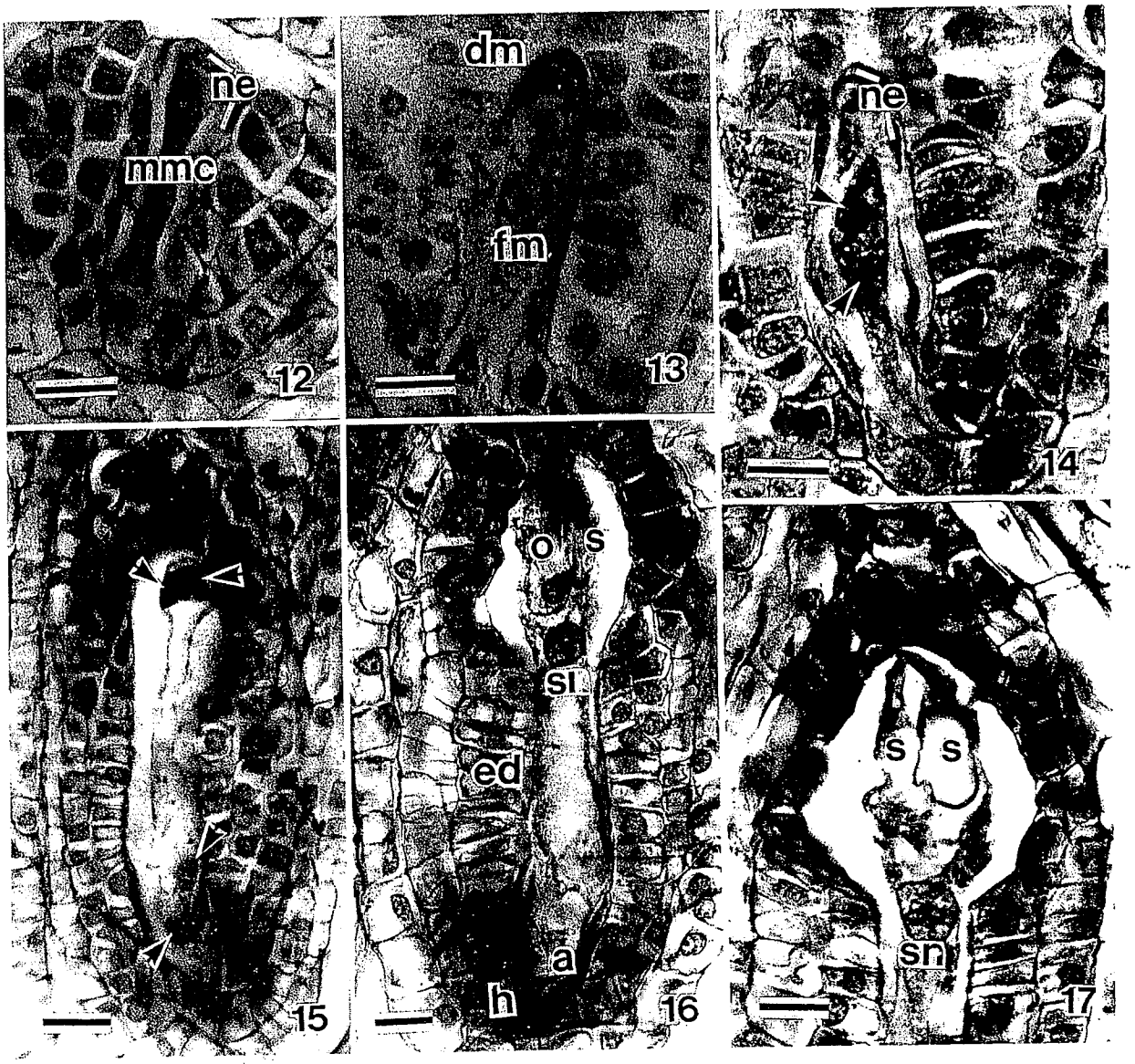


Figs 8-11. Ovule development in *Sanango racemosum*. – Figs 8-10. Ovule morphogenesis. The integument originates from both dermal and subdermal layers (arrow). – Fig. 11. Anatropous, unitegmic and tenuinucellate mature ovule. – Scale bar= 10 µm. – f, funicle; i; integument; p, placenta.

The mitotic division in the functional megaspore produces the two-nucleate megagametophyte (Fig. 14). It contains two juxtaposed nuclei and numerous small parietal vacuoles. The appearance of a large central vacuole coincides with the micropylar growth of the two-nucleate megagametophyte. A second free nuclear division produces the four-nucleate megagametophyte (Fig. 15) and it is followed by a third one producing the eight-nucleate megagametophyte. Before the formation of the cell walls the enlargement of the embryo sac is completed. The embryo sac development is according to the Polygonum type (Figs 13-16).

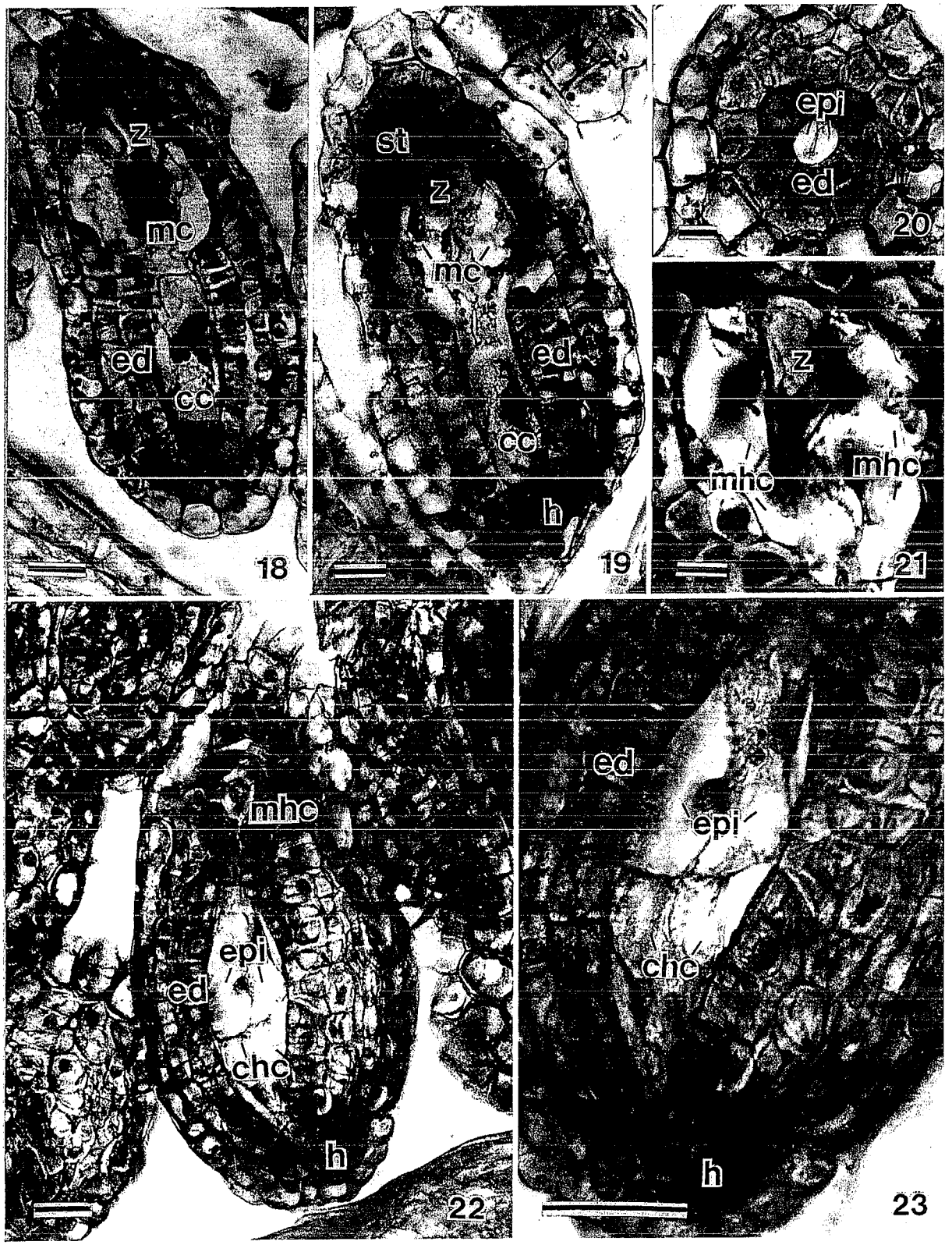
The mature embryo sac is characterized by two portions (Figs 16, 17); the narrower chalazal one is surrounded by the endothelium and encompasses the three antipodal cells and the chalazal portion of the central cell. The micropylar portion is broader, enclosed by a layer of flattened and starch storing cells and contains the egg apparatus and the micropylar portion of the central cell. The synergids are pyriform and prominently hooked (Fig. 17). A filiform apparatus has not been recognised in PAS tests. The nuclei of the synergids are located in the micropylar end and the vacuoles toward the central cell of the megagametophyte (Fig. 17). The micropylar two thirds of the egg cell is occupied mostly by a vacuole with the nucleus occurring chalazally (Fig. 16). The central cell occupies the bulk of the embryo sac (Fig. 16). The two polar nuclei are the largest nuclei in the megagametophyte, one of them is situated near the antipodals and the other one near the egg apparatus. The central cell is highly vacuolate with its cytoplasm confined to a thin layer along the embryo sac wall. In the mature megagametophyte the two polar nuclei are positioned in the micropylar portion, immediately to the egg apparatus. Fusion of the polar nuclei starts in the mature embryo sac but is completed only after the arrival of sperm nuclei. There is starch in the cytoplasm surrounding the polar nuclei. The starch is depleted during the first steps of endosperm development (Figs 24-26). The antipodal cells are small (Fig. 16). They degenerate shortly after fertilization. The pollen tube shows porogamous entrance and discharges its contents into one synergid. Syngamy and triple fusion occur almost simultaneously.



Figs 12-17. Megagametophyte development in *Sanango racemosum* (micropylar end up in all figures). – Fig. 12. Megaspore mother cell stage. – Fig. 13. Megaspore tetrad stage. – Fig. 14. Two-nucleate megagametophyte (arrows indicate the nuclei). –Fig. 15. Four-nucleate megagametophyte (arrows indicate the nuclei). – Fig. 16. Mature megagametophyte. – Fig. 17. Micropylar end of a mature megagametophyte. – Scale bars = 10 µm. – a, antipodal cells; dm, degenerated megaspores; o, egg cell: ed, endothelium; fm. functional megaspore; h, hypostase; mmc, megaspore mother cell; ne, nucellar epidermis: s. synergid; sn. secondary nucleus.

Development of the endosperm

Division of the primary endosperm nucleus precedes that of the zygote. Division is followed by wall formation resulting in two cells linearly arranged: a narrow chalazal and a broad micropylar one (Figs 18, 24). The second division is vertical (Figs 19 and 25) and it occurs almost synchronous in both cells resulting in four endosperm cells in two tiers. The chalazal tier functions as a two-celled haustorium. The two cells of the micropylar tier divide transversely and the two cells cut off toward the micropylar end also divide transversely. The four cells next to the zygote (Figs 21, 26) differentiate into a non aggressive micropylar haustorium while the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remaining two act as progenitors of the endosperm proper (Figs 22, 23, 26). Fig. 27 shows in a diagram the first four divisions in the endosperm. Endosperm development is thus ab initio cellular and it follows the cellular heteropolar type with longitudinal subtype, according to the typology of EODP system (Di Fulvio 1983)[1].

Discussion

Table 1 summarises the main embryological features that have been reported for genera belonging to the Loganiaceae s. l., *Desfontainia* differs from all other genera studied in having three or four middle layers in the anther wall, a crassinucellate ovule and cellular isopolar type of endosperm development, all features considered primitive. In recent literature *Desfontainia* has been considered the monotypic genus of the family Desfontainiaceae; the embryological studies (Maldonado de Magnano 1986 a) and the gene sequences (Bremer et al. 1994) both show that is not related to Loganiaceae and that *Desfontainia* definitely should be included in further and more extensive analysis of the Dipsacales. *Mitrasacme* also differs from other genera because endosperm development is derived from the transversal subtype of the cellular isopolar type and the haustoria are lateral, according to the typology of EODP system (Di Fulvio 1983). Because endosperm development is almost identical to that of *Phacelia parryi* (Hydrophyllaceae). *Mitrasacme* had been associated to Solanales (Maldonado de Magnano 1986 a). *Sanango*, like *Buddleja* and *Retzia* differs from *Fagraea*, *Spigelia* and *Strychnos* of the Loganiaceae where features such the presence of an hypostase, endothelium, haustoria and cellular development of the endosperm have never been recorded (excepting the doubtful reference about the presence of an endothelium in *Strychnos potatorum* and *Nicodemia diversifolia* made by Maheshwari Devi & Lakshminarayana (1980). All of these features have been found in the genera of the families Scrophulariaceae and Gesneriaceae that have been studied to date.

Endosperm development in *Sanango*, *Buddleja* and genera of Scrophulariaceae and Gesneriaceae all fall into the same general developmental group. *Sanango* differs from *Buddleja*, in that the cell divisions of the third and fourth mitotic cycles are transverse in the two micropylar cells of the four-celled stage. After the fourth mitosis, the endosperm is made up of eight cells: four cells disposed in two tiers that form the micropylar haustorium, one tier of cells which are the initials of the endosperm proper and finally a chalazal tier which constitutes the two-celled chalazal haustorium. By contrast, in *Buddleja* the third cycle is characterized by cell divisions which are longitudinal in both chalazal and micropylar cells. The fourth division occurs transversely in the four micropylar cells. Therefore, the micropylar haustorium is made up of four cells disposed in one tier: the endosperm proper constitutes four initial cells in one central tier and the chalazal haustorium is four celled. *Sanango* also differs from *Buddleja* in that its micropylar haustorium is non aggressive as compared to its being aggressive in *Buddleja*. Following Banerji (1961) *Buddleja* belongs to the *Verbascum* series and *Sanango* to the Pro-*Pedicularis* series, both series belonging to the Scrophulariaceae. The same pattern of endosperm development is found in the genus *Haberlea* of the Gesneriaceae (Johri et al. 1992).

In two other genera of which the taxonomic affinities are uncertain, *Polypremum* and *Retzia*, endosperm development is not fully known. Moore (1948) described the two first divisions in *Polypremum* and reported both chalazal and micropylar haustoria. Details were not included. Engell (1987) found cellular endosperm and terminal endosperm haustoria in *Retzia* but failed to work out the exact sequence of early endosperm formation. In order to determinate the position of either *Retzia* or *Polypremum* in Scrophulariales, an embryological investigation is needed.

The shape of the embryo sac in *Sanango* with a narrow chalazal portion and a broad micropylar portion is similar to what has been described for genera of Budlejaceae, Scrophulariaceae and Gesneriaceae (Arekal 1963a and b; Awasthi 1991; Bhandari 1976, 1985;

[1] The EODP system is based on: E, the cellular or nuclear nature of the endosperm development: O, the orientation of the wall formed during the first mitotic cycle; D, the destinity of the daughter cells produced by the first division; and P, the position of the walls produced during the second mitotic cycle.

Figs 18-23. First steps in the endosperm development of *Sanango racemosum*. – Fig. 18. Two-celled endosperm. – Fig. 19. Three-celled endosperm (chalazal cell has not divided yet). – Fig. 20. Transverse section of chalazal portion of the endosperm showing the two initial cells of the endosperm proper. – Fig. 21. Detail of the micropylar portion of a eight-celled endosperm showing the zygote and the four cells which become a micropylar haustorium (Other sections of the same ovule are shown in Figs 22 and 23). – Fig. 22. Longitudinal section of an eight-celled endosperm. – Fig. 23. Detail of Fig. 22. – Scale bar= 10 μm in Figs 20, 21; = 20 μm in Figs 18, 19. 22, 23. – cc, chalazal cell; chc, chalazal haustorium; ed, endothelium; epi, initial cells of endosperm proper; h. hypostase: mc, micropylar cells in the two-celled endosperm stage; mhc, micropylar haustorial cells: z, zygote).

Table 1. Embryology in Loganiaceae s. l.

Genera	*Buddleja*	*Desfontainia*	*Fagraea*	*Gelsemium*	*Geniostoma*
Anther					
Tapetum	secretory	secretory or ameboid	secretory		
Tapetal cells	1-nucleate	2-3-nucleate	multinucleate		
Middle layers	1 layer	3-4 layers			
Endothecium	1 layer	1 layer			
Microsporogenesis					
Tetrad type	decussate or tetrahedral	tetrahedral	decussate or tetrahedral		
Ovule					
Type	anatropous	anatropous	anatropous		
Tenui or crassinucellate	tenui-	crassi-	tenui-		
Hypostase*	+	0	0		
Endothelium*	+	+	0	0	0
Megasporogenesis					
Parietal cell*	0	+			
Tetrad type	linear	linear	linear		
Functional megaspore	chalazal	chalazal or subchalazal	chalazal		
Megagametophyte development					
Type	*Polygonum*	*Polygonum*	*Polygonum*		
Endosperm development					
Megatype	cellular	cellular	nuclear		
Type	heteropolar micropylar	isopolar			
Subtype	longitudinal	longitudinal			
Primary haustoria	terminal	0			
Secondary haustoria	terminal	terminal			
References	Dop 1913 Dop 1923 Crété 1942 Bendre 1975 Maldonado 1986 b	Maldonado 1986	Mohrbutter 1937a	Dahlgren 1922	Dahlgren 1922

+ present, 0 absent

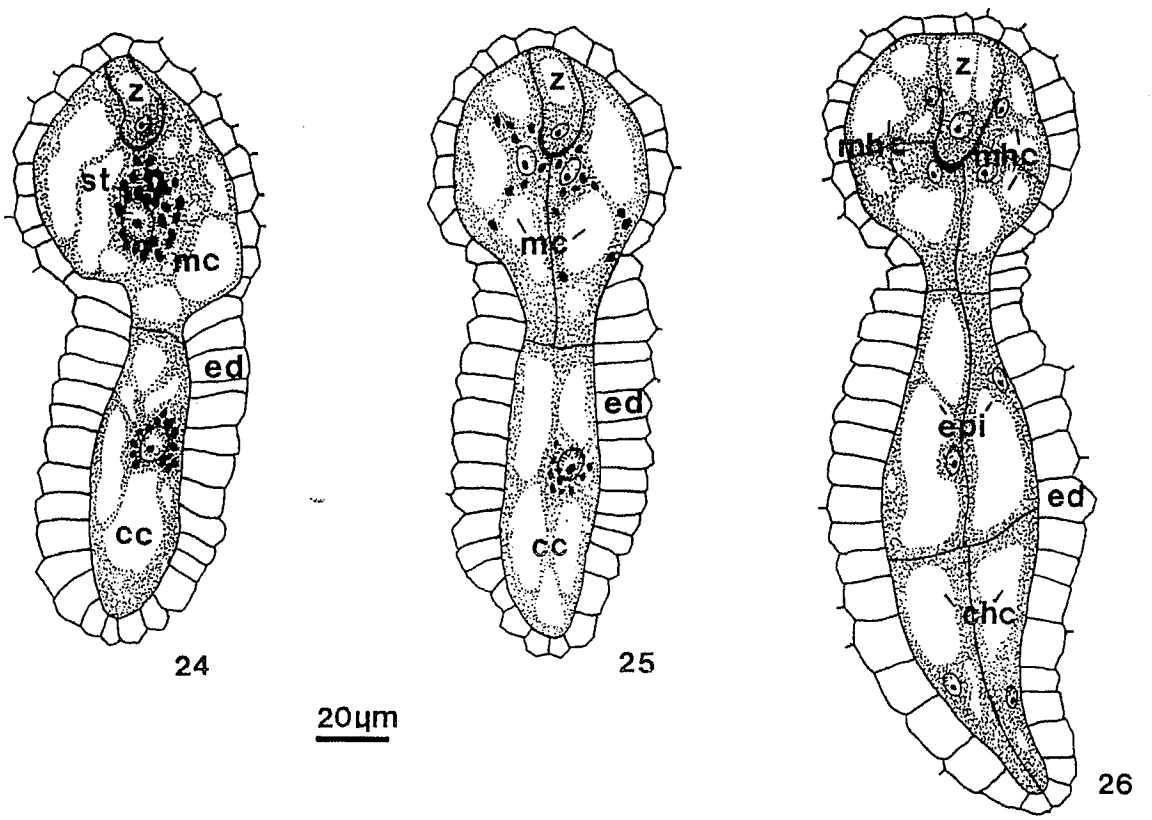
Crété 1951; Johri et al. 1992; Kapoor et al. 1976; Krishna Iyengar 1939, 1942; Maldonado de Magnano 1986b; Natesh & Bhandari 1975; Schertz 1919; Schmid 1906; Vijayaraghavan & Ratnaparkhi 1972; Vijayaraghavan & Prabhakar 1984). Examination of Engell's illustration of the embryo sac in *Retzia* shows a similar shape (Engell 1987).

With regard to the embryo and seed coat, development in *Buddleja* is identical with that in *Scrophularia himalensis*, one of the genera belonging to

Mitrasacme	*Nicodemia*	*Polypremum*	*Retzia*	*Sanango*	*Spigelia*	*Strychnos*
ameboid	secretory			secretory	secretory	secretory
	2-nucleate 1 layer 1 layer			2-4 -nucleate 1 layer 1 layer	1- nucleate 1 layer 1 layer	2 -nucleate 2 layers 1-2 layers
	isobilateral or tetrahedral			tetrahedral	tetrahedral	tetrahedral
hemianatropous	hemi- or anatropous	amphitropus	anatropus	anatropus	hemianatropus	hemi- or anatropus
tenui-	tenui-	tenui-	tenui-	tenui-	tenui-	tenui-
0	+	0 +	+ +	+ +	0 0	0 0
0 linear chalazal	0 linear chalazal	0 linear chalazal	0 linear	0 linear chalazal	0 linear chalazal	0 linear chalazal
Polygonum	*Polygonum*	*Polygonum*	*Polygonum*	*Polygonum*	*Polygonum*	*Polygonum*
intermediate derivative from cellular-isopolar	cellular	cellular	cellular	cellular	nuclear	nuclear
isopolar		heteropolar		heteropolar	isopolar	
transversal lateral		longitudinal terminal terminal	terminal	longitudinal terminal	0 0	
Yamazaki 1963	Maheshwari Devi & Lakshminaravana 1980	Moore 1948	Engell 1987	Maldonado et al., present study	Maldonado 1989 Dahlgren 1922	Dahlgren 1922 Maheshwari Devi & Lakshhiminaravana 1980

the *Verbascum* taxonomic series of Banerji (Maldonado de Magnano 1987). These aspects have not yet been studied in *Sanango*.

As far as the microsporangium is concerned, there is variation in the number of nuclei present in the tapetal cells. *Buddleja* (Bendre 1975) with 1-nucleate tapetum differs from *Sanango* and genera of Scrophulariaceae that have been studied, which all have two to four nucleate tapetal cells. A reinvestigation in this aspect appears necessary.



Figs 24-26. First steps in the endosperm development of *Sanango racemosum* (drawings corresponds to Figs 18, 19 and 21-23, respectively). – Fig. 24. Two-celled endosperm. – Fig. 25. Three-celled endosperm. – Fig. 26. Eight-celled endosperm stage. – Scale bar= 20 µm. – cc, chalazal cell; chc, chalazal haustorium; ed, endothelium; epi, initial cells of endosperm proper; mc, micropylar cells in the two-celled endosperm stage; mhc, micropylar haustorial cells; st, starch grains; z, zygote.

Conclusion

The studies of *Sanango racemosum* (Ruiz & Pav.) Barringer by Norman (1994) Dickison (1994) and Jensen (1994) have revealed morphological, anatomical and chemical features supporting the view that the genus is related to the Gesneriaceae of the order Scrophulariales and excluding a closer relationship with the Loganiaceae (Gentianales).

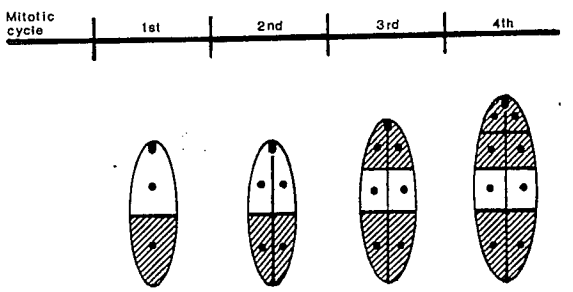


Fig. 27. Diagram of the first steps in the endosperm development in *Sanango racemosum*. Diagonal lines indicate haustorial cells.

The results of this embryological study confirm that *Sanango* is undoubtedly related to the Scrophulariales but it remains to be determined what the most satisfactory assignment of the genus should be. Actually, on the basis of early endosperm development *Sanango* appears as closely related to the Gesneriaceae as to the Scrophulariaceae. Further studies of embryonic stages and seed coat development would be helpful in clarifying this relationship. The other embryological features here studied seem not to have taxonomic importance.

Acknowledgements – The authors wish to thank Elianne Norman for bringing this problem to their attention and providing plant material.

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